THE EPIDEMIOLOGY AND CONTROL OF *ASCARIS LUMBRICOIDES* IN AN INDIAN FISHING COMMUNITY

by

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This thesis is dedicated to my wife Melissa -

friend, love and colleague.

ABSTRACT

The work reported in this thesis examined the epidemiology, population dynamics and control of Ascaris lumbricoides in a South Indian fishing village, Vairavan Kuppam (population=457). In January 1984, an initial collection of 24 hour stools was performed to determine pretreatment egg counts using the ethyl acetate/formalin concentration technique. All consenting villagers were then given anthelmintic treatment (pyrantel pamoate), and their stools were collected for 48 hours following treatment for the recovery of the expelled Ascaris. Surveys of 24 hour whole stools were completed every 3 months to monitor the process of reinfection. At the end of an 11 month period of reinfection, all cooperating villagers were again provided with treatment and the expelled Ascaris were collected. All Ascaris recovered from post-treatment stools were counted, measured, weighed and sexed.

The horizontal trends of Ascaris prevalence and intensity of infection with age recorded in this study are typical for intestinal helminths in communities with stable endemic infection. The prevalence increased rapidly with age and declined gradually in the teenage and adult classes. By contrast, following a rapid rise in intensity of infection up to 9 years of age, mean worm burdens fell sharply in adult age classes. The proportion of immature worms in the parasite populations recovered in each age class of the community did not vary with host age. There was no evidence that acquired resistance mechanisms influence the proportion of young worms in older people with long periods of past experience of Ascaris infection.

Following reinfection, similar patterns were observed for age prevalence and intensity, although the abundance had just reached 50% of the initial level. The stable age- and sex-determined pattern of infection supports the concept of targetted treatment. If treatment had been targetted toward infection in children aged 2 to 9 years, over 50% of the total Ascaris and the maximum source of egg production would have been eliminated. The rapid recovery of egg output in children indicates that treatment must be applied frequently. Treatment at longer intervals can actually increase egg output by altering the age distribution of the parasites and reducing the density-dependent regulation of egg fecundity.

The distribution of Ascaris within individuals in this community was highly aggregated, such that most people were lightly infected, and relatively few harboured the majority of the worm population in heavy infections. The similarity in the degree of parasite aggregation at initial treatment and following reinfection (as described by the negative binomial parameter k) for the total sampling population and for male and female hosts, was striking. At both sampling programmes, 20-24% of the population harboured 60-64% of the total worms of the community. There was a strong tendency for individual hosts to reacquire worm burdens of a similar magnitude relative to others. Analysis of individuals ranked by worm burden at both sampling points revealed a significant positive correlation between their initial and reinfection burdens. These correlations remained highly significant following standardization of the data by age group and sex. These observations confirm that individual characteristics, as well as group-shared factors, determine the intensity of Ascaris infection within the community.

Highly significant correlations between the scores for EPG, EPD and worm burdens of individuals were observed at both sampling points, supporting the use of egg counts as a relative method of identifying the most heavily infected people in the community for population-based anthelmintic treatment. Both positive and negative density-dependent relationships between parasite fecundity and parasite burden per host have been demonstrated for Ascaris infections in this community. Fecundity was reduced at low and high worm burdens as a consequence of mating probabilities and density-dependent restrictions, respectively.

The weight/length analysis of the recovered worms revealed a shift in the percent size distribution of the worm population at reinfection. In the absence of treatment children harboured a large number of low weight (young or stunted) worms while adult hosts carried worms of larger size. In contrast, the worms recovered following an 11 month period of reinfection from both children and adults were of consistently greater length and weight.

The value of field studies toward understanding the epidemiology of intestinal helminths in human communities and in the design of more cost-effective control programmes are discussed.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Defining the Research Problem

Ascaris lumbricoides, the human roundworm, is one of the largest and most prevalent parasitic nematodes of humans. Roughly one-quarter of the world's population is estimated to be infected with *Ascaris*, causing an annual global mortality of 20,000 people and an estimated morbidity of 1,000,000 cases (Walsh & Warren, 1979). The distribution of the diseases caused by this helminth extend throughout communities of poor and socio-economically deprived people in countries of the tropics and sub-tropics. Morbidity is particularly acute in children who are at greatest risk of heavy infection (Crompton, 1985). Nutritional deficiencies caused by poor diet are aggravated by high worm loads and these effects are particularly serious during the years of child development and growth (Tripathy *et al*, 1971; Stephenson, 1980; Stephenson *et al*, 1980; Brown *et al*, 1980).

Because *Ascaris* is primarily a source of morbidity as opposed to mortality, few attempts have been made to study the quantitative epidemiology of *Ascaris* relative to its high global prevalence and medical significance in the world today. Though the literature on *Ascaris* is substantial, the majority of the epidemiological research is largely descriptive in character with little emphasis on the population biology of *Ascaris*, despite the latter's significance to the design and implementation of control programmes. Recently a number of safe, low-cost, high efficacy anthelmintics have become

available (Seo et al, 1972; Migasena et al, 1978; Sinniah & Sinniah, 1981). These drugs can be used as tools, with due regard to ethical issues, in helping to understand the quantitative details of distribution, reinfection and population stability of intestinal parasites. In general anthelmintics cause rapid expellation of Ascaris from the host, allowing collection and analysis of worm burdens harboured by an individual host. This is a notable improvement over estimation of worm burdens by faecal egg output, due to the high degree of variability inherent in the latter measurement.

This thesis describes research undertaken to explore the epidemiology of Ascaris lumbricoides in village communities in South India, both in the natural endemic situation and after a period of reinfection following administration of blanket anthelmintic treatment. The principle aims of the study are threefold. The first is to gather basic information concerning the quantitative distribution of Ascaris in the community, its relationships to the host, and the dynamics of reinfection. The second is to determine whether certain groups or individuals are predisposed to heavy or light infection due to recurring non-random factors. The third, which will not be discussed in detail in this thesis, is to evaluate the relative efficacies of blanket, random and selective chemotherapy programmes in reducing the intensity of Ascaris in the community.

The remaining sections of this introduction will review the literature on Ascaris infection in humans to provide background information such as the medical importance, worldwide distribution and basic population ecology of the parasite in human communities. This information is of central importance in understanding the aims and conceptual issues underlying the research presented in this

dissertation. In Chapter 2, the overall design of the project, the field activities undertaken (stool collections, village meetings, health clinics, treatment regimes), the laboratory techniques used (egg counting, worm isolation) and methods of data analysis employed (data storage and analysis, statistical packages) are described. The procedures that were followed when solving typical problems which face an investigator setting up an active laboratory in India are explained with the aim of providing useful hints to future researchers interested in carrying out similar field work. Chapter 3 gives a basic description of the study area, including demography, social activities, cooperation levels and the major intestinal parasites detected in faecal analysis.

Results concerning the epidemiology of Ascaris in an endemic community, the distribution of Ascaris at reinfection and the recovery of egg output following treatment are presented in Chapters 4 and 5. Emphasis is placed on the age and sex of the host and on the individual with respect to their importance as determinants of worm intensity. Of particular interest in this analysis is the question of where individual hosts are predisposed to heavy or light infections with Ascaris. Chapter 6 describes the degree of density-dependent constraints on egg fecundity per mature female worm according to total worm burden. Chapter 7 presents data concerning the size distribution of male and female worms. These are examined within different sex and age groups at initial treatment and following 11 months reinfection. The general discussion examines the contribution of these results toward an understanding of the population dynamics of Ascaris lumbricoides in human communities. The implications of the research for the design of control programmes, particularly those based on selective and age-targetted anthelmintic treatment, are discussed.

1.2 Literature Review

1.2(i) The Parasite

Most of what is known about the general biology, physiology and biochemistry of *Ascaris lumbricoides* in man is based on research on *A. suum*, the roundworm of pigs. Research on methods of differentiating the two ascarids has been in progress since 1921, yet disagreement persists on whether they are two separate species, variants, or two biological races of a single species (Morishita, 1972; Gibson, 1983; Crompton & Pawlowski, 1985).

Sprent (1952), Ansel and Thibaut (1973) and Maung (1973) concluded that *A. lumbricoides* and *A. suum* are separate species based on morphological differences in dentition and the spicules of the male worms. These differences, however, are not always observed and may be dependent on the size and age of the worm (Morishita, 1972; Baird *et al*, 1986). Takada (1951) reported that 7 out of 9 volunteers began excreting fertile eggs in the faeces between 25 to 73 days following ingestion of *A. suum* eggs. Other researchers have repeated these experiments but have been unable to detect the development of the mature intestinal stage (Morishita, 1972). Taffs and Voller (1963) reported that the two ascarids share common surface antigens on both eggs and third stage larvae, and differentiation is not possible by immunofluorescence techniques using polyclonal antisera. However, recent biochemical analysis of the larval secretory and excretory antigens of the two ascarids have detected unique products in human *Ascaris* not shared by *A. suum* (Kennedy, Qureshi, Haswell-Elkins & Elkins, 1987; see Enclosures). Until *Ascaris* material from a variety of geographical regions is examined, it is not possible to determine

whether these differences are species-specific or reflect genetic variation within the same strain. Little is known about the role of A. suum in the epidemiology of human ascariasis, though it has been implicated in recent cases of ascariasis in Britain (Denham, 1984).

The life cycle of parasites belonging to the Ascaris genera are characterized by the lack of an intermediate host. Following the passage of unembryonated eggs in stools, the larvae undergo gametogenesis for 10 to 20 days after which the first larval moult occurs. Normal development of the Ascaris eggs occurs at temperatures ranging from 15°C to 34°C, though the speed of development varies with the incubation temperature (Morishita, 1972). Ascaris eggs have an almost legendary reputation for their ability to withstand a variety of chemical agents and temperatures as low as -30°C (Yamazaki et al, 1954; Schwartz, 1960). As temperatures rise above 35°C, the rate of desiccation increases exponentially, probably as a result of the destruction of the permeability barrier of the 4-layered egg shell. Rapid water loss occurs within seconds of exposure to 63° to 65°C, the probable melting point of the entire lipoprotein complex (Wharton, 1979). This tough barrier not only reduces desiccation at most natural temperatures, but it also prevents the penetration of various chemicals and anthelmintic agents applied to contaminated soil in attempts to inhibit transmission (Arfaa, 1978).

During embryonation the stool decomposes and the eggs mix with the surrounding soil. Eggs can also percolate into the soil profile as a result of rainwater (Storey & Phillips, 1985) or they can be mechanically mixed into the earth when nightsoil is used as fertilizer. Insects, such as dungbeetles, can also bury the stool a few millimetres deep within hours and prevent destruction of the eggs

by ultra-violet rays of the sun (Tadano et al, 1959). Alternatively, the faecal matter may dry, break up and become wind-borne, though information on the frequency of dust-borne infection with Ascaris is inadequate (WHO Expert Committee, 1967; Muttalib et al, 1980). In areas where human waste material is not used in farming, the persistent use of preferred defecation sites may assist in the establishment of infection where otherwise transmission would be hampered due to harsh environmental conditions (Chandler, 1927b; Elkins, 1984). The "seeding" of the soil around the households by the defecation of infected young children (commonly called the 'dooryard infection') also creates areas of intense transmission (Beaver, 1952; Faust et al, 1970; Chai et al, 1983).

When the human host ingests infective eggs, generally through contaminated hands, food or drinking water, the larvae begin a migratory transition from the second stage larvae to immature worm within the host, requiring a development time of approximately 14 days. A series of conditions in the host gut, such as elevated temperature, pH of the alimentary tract, and the relative concentrations of carbon dioxide and bicarbonate, stimulate the larvae to release a hatching fluid. This fluid weakens the internal shell, allowing the larvae to break through and escape into the gut environment (Rogers, 1960). The newly emerged larvae measure 0.2 to 0.3 mm in length (Morishita, 1972) and immediately penetrate the intestinal mucosa, migrate to the liver via the portal vessels and pass to the lungs. Research with A.suum in pigs has shown a negative relationship between the number and frequency of larvae ingested and the number which survive migration to become adults (Anderson et al, 1973). This is believed to be due to a dose-dependent host immune response, which can reduce but not eliminate worm establishment.

Following passage into the lungs the larvae break through the pulmonary capillaries into the air sacs, pass up the bronchioles, bronchi and trachea to the epiglottis where they are reswallowed by the host. During the lung passage the larvae moult twice, reach a length of 1.0 to 2.1 mm, and acquire resistance to gastric juice before they reach the stomach (Watson, 1965). Upon establishment of the fourth stage larvae in the duodenum the final moult occurs and the larval phase is complete.

After this moult, the immature worm undergoes a rapid increase in length during the initial 2 to 3 months in the intestine (Seo et al, 1980b). With sexual maturity, growth is characterized by a more pronounced increase in weight (Cho, 1977). Females exhibit a faster growth rate relative to males after maturity, and egg production is first detected when females reach 12.5 to 13.5 cm in length (Seo & Chai, 1980; Seo et al, 1980b; Seo, 1981). The quantity of eggs produced by females increases with body length up to approximately 25 cms. Egg production then plateaus and deteriorates after the worm reaches 28 cms in length (Chai et al, 1981). Length is closely correlated with age, hence fecundity is likely to be age-dependent. The capacity of A. lumbricoides for reproductive success is enormous; average egg output for females has been estimated at approximately 200,000 eggs per day (Faust et al, 1970). In terms of time, an increase in egg output per female occurs from 3 months following entry into the intestine to about 8 months, which is thought to be the average lifespan of female Ascaris (Seo, 1981; Chai et al, 1983). Variability in egg output can occur due to crowding. Female worms in lightly burdened hosts may produce fewer eggs per capita than females in heavily burdened hosts (Croll et al, 1982; Anderson, 1982; Anderson & May, 1985; Thein-Hlaing, 1985). Egg production by female worms also

fluctuates irregularly with time within individual hosts as a consequence of unknown factors, although host nutrition is thought to be important (Sinniah et al, 1980). This inherent variability in egg output from females, combined with the uneven distribution of eggs within a faecal sample, contributes to the inaccuracy of estimating worm burdens from egg counts (Martin, 1965; Sinniah et al, 1980; Sinniah, 1982).

1.2(ii) Ascariasis - The Disease

The detrimental effects to the host which result from infection by Ascaris arise from both the larval migratory stages and the mature worms in the gut. Though the majority of infections (approximately 85%) appear to be symptomless (Muller, 1975), the absolute number of fatal and diseased cases worldwide is high. In some countries Ascaris infections account for up to 5% of all surgical operations for acute abdominal disease with a mortality rate of 6 deaths per 100,000 (WHO Expert Committee, 1981). Symptoms of ascariasis are usually due to allergic reactions to the adult and larvae, the abrasive action of the adult worms on the intestinal tract and the wandering of the adult parasite from the gut into the biliary tract.

Allergic reactions, commonly called Loeffler's syndrome or pulmonary ascariasis, are first seen about one week following ingestion of eggs containing the infective second stage larvae. A pneumonitis with cough, dyspnea, substernal pain, fever, and blood stained sputum can occur, lasting approximately 10 days (Faust et al, 1970). These symptoms are followed by an eosinophilia in the peripheral blood which disappears after 5 to 6 days (Gelpi & Mustafa, 1967).

The severity and duration of the symptoms depends on the number of eggs ingested and the consistency of exposure to infection. The above symptomology generally occurs where an interrupted transmission exists. For example, Gelpi and Mustafa (1967) found a seasonal respiratory disorder among Saudi Arabs which occurred annually after the onset of a short rainy season following near drought conditions. The sharp seasonal incidence of the syndrome was found to be associated with the unusual climatic factors causing an interruption of the transmission of Ascaris. The authors suggested that the pathogenesis of the syndrome was due to hypersensitivity resulting from recurrent migrations of larvae. In areas where Ascaris is endemic throughout the year, the characteristic pneumonitis has not been observed (Cheah & Kan, 1972; Spillman, 1975), possibly due to a desensitization of the host, a phenomenon also seen in pigs (Kelly & Nayak, 1964).

Adult worms cause the symptomatology of ascariasis in the gut which can occur in moderate, massive, or very light infections. The most common complaints of moderate infections are abdominal pain, loss of appetite, colic, nausea and vomiting (Maki, 1961). More severe consequences of ascariasis are found when masses of worms form a bolus and block the intestine causing intussusception (in children), volvulus, hernial strangulation and torsion of the bowel requiring immediate surgery (WHO Expert Committee, 1967; Beaver, 1975)(Plate 1.1). Large numbers of Ascaris have also been found in the biliary ducts and liver (Lloyd, 1981), and in extreme cases migration continues to the heart, lungs and other organs (Daya et al, 1982; Baird et al, 1986). Rarely, infections of one to 5 worms can be dangerous if they penetrate the ampulla of Vater, the common bile duct or the pancreatic duct (Pinus, 1982). An analysis of biliary

Plate 1.1

Scars resulting from an operation to remove a bolus of Ascaris from the intestine of a young boy. The mother of this twelve year old boy from Lighthouse Kuppam informed the field team that the scars on his abdomen remained from the surgical operation performed in a government hospital when he was 4 years of age.



ascariasis cases from Formosa, West Germany and the Philippines found that female patients have a higher frequency of this condition than male patients (Morishita, 1972; Arcilla & Varilla, 1978). The reason for this migration has been explained as a consequence of the female parasite searching for a mate (Schmidt & Roberts, 1981). However, in 194 patients with biliary ascariasis in Japan, male worms were incriminated in a substantial proportion (39%) of the cases (Morishita, 1972). Studies of the behaviour of adult Ascaris in vitro reveal that the worms seem to have an instinctive tendency to penetrate small openings and may not require a definite external stimulus, such as anthelmintic drugs, spicy foods and fever, to enter excretion ducts (Morishita, 1972). Perforation of the bowel has also been reported with accompanying peritonitis. The outcome of the above conditions depends on the rapidity of diagnosis and the speed and skill of the surgeon, though the prognosis tends to be less favourable in cases of invasion of the biliary tract or perforation of the bowel (Faust et al, 1970; Pinus, 1982). A complete review of ascariasis and its clinical complications can be found in Craig and Faust's Clinical Parasitology (1970). The fatality rate due to Ascaris infection has been estimated at 0.02% (Muller, 1975), though such statistics may be highly unreliable due a lack of consistency in the reporting of cases in different hospitals, regions and countries (Peters, 1978).

1.2(iii) Relationship with Malnutrition

Malnutrition is a disease of extremely complex aetiology. The quantity and quality of diet, the ability to digest and absorb nutrients from the gastro-intestinal tract and the presence of concomitant infection influence nutritional status and hence growth and development of children (Taylor et al, 1979; Keusch, 1982;

Calloway, 1982; Beisel, 1982). Regions of the world where malnutrition is common are often typified by endemic diarrheal diseases, Ascaris and other intestinal helminths and protozoa. In contrast to the clear relationship between hookworm and iron deficiency anaemia, no conclusive evidence for human nutritional deficits directly related to Ascaris infection has been reported (Schultz, 1982; Pawlowski, 1982). In laboratory models few well controlled studies of experimental ascariasis have been carried out (Nesheim, 1985). Reduction of growth, food intake, nitrogen retention and lactase activity caused by A. suum infection in pigs has been demonstrated by Forsum et al (1981). The degree of impairment is correlated with both the magnitude of worm burden and the extent of protein deficiency in the diet. These results are based on a 16 infected and 6 uninfected swines on controlled diets. Veterinary studies of swine herds have clearly shown that in order to demonstrate a significant reduction in growth due to A. suum, pigs must be both heavily infected and on a low nutrition plane (Zimmerman et al, 1971; 1973; Nilsson, 1982).

In humans, most studies have been poorly designed with insufficient sample sizes and too little attention paid to statistical issues (Cerf et al, 1981; Schultz, 1982; Pawlowski & Arfaa, 1984). This is especially important considering the large individual variation in the indices of malnutrition (i.e. body weight and height, dietary intake, skinfold thickness, etc.) as well as the many disparate factors contributing to growth. Although the conclusions drawn from these studies are tenuous and often conflicting, in general they reflect the trends of animal experiments. Many authors have concluded that light Ascaris infections in well nourished children do not adversely affect growth (Tripathy et al, 1971; Layrisse & Vargas,

1975; Blumenthal & Schultz, 1976; Cerf et al, 1981; Kloetzel et al, 1982). It has also been suggested, and supported by A. suum studies, that even heavy Ascaris infections in well nourished children do not interfere with nutritional status (Cerf et al, 1981). Recent studies suggest that the converse is not true. Malnourished children with only light or moderate infections have been shown to benefit from anthelmintic treatment as measured by weight gain (Gupta et al, 1977; Willett et al, 1979) and improvement in triceps skinfold thickness (Stephenson et al, 1980).

Clinical studies have implicated Ascaris as detrimental to vitamin A absorption (Sivakumar & Reddy, 1975; Mahalanabis et al, 1976; 1979), riboflavin nutrition, serum albumin and Vitamin C levels (Blumenthal & Schultz, 1976), nitrogen and fat absorption (Venkatachalam & Patwardhan, 1953; Tripathy et al, 1971; Brown et al, 1980) and lactose digestion (Crompton, 1985). In some of these studies the severity of the defects are correlated with the intensity of infection. Improvement in the measured levels occurred following anthelmintic therapy (Crompton, 1985). The postulated mechanisms of these possible parasite-induced deficiencies are (1) reduction of host food intake due to abdominal pain and loss of appetite, (2) consumption of vital nutrients by the worms and (3) interference with absorption of nutrients from the jejunum through physical blockage and mucosal damage caused by the secretion of parasite enzymes which destroy host digestive enzymes (Jelliffe, 1953).

A clear definition of the relationship between childhood nutrition and ascariasis is regarded as a high priority by the WHO and in great need of further well conducted research (WHO Expert Committee, 1981; Pawlowski & Arfaa, 1984). More reseach is needed both at the clinical and the community level using large sample sizes to provide

statistical rigour. Also of interest is the investigation of the reciprocal effects of poor host nutrition on susceptibility to Ascaris infection and the growth and fecundity of worms.

1.2(iv) Epidemiology

Several large field studies of the epidemiology of intestinal helminths were carried out in the 1920's in India and other endemic countries by workers supported by foundations such as the International Health Board (International Health Board, 1922; Payne et al, 1923; Augustine et al, 1928; Chandler, 1927a; 1927b). Intensive interest in the global prevalence, intensity, egg count techniques, egg and larval ecology in the environment and pharmacology of anthelmintics was sparked by large-scale campaigns with the goal of controlling and eventually eradicating hookworm. Smillie (1922) and others advocated the importance of controlling the heaviest hookworm infections and therefore eliminating hookworm disease as opposed to the eradication of the parasite. This suggestion underpins the basis of selective chemotherapeutic treatment, a control strategy being considered today by many authors (Warren, 1981; Anderson & May, 1982; Pawlowski, 1984; Anderson, 1985). Of particular note in epidemiological studies is the series of articles by A.C. Chandler presenting the results of his extensive surveys of intestinal worms in India. Although his first interest was hookworm, Chandler put forward many ideas concerning the modes of transmission of Ascaris. Comparing house arrangements in villages, climatic factors, social interactions, defecation habits, soil compositions and water supplies in heavily and lightly infected regions, Chandler advocated that faecal contamination of the water supply is the main source of Ascaris transmission in South India (Chandler, 1927a; 1927b).

When it became clear that the control measures being employed were not effectively interrupting hookworm transmission and that eradication would be much more difficult than first believed, interest in helminth epidemiology waned in Western nations. In Japan, however, intensive analytical investigation of Ascaris biology, biochemistry and epidemiology was undertaken in the 1950's. The prevalence of Ascaris in Japan increased during World War II to over 60%, and the intensity was high in some areas as judged by autopsy reports (Morishita, 1972) and faecal collections following anthelmintic treatment (Yabumoto, 1948; Morishita et al, 1949). Yabumoto (1948) treated 1,028 individuals in one village and collected a mean of 42 worms per host from post-treatment faeces, with 89 cases passing over 100 worms. Following these early surveys in Japan, a large body of data concerning the effectiveness of various control strategies and local sources of infection was collected. A national control campaign based on the findings of this research was composed of three basic components: (1) administration of mass anthelmintic treatment to communities repeatedly, (2) destruction of helminth eggs in nightsoil before use as fertilizer and (3) encouragement of the population to wash vegetables with detergent before consumption (Morishita, 1972). This rigorously undertaken control programme was highly successful, eventually reducing the nationwide prevalence of Ascaris to approximately 1% in the late 1970's.

Following the founding of the Japanese Association of Parasite Control in 1955, a trio of workers, Fushimi, Komiya and Masuda published a series of articles on Ascaris epidemiology. Fushimi (1959a; 1959b; 1962; Fushimi & Nishimura, 1959) constructed a statistical model of the correlation between quantitative egg counts and the worm infection density within a community based on data from

faecal smear examinations. Komiya (1959; 1962) produced a series of papers which described some simple empirical models of the dynamics of reinfection through several rounds of mass chemotherapy. Masuda (1959) examined the theoretical and practical considerations of measuring the prevalence of unfertilized eggs as an index of reduced worm burden, an important consideration during the years 1955 to 1965 when the prevalence in Japan fell from 37% to 5%.

In 1964 the Republic of Korea formed a civilian health organization called the Korean Association for Parasite Eradication (KAPE) whose purpose was to control parasitic infections throughout the country. Between 1975 and 1985 Professor B.S. Seo and colleagues published a series of papers on Ascaris control and reinfection following chemotherapy based on egg and worm counts. These excellent community-level studies were based on worms counts collected following anthelmintic treatment with pyrantel pamoate. Using this technique, precise population-based studies were carried out to examine seasonality, optimal intervals between application of mass chemotherapy, duration of control efforts needed to eradicate Ascaris, worm growth and maturation time, reinfection rates by age and village prevalence and drug efficacy (Seo et al, 1969; 1979; 1980a; Cho, 1977; Seo & Chai, 1980). The efforts of KAPE have successfully reduced the prevalence of Ascaris in Korea from 55.4% in 1969 to 10.2% in 1981, primarily through a biannual national treatment programme directed toward students of the ages 6 to 16 (Seo, 1981).

Since 1978, a large body of research concerning the population dynamics and control of direct life cycle helminth parasites has been published (Anderson, 1980; 1982; Warren, 1981; Anderson & May, 1982). A theoretical framework has been developed through mathematical models to aid in the epidemiological study of helminth infections (Anderson &

May, 1982; 1985). This has been applied to research and control programmes for Ascaris in Iran (Croll et al, 1982), Burma (Thein-Hlaing et al, 1984) and India (Elkins et al, 1986). New theoretical and statistical techniques have helped to re-analyze previously published data to improve our understanding of the implications of the results. The models of Anderson and May (1982; 1985) are based on assumptions that the following five principal factors influence the population dynamics of the major helminths of man:

1) The average life expectancies of the host and parasite determine the relative time scales on which the dynamics of the host and parasite operate. In the environment the average lifespan of the mature Ascaris is believed to be longer than the infective eggs. Though the eggs may survive under ideal conditions (moist, shady areas) up to 14 years (Krasnonos, 1978), the average lifespan in less favourable environments may be only a few months (Croll et al, 1982; Anderson, 1982). The human host, in turn, has a lifespan several times that of the adult worm.

2) The growth of parasite populations within the host is restricted by density-dependent factors which reduce fecundity, survival and/or establishment as the parasite burden increases. These density-dependent constraints contribute to the stability of the parasite population by limiting growth at high transmission levels and may arise via host responses or limitation of resources.

3) The distribution of the parasites within the host population is highly aggregated such that the majority of the worms are harboured by a minority of individuals in the community (Li & Hsu, 1951; Crofton, 1971a; 1971b). The severity of density-dependent constraints on the parasite population is increased in highly aggregated distributions (Anderson, 1982), since a majority of the total worms are found in heavily burdened individuals.

4) Ascaris is dioecious and so the production of fertilized eggs is dependent upon the probability that female worms are successfully paired with mature males. Due to the polygamous nature of the parasite and its aggregated distribution, the reproductive success of the parasites is high even when the worm density is low. Thus, mating probabilities may play a minor role in contributing to population stability in endemic communities. During the successful control programmes of Japan and Korea where the mean worm burden was reduced to a low level, however, an increase in numbers of individuals passing only unfertilized eggs in the faeces was detected concomitantly with a decrease in those passing fertilized eggs (Masuda, 1959; Seo, 1980).

5) The basic reproductive rate (R_o) defines the transmission potential of the parasite in the community (Anderson, 1980; Anderson & May, 1982). This parameter is an estimate of the average number of female offspring produced throughout the lifetime of a mature female parasite which themselves achieve reproductive maturity in the absence of density-dependent constraints. When R_o is greater than one, density-dependent

regulation of fecundity and survival operate to stabilize the population. When R_o equals one, the population is at an equilibrium since each female exactly replaces itself in the next generation. This value, $R_o=1$, represents a threshold value since if $R_o<1$, the parasite is unable to maintain itself, and the population declines.

The mathematical relationships between these 5 main parameters can be used to predict reinfection rates and the potential success or failure of various chemotherapy regimes through the development of theoretical models (Anderson & May, 1982; Anderson & Medley, 1985). Provided they are based on sound, quantitative epidemiological data, these models can be used as valuable tools for strategy design and making decisions concerning interval of treatment, duration of chemotherapy, target population, etc., for large-scale control programmes.

There is a need for more information from field studies to expand our practical knowledge about the factors which determine the distribution of worms in the human community, both prior to treatment and following a period of reinfection. The data presented in this thesis are a result of investigations designed to increase our knowledge of the population dynamics of Ascaris lumbricoides within their human hosts.

CHAPTER 2

MATERIALS AND METHODS

2.1 Study Design

To fully investigate the objectives outlined in Chapter 1, a series of villages were chosen to undergo different treatment programmes. Repeated 24 hour stool collections to quantify egg output and 48 hour post-chemotherapy stool collections to determine worm burdens were carried out to monitor the success of the control strategies and to provide information on the distribution of worms by individual, sex and age groups, reinfection rates, relationships between egg production and worm burden, and predisposition to heavy and light helminth infection.

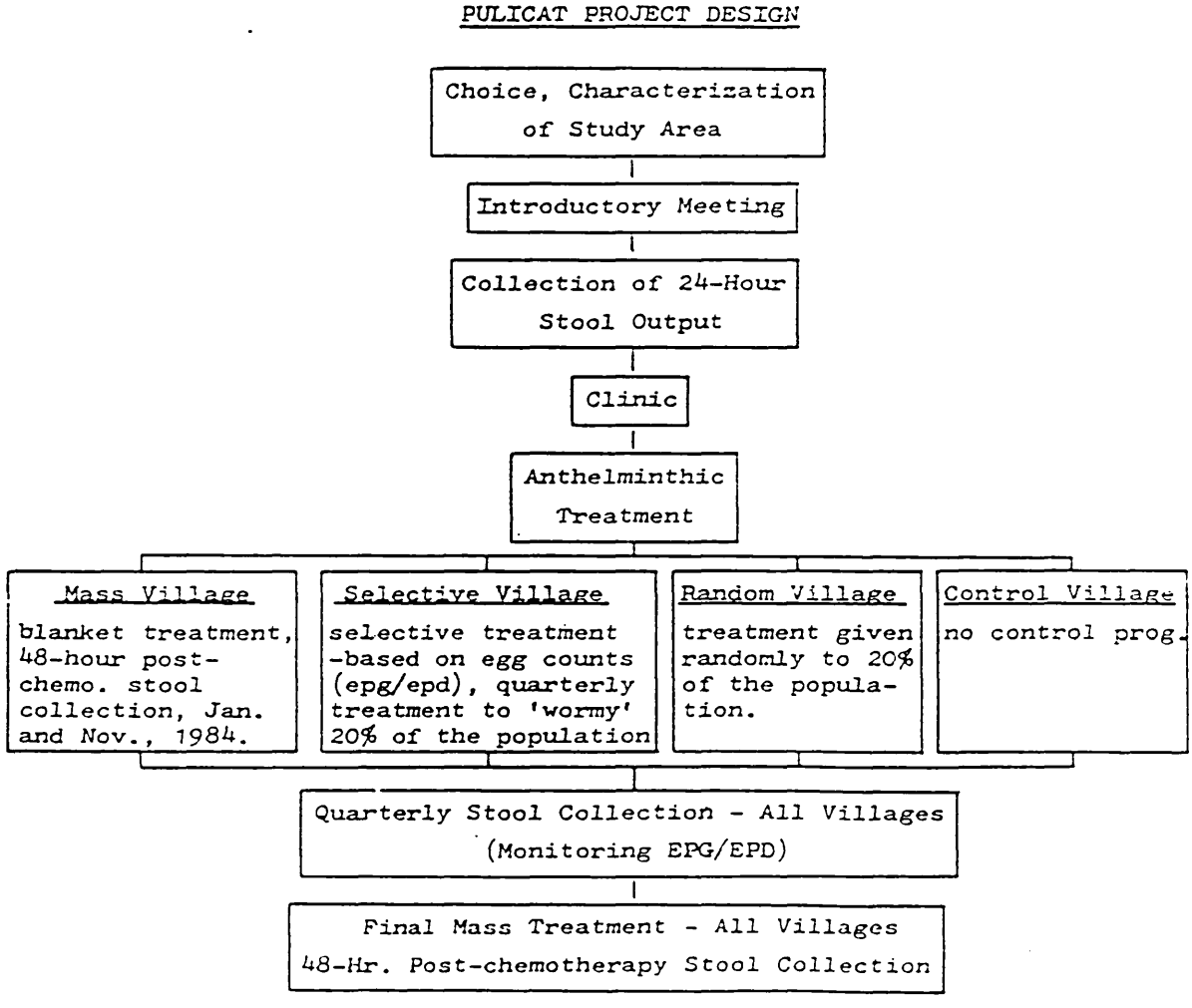
After assignment of the type of control to be administered to each village, the following plan of investigation was implemented (Figure 2.1):

1) An introductory meeting and slide show was held in the evening in each community to explain the programme and seek cooperation by majority consent.

2) Complete 24 hour faecal output and clinical information, emphasizing nutritional disorders (i.e. angular stomatitus, anaemia, Bitot's spots, night blindness, protein energy malnutrition), illnesses (such as diarrhea, respiratory disorders, digestive problems), and height and weight were recorded for each cooperating resident. Medications, including vitamin supplements, were dispensed by the doctors.

Figure 2.1

The plan of investigation adopted to examine the epidemiology of intestinal helminths and the effects of community-based anthelmintic treatment programmes.



3) Anthelmintic treatment was applied in the villages according to the following schedule:

a) Mass Village: All consenting residents (both egg positive and egg negative) were given anthelmintics initially, and the treatment was repeated when the overall egg output attained pre-intervention levels.

b) Selective Village: Treatment was applied exclusively to the most heavily infected 20% of the population (as determined by eggs per gram of faeces (EPG) and eggs per day (EPD)) and repeated at two month intervals.

c) Random Village: Randomly chosen individuals were given anthelmintic treatment. For selection, a number was assigned to each person, and a set of random numbers within the appropriate range was generated. Treatment was given to 20% of the population whose numbers were generated by this procedure.

d) Control Village: Treatment was delayed until the completion of the study.

4) Stools were collected over a 48 hour period immediately following administration of anthelmintic to allow the measurement of worm burdens from each cooperating individual, to examine the distribution of worms in the community, to identify the individuals harbouring the heaviest levels of infection by pre-treatment egg count scores and worm expulsion counts and to evaluate the reliability of faecal egg counts (EPG and EPD) as estimates of worm burden.

5) Further observations were carried out over a one year period comprising:

a) Quarter-yearly 24 hour stool collections from all residents in each village. This served different purposes according to the type of treatment administered. In the mass chemotherapy village, reinfection rates were monitored by egg output. In the control village it allowed estimation of seasonal variation in egg output in an undisturbed community. Monitoring egg output was also one measurement used to evaluate the efficacy of selective versus random chemotherapy in the respective villages.

b) Quarter-yearly medical clinics to monitor recovery or appearance of nutritional disorders and height and weight changes among residents of each village and to encourage participation in stool collection.

2.2 Sampling Schedule

Twenty-four hour stool collections were initiated in control Village I (see Table 2.1) in September 1983 and repeated in February, July and October 1984 and February and March 1985. The control village programme was completed in March 1985 with mass treatment and 48 hour stool collections. A mass treatment programme was initiated in Village II in November 1983 but was discontinued following 24 hour stool collections in February and August 1984 due to a low prevalence and intensity of *Ascaris*. A second mass chemotherapy Village III was initiated in January 1984, and follow up collections were carried out in March (to assess drug efficacy), July and November 1984. Mass chemotherapy and 48 hour collections were repeated in November 1984.

Initial collections in selective Villages IV and V were undertaken in March 1984. Selective treatment began in July 1984 together with a general 24 hour stool collection to establish the initial intensity of

Table 2.1

Description of the seven villages adopted for this epidemiological study.

Village	Reference Number	Pop.	No. of Houses	Method of Control	Location/ Livelihood
Korrai Kuppam	I	217	40	No Control	Seaside/Sea Fishing
Thonirevu	II	448	95	Mass	Lakeside/Lake Fishing
Vairavan Kuppam	III	462	70	Mass	Seaside/Sea Fishing
Nadu Kuppam	IV	198	37	Selective	Seaside/Sea Fishing
Lighthouse Kuppam	V	445	92	Selective	Lakeside/Lake Fishing
Kolathumedu	VI	245	55	No Control	Inland/Prawn Picking
Kattupulli	VII	153	31	Random	Seaside/Sea Fishing

egg output. Treatment was repeated bimonthly thereafter, with quarterly village-wide stool collections carried out in November 1984 and March and April 1985. The programmes were completed with a mass treatment and 48 hour stool collection in April 1985. An initial random chemotherapy Village VI was initiated in July 1984, but immediately terminated due to low prevalence of Ascaris. The second random Village VII was begun in October 1984. The programme was terminated in November 1984, however, due to complete destruction of the village by a cyclone. The residents relocated to a large village from which they had originally separated. Unfortunately due to these problems and time constraints, it was not possible to evaluate the effects of random treatment as a means of community control of Ascaris.

2.3 Establishment of a Field Laboratory

The first three months of the project was spent in establishing a field laboratory. The main activities included the hiring of staff, purchasing locally available equipment, cleaning and preparing the laboratory building for working and living, and clearing a shipment of imported scientific equipment through customs. Because these activities are essential to beginning a field epidemiological study, the methods used in overcoming these problems for this particular field study are described in detail.

2.3(i) Hiring Staff

On the advice received from several diagnostic laboratories in the Madras area, an advertisement for medical technicians was posted in three local teaching institutes for medical technology and laboratory staff (Figure 2.2). A pay scale was established at 20 to 30% above

Figure 2.2

An example of notices posted in institutes of medical technology in Madras (lower) and the Madras daily newspaper (top) to advertise the positions for field and laboratory staff.

THE HINDU, Monday, August 15, 1983.

Classified Advertisements

SITUATIONS VACANT

WANTED MEDICAL Technicians for 12-month field study concerning intestinal helminths. Medical Technology degree or comparable experience required. Must be conversant English and Tamil. Salary Rs. 750—850. Send details David Elkins, Dept. Zoology, Madras Christian College, Tambaram, Madras-600 059.

WANTED FOUR MEDICAL TECHNICIANS

For 12-14 month Research Project Concerning Intestinal Helminths. Must be able to work in both field and laboratory conditions. Applicants should hold Degree in Medical Technology or comparable Experience and be fully conversant in both Tamil and English. Consolidated salary Rs. 750-850 commensurate with qualifications and experience. Send full details to David Elkins, Dept. Zoology, Madras Christian College, Tambaram, Madras-600 059.

the basic starting pay offered in the local hospitals and private diagnostic laboratories. This was done to increase the attractiveness of the positions since they were of short duration and required the location of staff in an isolated area. At the same time, advertisements were placed in the major English newspaper of southern India to provide a greater circulation to potential applicants.

The response to the advertisements was poor with a total of 15 applicants received. During the interview process, the difficulty of requesting an employee to move residence became clear because of the great importance of the extended family in India. Married applicants were not interested in moving to Pulicat where few cement houses were available, and commuting time from Madras was long and the service unreliable. Single applicants were inclined to remain living at home in more secure jobs despite lower salaries. Short term work is undesirable because establishment, job stability and marriage are very strong influences on young graduates. An additional problem was the nature of the work which did not appeal to educated individuals. In India, the handling of faeces is an occupation reserved for the lowest castes and could potentially damage a woman's marriage prospects. Despite these obstacles three technicians were hired, two women with degrees in Zoology from Madras Christian College and one male with a medical technology degree. The employment of the two women, aged 24 years, required several personal visits to their homes to guarantee their safety and to confirm responsibility for their welfare.

It is difficult to suggest improvements in the method of hiring laboratory technicians when both field and lab skills are required. In this case help from Dr. P.J. Sanjeeva Raj at the Madras Christian College, Department of Zoology, in recommending personnel and in encouraging their participation was of great value.

2.3 (ii) Importation of Scientific Equipment

Due to information received at the Indian Embassy in London, all imported equipment, including plastic containers for stool collection, microscopes, centrifuge, brass sieves and expendables, was directly shipped to Madras. The expected procedure for release from Customs was by listing the items on the investigator's passport and confirming of the intent to export the goods from India upon departure. This advice proved to be misleading. In order to release the equipment it was necessary to execute a bond of assurance that the equipment (valued at £20,000) would be re-exported within a specified time limit. The execution of this bond required over six weeks of frequent negotiations between the customs authorities, Madras Christian College and Indian Bank. The latter two were co-signers of the bond. As an assurance to the college, an additional agreement was made between Imperial College, London and Madras Christian College. This agreement stated that if the equipment was not re-exported, Imperial College would make the £20,000 available to the Indian Customs authority, and Madras Christian College would not be financially responsible. The bond expired every six months and was re-negotiated and renewed four times during the project.

A more appropriate procedure for the importation of equipment into India would be to request the bankers of Imperial College, London to negotiate directly with an affiliative bank in India. A lien would be placed on the bonded amount in the Imperial College accounts with the agreement that if the conditions of the bond were violated, the money would be transferred to the bank in India for payment to the Custom's authorities.

2.3(iii) Development of the Laboratory

This project operated from a laboratory built in 1967 for the Department of Zoology of Madras Christian College. The building was intended to be a laboratory for estuarine biological research and teaching for undergraduate and post-graduate students. Due to the long and uncomfortable three hour journey from the college to the field laboratory, the college staff and students made little use of the facility. In addition, the building had no electricity, running or stored water. The facility consisted of four sections: a living quarters, laboratory, office and a student dormitory. During the two years of the project, three technicians stayed in residence, with the two women in the living quarters which included bedroom, toilet and bathing room, dining area and kitchen. The male staff member stayed in the dormitory. The two investigators resided in the office room which had a small toilet.

The first tasks undertaken in preparing the laboratory were renovating the wiring to restore electricity and acquiring permission to build a direct connection from the lab to the community water system. The local electrician was helpful in both activities, although it took a great deal of time and trial and error before the systems were fully functional. The Pulicat water system consisted of several public taps supplying groups of houses for approximately one to two hours per day at irregular times. Women from nearby houses had developed an elaborate set of rules concerning the number of water pots allowed per household and a cueing procedure in an effort to reduce disagreements over the use of the taps. Under these conditions it was impossible to obtain sufficient water for laboratory and personal use. After three weeks of meeting with local officials, permission was granted to lay an underground pipe from the nearest

public water tap to a storage tank at the lab. In addition, a large open well was dug to attain fresh water supplies. The well produced brackish water but its water supply proved essential for the task of flushing post-chemotherapy stools through sieves to isolate the helminths expelled by drug treatment. Well water was also vital for general use during the frequent cessations of town water supplies. A 0.5 horsepower water pump (Susi Suguna, Bombay) was purchased and connected to both the well and the lower fresh water tank leading to an overhead storage tank on the roof of the building. The pump carried water up to the overhead tank where it would be pressurized for use through taps in the laboratory. Water was also supplied through a plastic hose from the rooftop to an outbuilding which was constructed of logs and coconut leaves for sieving post-chemotherapy stools.

For transport to the study villages, a locally made fishing boat approximately 20 feet long was purchased and a fisherman was employed for poling and sailing the boat (Plate 2.1). A paddling system was also designed in order to cross deep channels to reach one of the study villages, Thonirevu. An old outboard motor that had been donated to the laboratory was renovated to travel the 12 miles to the farthest village, Kattupalli. One village, Kolathumedu, was located across a very shallow area of water, and an oxcart and driver were hired for the weeklong programme (Plate 2.2). The cargo of stools had to be well concealed in the cart to preserve the dignity of the oxcart owner's family.

Public buses and trains were used for all travel beyond Pulicat. Approximately two trips per week were taken to Madras (consuming 4 to 7 hours in travel time) for the purchase of chemicals, lab supplies and medications, which could not be carried in large quantities by



Plates 2.1 and 2.2

Modes of transport, including country boat (top) and oxcart (lower), adopted by the field team to travel to the villages and to carry the faecal material to the laboratory.



public transport. In times of negotiations, i.e. for import-export matters, visas, meeting with Madras Christian College, financial concerns and arranging collaboration with doctors, daily trips to Madras were often required for durations extending three to four weeks.

2.4 Initial Meetings in the Villages

The activities of the research revolved around a five to six day programme in the study communities (Figure 2.1). The first contact with each village was a short one-on-one meeting with the leader of the village to explain that we were interested in helping to establish a basic health programme for the village. The leader was informed that this would involve regular clinics with three attending doctors (attendance at no charge) and an emphasis on stool examination for intestinal worms. The villages were particularly aware of the abundance of roundworm in children who frequently vomited or passed worms in their stools. Interest in such a programme was expressed, and a village meeting was planned for a Monday evening. The date had to be carefully considered because there were national, state or local holidays averaging one every three days of the year. On the morning of the meeting, a messenger would visit the village to remind the village head of the planned meeting and to talk with other influential members of the community to ensure that news of the meeting had been spread throughout the households. Many times plans for the meeting were never communicated beyond the village leader, and the rest of the people were completely unaware of our proposals. A portable loudspeaker and tape recorder system were rented locally, and a slide projector was borrowed from Madras Christian College to enliven the presentation. At dusk, these machines were connected to the wiring of

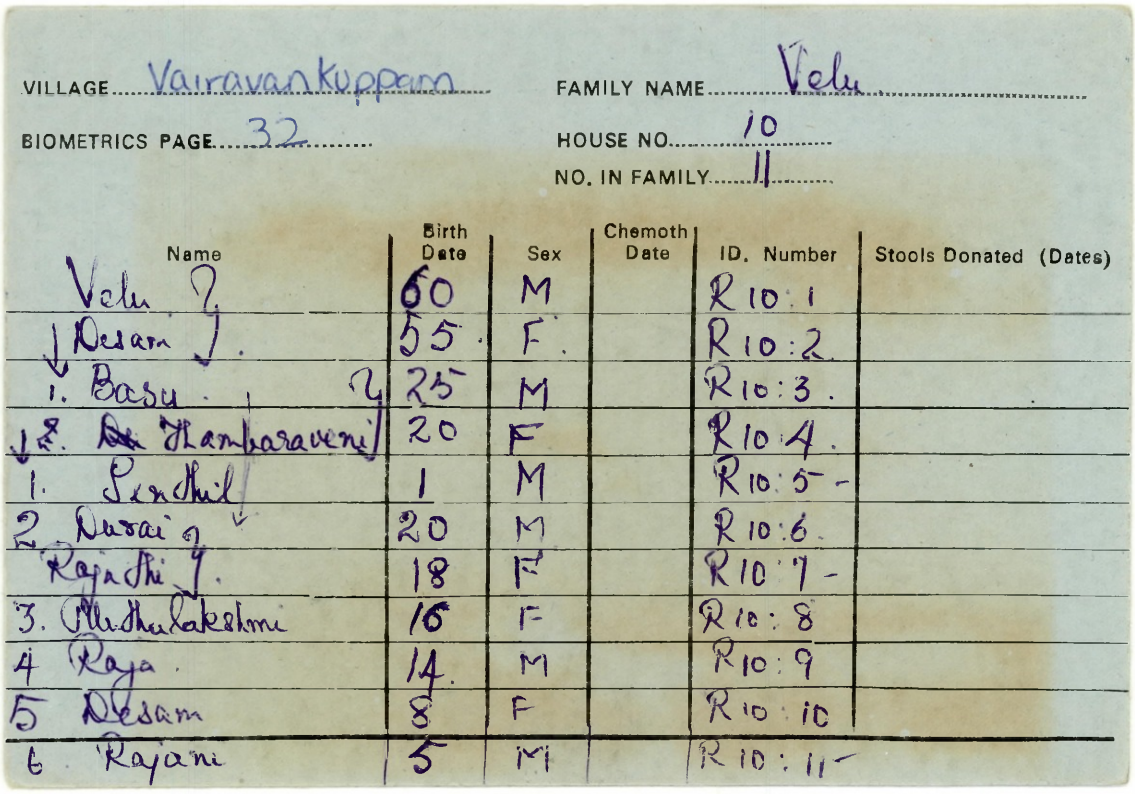
the Hindu temple, which was often the only building with an electrical connection. Popular Indian cinema music was played for ten to fifteen minutes over the loudspeaker to attract the villagers to the temple. A prepared talk explaining the programme was given by the investigator and translated into Tamil by a lab technician. Following this introduction, each member of the field team was introduced and brief lectures on intestinal worms were given with emphasis on their life cycle and possible effects on health and nutritional status. The lectures were illustrated with posters and slides taken at government hospitals or in other villages. This proved to be useful since it was a surprise for the community to see their friends and neighbours on the screen and to realize that other villages were participating in the programme. At the close of the meeting the village leader was requested to speak and to call for a general vote in favour or against participation. Every community responded with full agreement, and enthusiasm at this point in the programme was high.

2.5 Biodata and 24 Hour Stool Collections

On the morning immediately following the meeting, while enthusiasm was still evident, the research team returned to the village to conduct a house-to-house population survey, recording the names, ages, sex and family relationships of each member of the household on specially printed family cards (Figure 2.3). A map was drawn to show the location of each house by code number as well as public buildings, water pumps, rubbish heaps and tree covered areas (Figure 2.4). The initial data collection was time-consuming, and the maximum number of houses visited was 40 or 50 per day. For the first programme, the larger villages were divided into three groups covered in seperate weeks, however the third group inevitably lost enthusiasm

Figure 2.3

Example of the Family Cards filled out during the initial house-to-house survey of the community. The age, sex, I.D.# and family relationships were shown on the cards. Births, deaths and movements in and out of the community were recorded on subsequent visits.



VILLAGE Vairavankuppam FAMILY NAME Velu

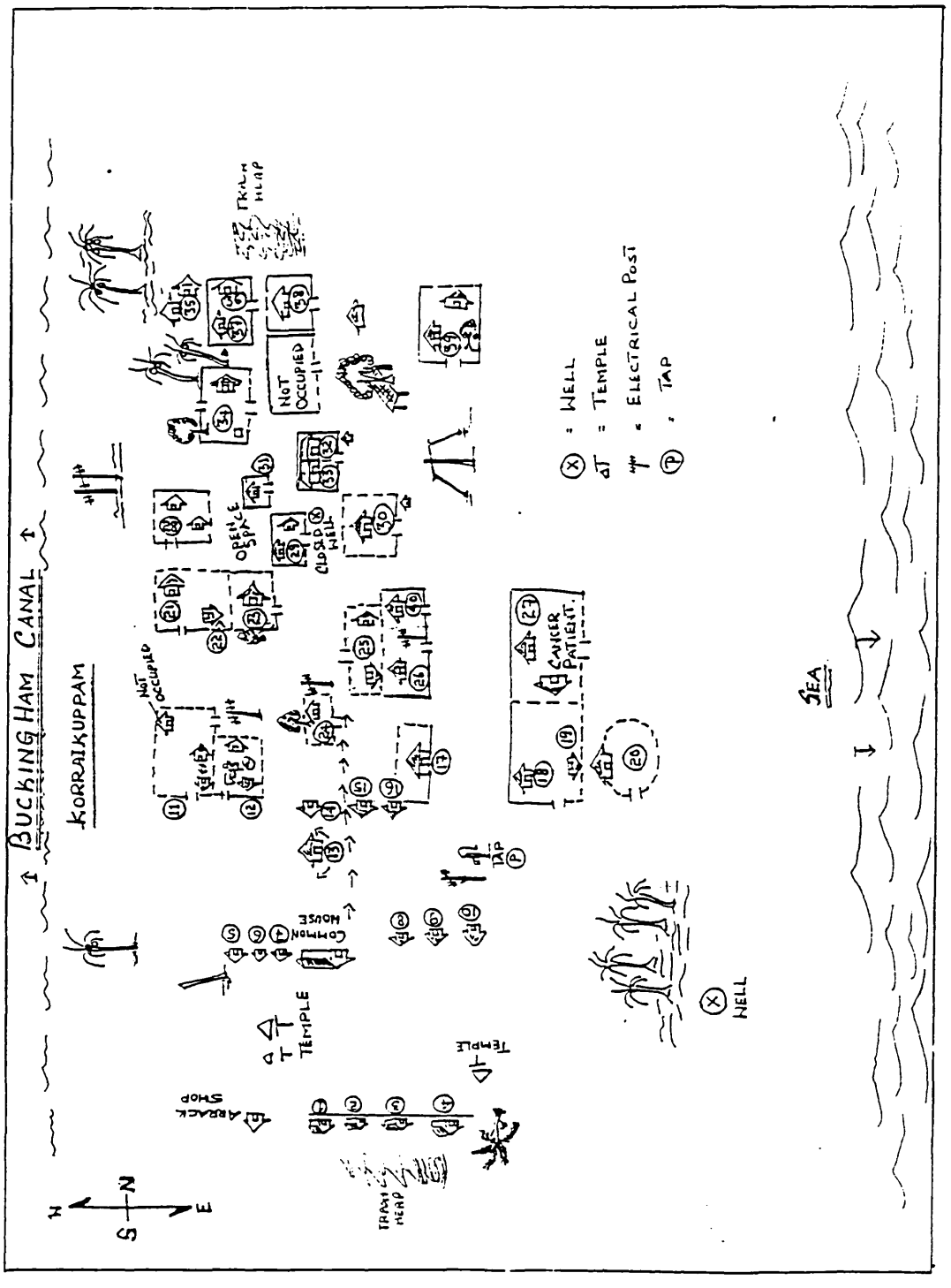
BIOMETRICS PAGE 32 HOUSE NO. 10

NO. IN FAMILY 11

Name	Birth Date	Sex	Chemoth Date	ID. Number	Stools Donated (Dates)
Velu ?	60	M		R 10 : 1	
Desam ?	55	F		R 10 : 2	
1. Basu	25	M		R 10 : 3	
2. Thamaraveni	20	F		R 10 : 4	
1. Senthil	1	M		R 10 : 5 -	
2. Murai ?	20	M		R 10 : 6	
Rajathi ?	18	F		R 10 : 7 -	
3. Muthulakshmi	16	F		R 10 : 8	
4 Raja	14	M		R 10 : 9	
5 Desam	8	F		R 10 : 10	
6 Rajani	5	M		R 10 : 11 -	

Figure 2.4

Hand-drawn map of Korrai Kuppam detailing house locations, trees, water pumps, well, public buildings, etc.



by our daily presence and showed a lower participation level. To avoid this, the time taken in data and stool collection of the entire village was minimized as much as possible with the health clinics held at a later date if necessary.

All members of each family were issued a one litre resealable plastic container in different colours. The lid of each box was labelled with an identification number consisting of a one or two letter abbreviation of the village name, and a four digit number describing the house number and the individual's rank number in the family based on age. The name of the individual was written on the label in English and in Tamil, and a small symbol, such as a cooking pot, fish, crab, boat, umbrella, etc. was drawn to individualize each box (Plate 2.3). An identical picture was drawn in indelible ink on the forearm of each individual to facilitate identification of the correct box. This technique was very popular with all ages, and it was indispensible for children and older people who could not read their names to positively identify their personal container. This also relieved the parents of some of the responsibility of ensuring that each child used the correct box. Members of each family were given detailed instructions on how to use the stool containers. This included the importance of giving full cooperation, explanations of the upcoming clinic, the necessity of using only the correct box assigned to the individual family member and that all stools were to be passed in the container until the team returned on the following day.

Upon collection twenty four hours later, each person was carefully questioned concerning which stool box he/she had defecated in and if any stools had been passed outside the container. All filled containers were collected, and individuals not able to or unwilling to

Figure 2.5

Pink "admission tickets" issued to individuals who donated 24 hour stools to enable them to attend the upcoming medical clinic.

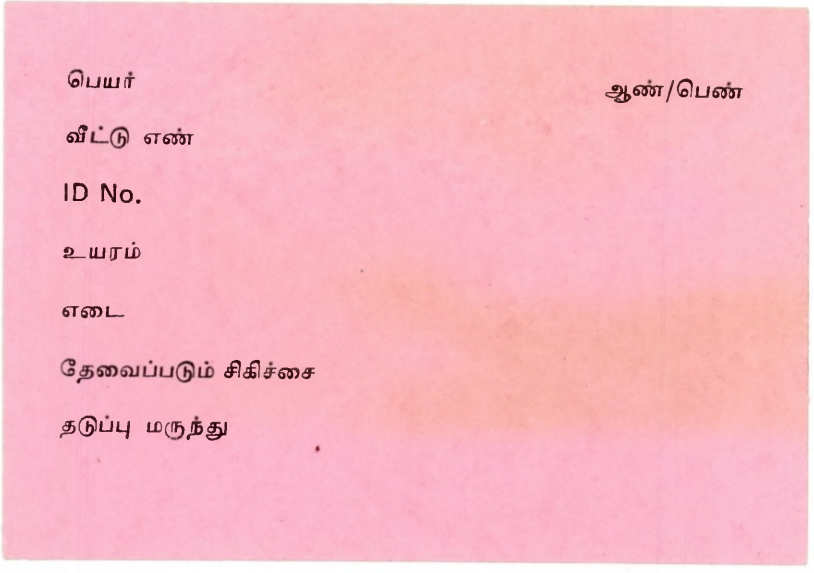


Plate 2.3

Labelling of 24 hour stool containers: individual identification number, name in English and Tamil, rank number within the household and an identifying picture also drawn on the forearm.



provide a stool were given a further 24 hours to cooperate after receiving a further explanation of the importance of their participation. All villagers donating stools were issued a pink card (Figure 2.5) with their name and ID# which allowed them to attend the forthcoming medical clinic arranged by the project team.

2.6 The Village Clinics

Acting on the advice of the District Medical Officer, three medical doctors from Madras were employed to carry out medical examinations and prescribe basic medications at the village clinics on prearranged dates. Before each clinic, the doctors supplied a list of necessary medications and vitamins (Appendix 2(a)) to be purchased wholesale in Madras written on prescription paper designed for the project and signed by the doctors involved. In the early morning before the clinic, the equipment needed (Appendix 2(b)) was transported to the village and set up in the school or at the temple by the investigators, boatman and helpful residents. The clinics were usually underway by 10:00 AM and closed at 3:30 PM with 80 to 180 people attending. Individuals who had not donated a stool but required an examination were allowed to see the doctors during the last half hour of the clinic. The average cost per patient was 50 pence to £1.50 depending on the number attending.

The procedure in the clinics was designed to maintain order and efficiency. Each person presented their pink admission card to a registration desk occupied by a field technician who double-checked the name against a list of those who had donated stools. The technician then addressed a health form (Figure 2.6) with the patient's name, ID#, age and sex. The patient proceeded to the weighing area where their body weight was determined on a beam

Figure 2.6

Health form designed for use by the team of doctors during examination of the patients attending the medical clinics.

PRIMARY HEALTH CARE PROJECT FOR FISHERMEN.
PULICAT.

VILLAGE: NAME:
I.D. NO.
Age: SEX:
Religion: Community:
Height: Weight:

When was the last treatment for worms given?
What medicine was taken?

IMMUNIZATION STATUS:

BCG:
Smallpox:
Triple Antigen: Pregnant now?
Polio:
Measles:

CONGENITAL MALFORMATIONS:

Face and Head Hands Legs. CVS RS

GENERAL IMPRESSIONS:

Apathy
Pallor
Irritability

HAIR

Dry
Dyspigmentation
Thin
Easily Pluckable.

EYES

Night Blindness
Conjunctival Dryness
Bitots spots.
Corneal Dryness
Keratomalacia.

LIPS

Angular lesions
Cheilosis.

GUMS

Swollen and Diffuse Bleeding.

TONGUE

Piliform Papillary Atrophy
Mild/Moderate/Severe Glossitis

ANEMIA.

ABDOMEN.

Hepatomegally
Splenomegaly
Pot-Belly

LOWER EXTREMITIES

Bilateral edema
Calf tenderness

SKELETAL

Cranial front.
Bossing Parietal
Craniotabes
Costochrondral bedding.
Enlarged joints.
Bone tenderness
Muscle wasting.

INFECTIONS FREQUENTLY SUFFERING FORM:

a) Diarrhea.
Upper Respiratory Petechiae.
Lower Respiratory Infections
Infection type.
Stomach pain.
Loss of Appetite.

HAVE YOU EVER HAD:

b) T.B.
Whooping Cough
Measles.
Hepatitis.
Malaria.
Leprosy.

c) CSOM Impetigo
Scabies. Furenculesis.

d) Abcess
Molluscum. Scalp Infection.
Glands.

CLINICAL IMPRESSION:

d) Marasmus.
Kwashiorkor
Marasmic Kwashiorkor.
Under nutrition.
PCM/Grade.

e) Breast feeding alone upto:
Breast + Bottle from:
Bottle feeding along from:
Solids introduced at:

DIAGNOSIS AND MEDICATIONS PRESCRIBED.

balance (Avery scale, accuracy to 50.0 gms) and recorded on the health form and the pink card. The latter was kept by the villagers as their own personal record since most had never had their weight or height measured. The height scale was a metal measuring tape (accuracy to 0.1 cm) attached to an 8cm x 8cm x 2m plank of wood and fixed to the wall. For height assessment, individuals were requested to stand with their back and legs straight, feet together and look straight ahead. A wooden headpiece was moved down the scale until it touched the top of the head through the hair. The corresponding measurement was recorded. For children too young to stand straight, crown-heel length was measured on a wooden-length board as described by Jelliffe (1966).

The next station was a medical examination by the doctors. A temporary cubicle made of white cotton cloth supported by rope was used for examinations requiring privacy. The doctors dispensed medications according to need in small packets labelled with name and daily dose pattern (Plate 2.4). Patients with illnesses requiring complicated treatment, hospitalization or surgery were issued letters of referral to government hospitals in Madras and urged to obtain treatment there. Illnesses and nutritional disorders as well as medications dispensed were recorded for each patient on the health forms.

If anthelmintic treatment was scheduled, the doctors calculated the correct dosage of pyrantel pamoate required based on 10 mg per kg body weight and recorded the number of tablets to be dispensed on the health form. Children less than one year of age, pregnant women and people over 70 years old were excluded from treatment. The drug was administered at the final station according to the doctor's recommendation. Each person was encouraged to eat a large meal which was found to reduce complaints of dizziness and vomiting, particularly

Plate 2.4

Dr. Ravishankar administers a Vitamin B_{12} injection to a fisherman during a village clinic in Vairavan Kuppam.

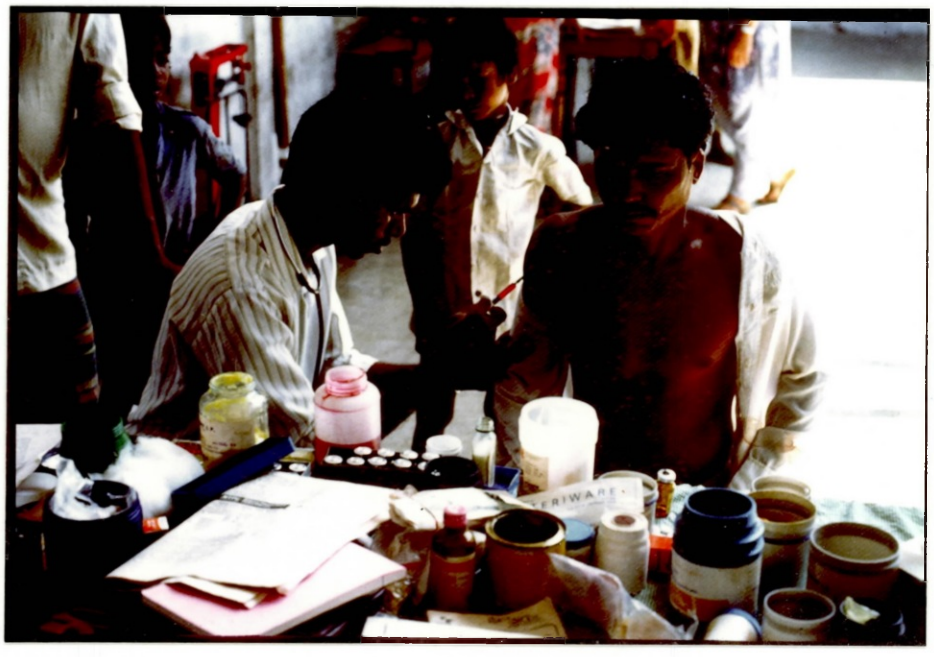


Plate 2.5

Child receiving pyrantel pamoate. Labelled white containers were provided to every treated individual for collection of complete 48 hour post-chemotherapy faeces.



in older women. For children unable to swallow tablets, the medication was powdered and dissolved in sugared water. At the treatment station, each individual was issued a 2 litre resealable plastic container (Peerless Plastics) labelled as previously described (Plate 2.5). Individuals were carefully instructed to pass all stools in the correct box until collection by the field team. Before leaving the village, a house-to-house collection of filled containers was carried out, and stools passed within eight hours of treatment were labelled as Day 0. At the conclusion of the clinic, the doctors received a meal and Rs. 100 (£6.00) for the day.

Following treatment, the village was visited in the mornings and evenings for a period of at least 48 hours to collect filled containers, to distribute fresh ones and to re-emphasize the importance of using the boxes for the full period of time. Each individual was interviewed to find out if any stools were passed outside the container. It proved to be more effective to ask friends of small children rather than their parents if they had passed any stools outside. Aspirin or stomach antacids were given to anyone suffering from dizziness or stomach pains. The filled containers were placed in the shade during collections to slow the degradation of the expelled worms. All stool collecting and carrying was done by the investigators. Upon returning to the lab, containers were marked to indicate the date of collection. Stools were preserved in an equal part of 10% formalin and stored for at least 48 hours before further processing.

2.7 Anthelmintic Drug

The originally selected drug of choice for anthelmintic therapy was mebendazole in a single dose of 200 mg. This anthelmintic is

highly effective for Ascaris (Chavarria et al, 1973; Seo et al, 1978) and was provided free of charge from Pfizer (England). However, in the first village where it was administered, severe side effects were reported. Twelve women were violently ill with complaints of continuous diarrhea, vomiting, cramps, dizziness and fainting. One woman was unable to pass fluids while another complained of incontinence. Though some minor side effects have been reported in the literature (Chavarria et al, 1973; Bunnag et al, 1976; Migasena et al, 1978), the unusual severity of the complaints was unexpected. The causes may have been due to a combination of alcohol, poor diet and psychosomatic fears. Due to these problems the programme in this village (Gunan Kuppam) was cancelled.

A second problem with mebendazole for this study concerns the fact that the drug causes starvation of the worms by blocking the uptake of glucose, a process which takes up to seven days (Van den Bossche, 1976). Thus, a much longer period of post-chemotherapy stool collection would have been required to obtain accurate estimates of worm burdens.

Pyrantel pamoate was therefore adopted as the drug of choice for the project. This highly effective, broad spectrum anthelmintic is manufactured in India (IPCA, Bombay), and it has a similar efficacy as mebendazole against Ascaris, hookworm and Enterobius in a single dose (Bumbalo, 1969; Yokogawa et al, 1970; Rim & Lim, 1972; Seo et al, 1972; 1980; Sinniah & Sinniah, 1981; Davies, 1985). The majority of Ascaris are expelled within 48 hours of treatment with pyrantel pamoate (Seo et al, 1972). The drug was purchased at £95.00 for 1000 tablets (or approximately 400 doses).

2.8 Processing Stool Specimens for Helminth Egg Count Determinations

Twenty-four hour stools were immediately transported to the laboratory following collection and weighed on a portable electronic scale (Port-o-gram, O'haus, Germany) to determine whole day faecal output to the nearest gram. After thorough homogenation by manual stirring, samples of faeces of known weight, ranging from 1.8 to 2.6 grams, were placed in 8 mls of 10% formalin in screw top glass tubes (Corning), shaken vigorously and stored.

Samples were processed according to a modified quantitative formalin ether sedimentation technique described by Hall (1981a; 1981b) to concentrate helminth eggs and larvae. After a complete literature review of all available egg counting techniques, this method was chosen because it allows for preservation of eggs as well as quantification. The preserved samples were poured into 15 ml conical tubes and washed once. The pellets were resuspended in 3 mls of water, then passed through copper mesh (34 gauge) and flushed vigorously with approximately 12 mls of water using a pressurized spray bottle. After centrifugation, the pellets were shaken for 60 seconds in 7 mls water plus 3 mls of ethyl acetate. Ethyl acetate has been show to be functionally equivalent to diethyl ether in this technique (Truant et al, 1981) and was preferred due to lack of safety facilities required for the handling and transport of ether. Following centrifugation, the detritus rings and supernatants were decanted. The egg bearing pellets were resuspended in a small volume of 10% formalin and transferred to screw top tubes.

To perform helminth egg counts, the number of drops of sediment in the tube was counted and recorded, and after vigorous resuspension, one or two drops were placed on a microslide for examination. The total number of Ascaris (fertilized and unfertilized eggs counted

seperately), Trichuris and hookworm eggs were recorded and the presence of Hymenolepis nana, Taenia and Strongyloides noted. Eggs per gram (EPG) and eggs per day (EPD) were calculated by the formulae:

$$EPG = hV/vw \qquad (E2.1)$$

$$EPD = EPG \times W \qquad (E2.2)$$

where h= the number of eggs counted, V= the total volume of sediment in drops, v= the number of drops of sediment examined, w= the weight of the preserved faecal sample in grams, and W= the total 24 hours stool weight in grams. The data was stored using an Epson HX-20 portable computer which functioned well under hot and humid field conditions.

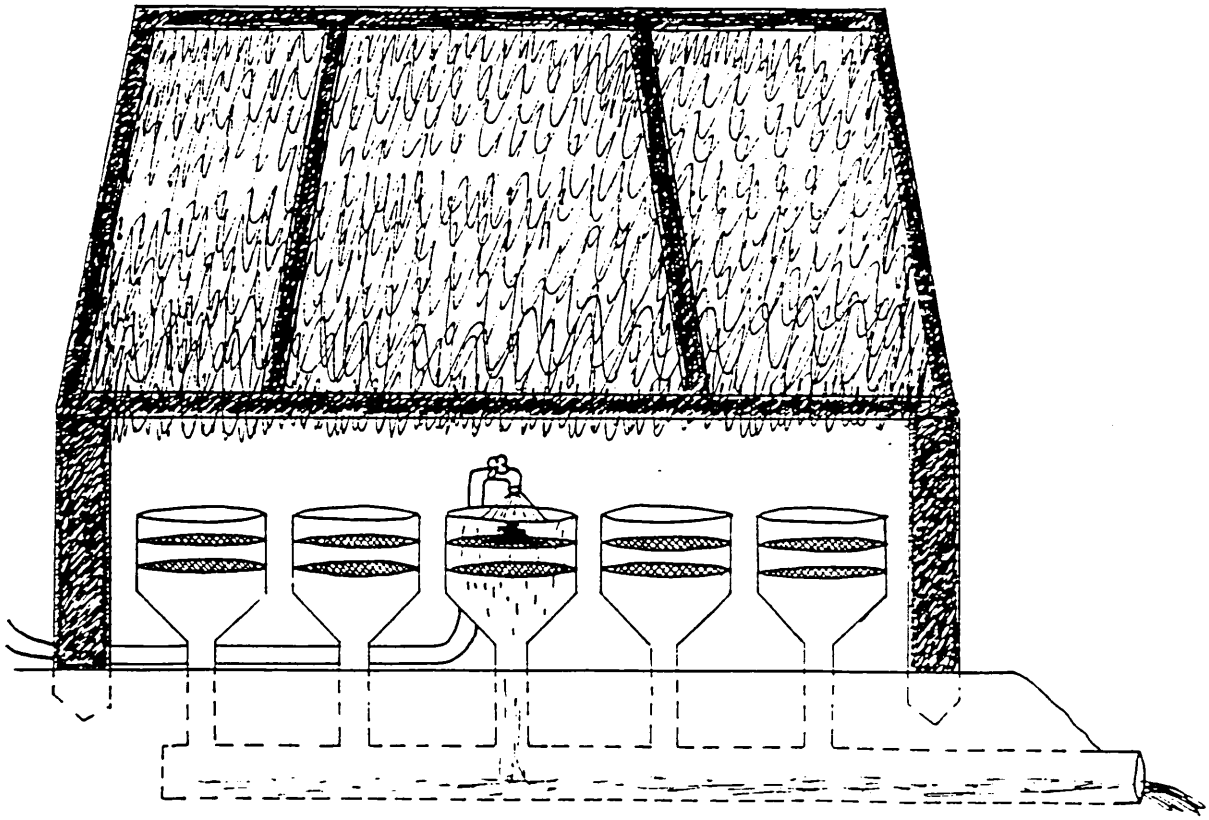
2.9 Isolation and Examination of Expelled Worms

Following preservation in 10% formalin and storage for at least 48 hours, post-chemotherapy stools were washed through two grades of copper sieves (1.0 mm and 300μm) to remove the bulk of the faeces. An A-frame shelter was constructed in the center of tall bushes near the laboratory in order to shield the worker from the sun and to avoid observation by local residents. A thin cotton curtain was placed around the poles to create breathable walls. A plumbing system was designed and constructed to transport the waste away from the work area. The two different sizes of sieves were joined together and placed into six plastic funnels which were glued to short collars connected to a larger main PVC pipe extending outside the shelter (Figure 2.7). It proved possible to sieve 100 stools per day using pressurized fresh or salt water delivered through a plastic hose from the storage tank on the roof of the laboratory.

Each stool was flushed until only expelled worms and larger faecal material remained in the sieve. The sediment, including worms, was

Figure 2.7

Schematic representation of the coconut leaf shelter and plumbing system built for washing the post-treatment stools to isolate helminths expelled following anthelmintic treatment. After flushing most of the faecal material through the sieves, the remaining sediment was rinsed back into the original container and preserved in formalin for later examination.



flushed back into the original container and preserved in 5% formalin. The empty sieve was then brushed, inverted and sprayed with water for cleaning before the next stool was flushed through.

To remove the helminths from the sediment, the technicians transferred the large Ascaris into fresh, labelled containers and poured the sediment into 6 to 10 eight inch petri dishes. The contents of each dish were examined against a black background with fine point forceps and a 10x magnifying glass. This procedure was laborious and time consuming (each stool requiring between 20 minutes to 4 hours with 3 to 5 people working together). After demonstrations of the range of sizes (from 2 to 35 cm) and appearances of the different species of helminths, the technicians were instructed to place anything resembling a worm, including fibers, hairs, etc., into a labelled tube filled with 5% formalin (Plate 2.6). During the sediment analysis, ventilation was maintained using an overhead fan. This system could have been improved by placing reverse flow fans in the windows to draw formaldehyde fumes out of the building.

The test tubes containing potential helminths and the boxes containing the large Ascaris were examined individually. Ascaris were weighed to the nearest 0.1 gram, and body length was measured. Ascaris longer than 7 cm were sexed by dissection when necessary. Trichuris and hookworms were counted and sexed, and mature and immature Enterobius were counted. Sexual and species differentiation of Enterobius and hookworm were performed in London. The counting and identification of small helminths were carried out under a dissecting microscope using high magnification.

Plate 2.6

The laboratory team examining washed post-treatment faecal sediment for small helminths, including immature *Ascaris*, *Trichuris*, hookworm, *Enterobius* and *Hymenolepis* segments.



2.10 Data Organization and Analysis

Organization and analysis of data in the field has unfortunately been a weak point of many field parasitological investigations due to poor recording procedures, improper storage of data and lack of facilities for data processing. In an attempt to avoid this, a series of recording techniques were devised for use in the village and in the laboratory. To avoid mistakes concerning houses and their members, 'family cards' were printed for use during personnel data collection and subsequent visits to the village (Figure 2.3). The village name, head of the family's name, the number assigned to the house, and the number of members living in the house were recorded on the heading of each card. Listed below were the names, identification numbers, ages, sex and dates of chemotherapy and stool donations of each individual. Family relationships and births and deaths were also described on the cards. This clear, portable record was carried on village visits to avoid mistakes in giving chemotherapy and addressing stool boxes. In general, raw data (24 hour stool weight, raw egg counts, etc.) were recorded on labelled sheets of paper, then transferred to a large book file at the end of the day. For processing and storage of egg count data, the raw counts were typed onto a computer (Epson HX-20 Portable Computer) and run through a programme written in BASIC which calculated EPG and EPD. A programme was also written to perform Kendall's non-parametric rank correlation tests on worm burden data that had been ranked according to magnitude. A programme which determined the goodness of fit between observed frequency distributions and the negative binomial distribution and calculated the dispersion parameter k (an inverse measurement of worm aggregation) was run on a programmable calculator (Hewlett-Packard, HP11-C). The information recorded on data cards (Figure 2.8) and the

Figure 2.8

Two-sided data cards used to record information concerning egg counts (upper) and worm burdens (lower) by individual throughout the year.

ID. NO. ..... VILLAGE .....

24 HR. STOOLS

DATE		STOOL WT		VOLUME	SEDMT	EGGS				INIT
COLL	EXAM	WHOLE	SAMPLE	TOTAL	EXAM	ASC. F	ASC. U	TRI	HOOKW	
1										
2										
3										
4										
5										
6										
7										

OTHER .....

ID. NO. ..... VILLAGE .....

48 HR. STOOLS

DATE		ASCARIS			TRICHURIS			HOOK WORM			DATA ON PAGE	NO. STOOLS PASSED
COLL	EXAM	M	F	TOT.	M	F	TOT.	M	F	TOT.		
1												
2												
3												
4												
5												
6												

OTHER .....

computer printout was also detailed in large databooks as a safety precaution. In London, the data were transferred from the databooks onto the Imperial College mainframe employing a standard database software package, RIM (Relational Information Management, Boeing Corporation) for statistical analysis and data manipulation. BMDP Statistical software package (Biomedical Computer Program, University of California) was used for one- and two-way analysis of variance, Student's t tests, correlations and regression analysis. Programmes for distribution analysis, non-linear regression and non-parametric rank tests were kindly provided by Graham Medley (Imperial College). Three textbooks, Biometry (Sokal & Rohlf, 1981) and Some Methods for the Statistical Analysis of Benthic Invertebrates (Elliot, 1979) and Non-Parametric Statistics (Siegel, 1956) were frequently consulted for aid in making statistical decisions.

CHAPTER 3

THE STUDY AREA

3.1 Choice and Characterization

A preliminary survey of intestinal helminths in children among five different social communities near Madras, India in 1980 revealed that the fishing communities at Pulicat Lake were heavily infected with Ascaris lumbricoides and other helminth species (Elkins, 1984). The Pulicat location was adopted for the present project for the following reasons:

i. a high prevalence and intensity (mean eggs per gram) of Ascaris,

ii. the villages are small and isolated with limited between village contact,

iii. no previous control programmes, research projects or any effective local health care delivery system had been implemented in this area,

iv. the availability of laboratory facilities at Pulicat offered by Madras Christian College.

Informed consent from the individuals and parents or guardians of the children under study was obtained at all stages of the research.

The study villages lie 55 kms north of Madras, the capital city of Tamil Nadu, South India, along the coast of the Bay of Bengal (Figure 3.1). They are located on a narrow, sandy peninsula, bounded by the sea to the east and shallow backwaters of a large, brackish water lake to the west. The communities are isolated from each other by expanses

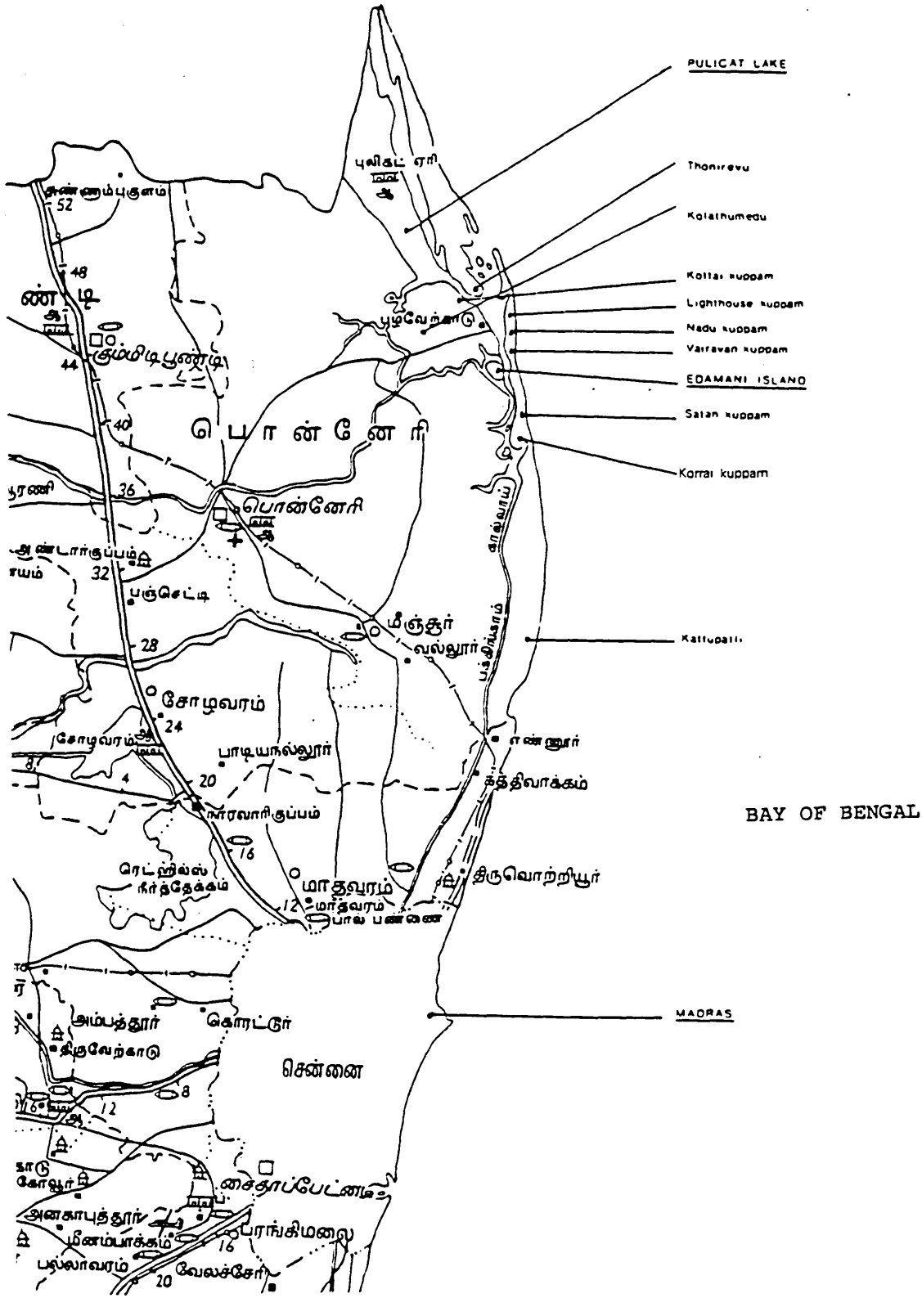


Figure 3.1

Map of the northeastern region of Tamil Nadu, South India. The study villages are located on a sandy peninsula seperating Pulicat Lake from the Bay of Bengal, approximately 55 kms north of Madras.

of sand and casuarina groves (Pinus khaysa) with no connecting roads.

The project was initiated at the end of a severe three year drought in South India. The average annual rainfall in Madras is 1215.3 mm falling mainly during the monsoon season in the months of October and November and during minor rains in August. The maximum and minimum mean daily temperatures are 32.9°C and 24.3°C, respectively, and the average annual relative humidity is 74%. A mild to strong breeze blows in from the sea throughout the year. Cyclones are not uncommon in the area, although damage is not usually severe. In November 1985, however, a three day storm of high winds and rain caused extensive damage to the Pulicat area. One of the study villages (Kattupalli) was totally destroyed, and the programme in this community had to be discontinued. The cyclone did not seriously interfere with the success of the data collection in other villages, particularly in Vairavan Kuppam where the programme was nearly completed.

3.2 Demography of Vairavan Kuppam

The age and sex structure of the population of Vairavan Kuppam is displayed in Figure 3.2. Of the total population of 462, 50% are below the age of 15 years, with an equal number of males and females overall. Life expectancy (from birth) is approximately 35 years and the net annual birth rate is roughly 34 per 1000 head of population (approximately 3 times the current birth rate in England and Wales and the United States). In 1983 (January to December) 13 births of 6 females and 7 males occurred in Vairavan Kuppam. In 1984, 15 births of 11 females and 4 males were recorded. In 1984, 7 deaths occurred and 4 were of children less than 3 years of age. The dates of births and deaths in the village during the time of the programme are

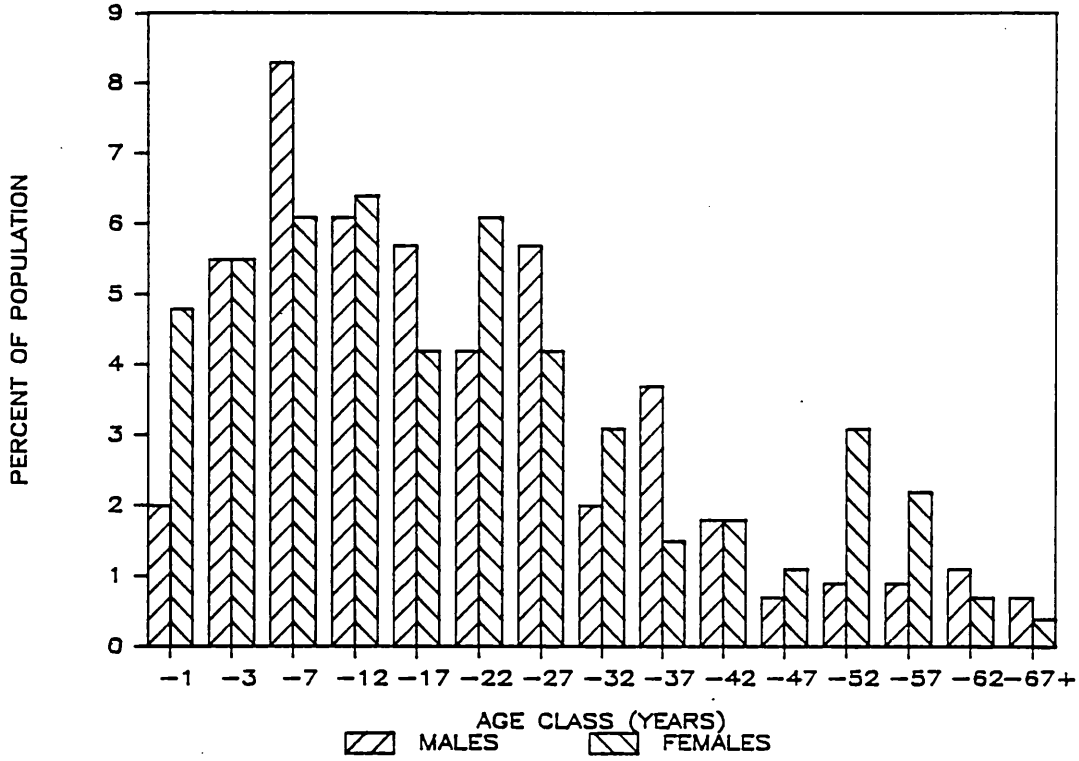


Figure 3.2

The age and sex structure of the population of Vairavan Kuppam. The total population in January 1984 was 462, and the median age was 15 years.

recorded in Appendix 3. The demographic pattern observed in Vairavan Kuppam is typical of many developing countries.

3.3 Social Aspects of the Community

The houses of the study villages are arranged in fenced compounds (see Figure 2.4) comprising living quarters, cooking and bathing areas. In general, large extended families dwell in cement houses with tiled roofs, while smaller families live in houses constructed from a mud and dung mixture with thatched roofs. The streets and the ground surrounding the houses is loose sand in all of the seaside villages, while the lakeside villages have a firmer soil. Coconut palm and shade trees are grown in some of the family compounds.

All villages have at least one Hindu temple for worship, and most have a government building which serves as a school. The majority of the population are Hindus, although small pockets of Christians and Muslims are found in some of the study villages. The villages are arranged in fairly discrete groups according to caste, with up to three subgroups of fishing castes often being present within one village. The language spoken in all the villages is Tamil, the dominant language of the state. Some of the larger villages have community televisions which are in frequent use in the evenings. Many children attend primary school, but most adults are uneducated.

Men in seaside villages fish in the ocean on log rafts with nets, while lakeside fishermen catch lake fish and prawns in wooden, human propelled boats. Generally men leave for fishing in the late afternoon and return the following morning. Income is highly seasonal, depending on the weather and the fish and prawn populations. Their free time is usually spent repairing nets, playing cards and sleeping on the beach. Women and their older daughters undertake the

responsibility for drying and selling the fish, childcare, cooking, shopping, cleaning and bringing water. Drinking and washing water is obtained from open public wells or private hand pump bore wells. Children attending school receive a midday meal consisting of rice and vegetables free of charge under a government scheme to improve child nutrition. Family meals consist of fish, rice, dahls and well-cooked vegetables taken 2 to 3 times a day. Home-prepared breakfast foods and snacks are frequently sold in small shops and by women in the village.

Women and men use separate preferred defecation sites located in the shady casuarina groves (women) or on the beach by the sea (men). Children defecate indiscriminately at the seaside, outside the house compound or in large rubbish heaps found around the perimeter of the villages (Plate 3.1).

Available health care in the Pulicat area at the time of this project was minimal. The government hospital was rarely visited due to the infrequent presence of the doctor. Three 'clinics' run by unqualified 'doctors' provided for most of the medical needs of the villagers. Some families travelled to a nearby town or the city of Madras to receive treatment at large government hospitals. A general description of each of the seven villages under study is given in Table 2.1. In this thesis, however, only the village of Vairavan Kuppam is considered in detail.

3.4 Cooperation and Faecal Production

In the first round of the 24 hour stool collection 71% of the total population cooperated, with 78% receiving anthelmintic treatment (4% were ineligible due to age or pregnancy). Following the treatment in January, 48 hour post-treatment stools were provided by 47% of the

Plate 3.1

Children defecating on a sandy mound which also serves as a rubbish heap for nearby houses.



community. The highest level of cooperation was provided by young children and elderly people. In the March and July 24 hour stool collections to monitor reinfection, 44% of the total population donated stools. In November, 56% of the villagers provided a 24 hour stool, with 90% of the village accepting anthelmintic treatment and 66% giving a complete 48 hour post-chemotherapy stool output. The basic incentive used to encourage cooperation was admission to the quarterly medical clinics. Cooperation for the final collection/chemotherapy in November was remarkably boosted by an offer of a free family photograph if every member of the family cooperated in the final 24 and 48 hour stool collection and chemotherapy. Table 3.1 shows the number of cooperating individuals by date and sex.

The average 24-hour stool weights by age and sex are recorded in Figure 3.3. In males, there is a highly significant (Student's $t=8.18$, $p<0.001$) rise in faecal output to 175 gm in the 20 to 29 age group, followed by an equally significant decrease to 128 gm in the 45+ age group. Females do not show a decrease in older age groups, and faecal output steadily increases to more than double the mean faecal weight of the 0 to 4 year age group. Between males and females there is a significant difference ($t=2.13$, $p<0.05$) in mean faecal weight in the ages 10 to 19 and in the 20 to 29 year olds ($t=3.31$, $p<0.001$). Variation in stool weight is high both within age groups and in individuals on a day-to-day basis.

3.5 Drug Efficacy

Within 8 weeks after mass chemotherapy in Vairavan Kuppam, a second 24 hour stool collection was carried out to establish (i) the conversion rate of egg counts from positive to negative following treatment with pyrantel pamoate; (ii) the post-chemotherapy prevalence

Table 3.1

Number of participating members in the community throughout each stage of the Vairavan Kuppam research programme.
Note: Counts recorded in March, July and November 1984 from individuals who received treatment in January 1984 are considered 'reinfection' data in this thesis.

	January 1984		March		July		November 1984	
Description	Males	Females	M	F	M	F	Males	Females
Total Population	225	237	-	-	-	-	229	239
24 Hour Stools	162	164	106	117	108	147	151	162
Anthelmintic Rx	183	177	-	-	-	-	212	208
Rx(Jan)+24hr stools	-	-	93	98	80	109	118	125
Full 48 Hr Stools	105	114	-	-	-	-	158	148
Rx(Jan)+48hr stools	-	-	-	-	-	-	131	117
Jan+Nov 48hr stools	86	88	-	-	-	-	86	88
Jan+Nov 24hr stools	107	110	-	-	-	-	107	110

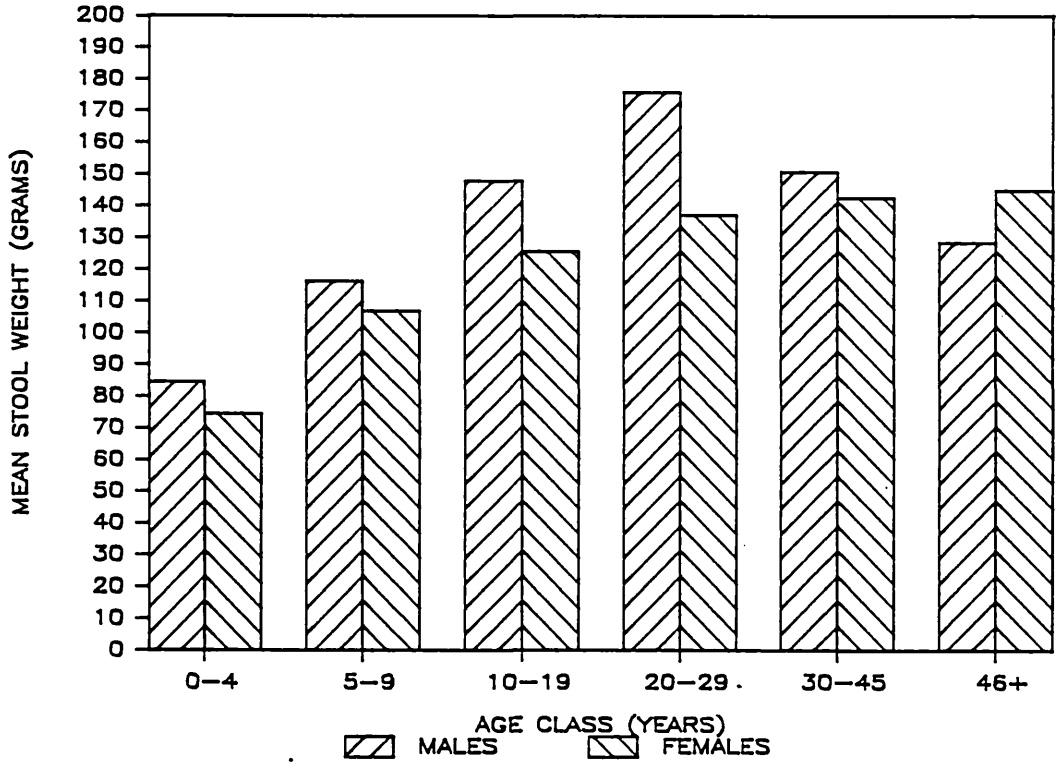


Figure 3.3

Average 24 hour stool weights donated during faecal collections throughout the study period in Vairavan Kuppam grouped by the age and sex of host. Sample sizes were as follows:

Age Group	Males	Females	Age Group	Males	Females
0-4	77	123	20-29	100	114
5-9	114	83	30-45	79	86
10-19	120	103	46 plus	42	85

A total of 1126 whole day stools were included in the analysis.

of Ascaris; (iii) the accuracy of worm expulsion counts as measures of total worm burden. Of the 223 stools collected, 191 were from treated patients and, of these, 95% were negative for Ascaris eggs. Of the 10 patients remaining egg positive for Ascaris after treatment, 5 passed only unfertilized eggs. The true efficacy of the drug may be lower than the 95% level recorded due to the inability to detect male worms and immature females by faecal egg count methods.

Of the 2284 Ascaris recovered from post-treatment stools in January, greater than 70% of mature and immature worms were found in stools collected during the first 24 hours (see Figure 3.4). The female to male ratios (F.M.R.) of mature worms expelled during the first and second 24 hours following treatment were 1:0.92 and 1:0.69, respectively, with a total F.M.R. of 1:0.85. In a subgroup of 53 individuals who provided complete 72 hours of faecal output, 71%, 23% and 6% of the 506 Ascaris expelled were recovered from stools donated on Day 1, Day 2 and Day 3 following treatment, respectively (Figure 3.5). Because of the small proportion of roundworms recovered on the third day after drug administration, a 48 hour period of stool collection for the estimation of worm burdens was adopted for this research.

3.6 Major Helminth Infections

At the pretreatment survey in Vairavan Kuppam, the prevalences of Ascaris, Trichuris, Enterobius and hookworm infection were 85%, 95%, 71% and 31%, respectively, within the total sample population. Strongyloides and Hymenolepis nana were also present with respective prevalences of 5.2% and 3.0%. Table 3.2 shows the initial age prevalence of these 6 helminths based on egg positivity, except for Enterobius which is based on worm expulsion studies. The age

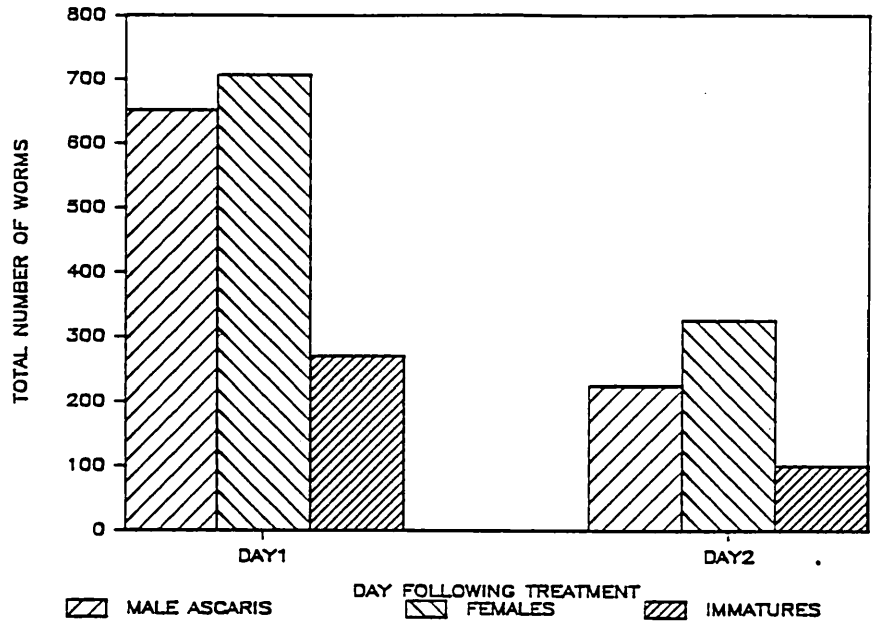


Figure 3.4

The number of male (length 10.5 cm or greater), female (12.5 cm or greater) and immature Ascaris recovered during the first (Day 1) and second (Day 2) 24 hours following treatment with pyrantel pamoate.

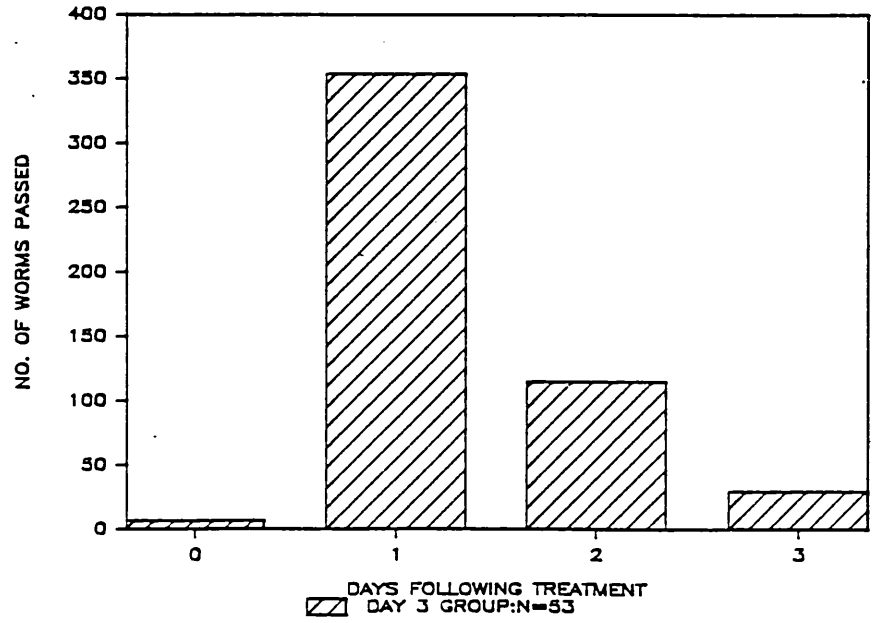


Figure 3.5

The recovery of Ascaris from stools collected during the first 8 hours (Day 0), 8 to 24 hours (Day 1), 24 to 48 hours (Day 2) and 48 to 72 hours (Day 3) following drug treatment. The group consisted of 53 members who donated full 3 days of faecal output.

Table 3.2

Helminth prevalence in Vairavan Kuppam in January according to egg positivity in pretreatment stools (n=326) or worm positivity (*Enterobius*, n=219) following anthelmintic treatment by host age.

	AGE GROUP IN YEARS						
	0-4	5-9	10-19	20-29	30-45	46+	TOTAL
PARASITE	No.+(%)	No.+(%)	No.(%)	No.(%)	No.(%)	No.(%)	No.(%)
Ascaris	38(73)	52(96)	65(90)	54(86)	43(84)	26(77)	278(85)
Trichuris	41(79)	54(100)	72(100)	54(100)	51(100)	30(88)	311(95)
Hookworm	3(6)	11(20)	30(42)	21(33)	22(43)	15(44)	102(31)
Enterobius	21(60)	30(65)	35(75)	29(78)	22(73)	18(75)	155(71)
H. nana	1(2)	3(6)	4(6)	2(3)	0	0	10(3)
Strongyl.	2(4)	5(9)	5(7)	2(3)	2(4)	1(3)	17(5)

prevalence profiles of Ascaris, Trichuris and Enterobius are similar with peaks found in the 5 to 9 year olds, followed by a slight decline with age. In contrast, the prevalence of hookworm rises steadily with age. Epidemiological information concerning helminths other than Ascaris in this community is presented in detail in a seperate thesis (Haswell-Elkins, 1987).

CHAPTER 4

THE EPIDEMIOLOGY OF ASCARIS LUMBRICOIDES

4.1 Introduction

Many epidemiological studies undertaken in endemic areas throughout the world have demonstrated that host age is an important factor in determining the distribution of Ascaris within communities. In most regions, with the notable exception of central areas of Iran (Arfaa & Ghadirian, 1977; Croll et al, 1983), intensity (estimated by either faecal egg or worm counts) is highest in young age groups and declines with age (Sadun, 1955; Yan et al, 1978; Thein-Hlaing, 1984). This generates a convex age intensity curve, a pattern also observed for a variety of other species of intestinal helminth infections (Sadun, 1955; Hayashi et al, 1959; Bundy, 1986; Haswell-Elkins, 1987). The observed consistency of this age pattern suggests that strategies for community control of Ascaris could be based on targetting treatment toward heavily infected age groups (Warren & Mahmoud, 1976; Anderson & Medley, 1985). As in all methods for parasite control based on chemotherapy, treatment must be repeated at short intervals since reinfection occurs rapidly. Few previous studies have examined the dynamics of parasite reacquisition in relation to host age following control interventions (Chai et al, 1983; Hlaing et al, 1985). Of particular interest is how the age intensity relationship is affected by chemotherapy, and whether the most heavily infected age groups prior to treatment tend to reacquire heavy burdens.

Field data describing reinfection in terms of both prevalence and intensity of *Ascaris* by worm counts among large sample groups have only recently become available (Seo, 1980; Chai *et al*, 1983; Thein-Hlaing *et al*, 1985). In previous studies, prevalence and estimates of egg output (EPG) have largely been used to describe rates of reinfection following anthelmintic treatment, but the relationship between these parameters and the distribution of worms within individual hosts after a period of reinfection has not been examined. This information is vital for the evaluation of control programmes based on age-targetted treatment of the most susceptible host groups, since the major difficulty in achieving effective parasite control is the rapid rate and persistence of reinfection following chemotherapy. The dynamics of reinfection are thought to vary according to local factors such as initial prevalence and intensity of the parasite, environmental and seasonal changes, and the extent and type of control administered. Quantitative data concerning rates and patterns of worm reacquisition from a variety of regions in which these parameters differ would be very useful for designing large scale control strategies (Morishita, 1972; Seo *et al*, 1981).

This chapter reports the analysis of initial and reinfection studies based on the prevalence and intensity of egg counts and worms recovered following chemotherapy in the village of Vairavan Kuppam. The influence of host factors, particularly age and sex, on the intensity of egg output and worm burden in January 1984 (when initial 24 hour stools and post-chemotherapy worms were collected) and November 1984 (when the programme was repeated) are examined. Sex and age-stratified analysis of faecal egg counts between interventions in March and July are shown to provide information on drug efficacy and reestablishment of egg output. The implications of these findings for

the design of strategies for the community control of Ascaris are discussed in this chapter and summarized in the General Discussion.

4.2 Population Dynamics of Ascaris

4.2(i) Prevalence and Intensity within Host Age Groups

Ascaris infections are acquired early in life in Vairavankuppam where 46% of the 0 to 2 year age class excreted eggs in the faeces (Figure 4.1). By age 9 years, the prevalence attained a peak value of 96%; thereafter a slight decline was recorded with a value of around 80% in the oldest age class. The age prevalence pattern was similar between male and female hosts.

Horizontal age-intensity of infection profiles are recorded in Figure 4.2. Graphs (a) and (b) denote pre-treatment values of EPG and EPD, respectively, while graph (c) records worm counts derived from the initial expulsion chemotherapy. All three graphs show similar trends, with a peak intensity of infection occurring in the 5 to 9 year old children followed by a decline in older age classes. This decline in intensity was more marked than that recorded for prevalence (Figure 4.1) and was considerably steeper in males compared to females. Females were more heavily infected than males in most age classes, and the overall difference is highly significant (One way analysis of variance (ANOVA) on log(x+1) transformed worm counts, $F=8.75$, $df=1,217$, $p<.005$). This trend was most striking in the 0 to 4 year olds where females had an average burden of 16.9 worms compared to the average burden of 5.8 worms in males. In general the intensity measures based on egg counts provided a good description of the age-related changes in average worm burden (compare Figures 4.2(a), (b) and (c)).

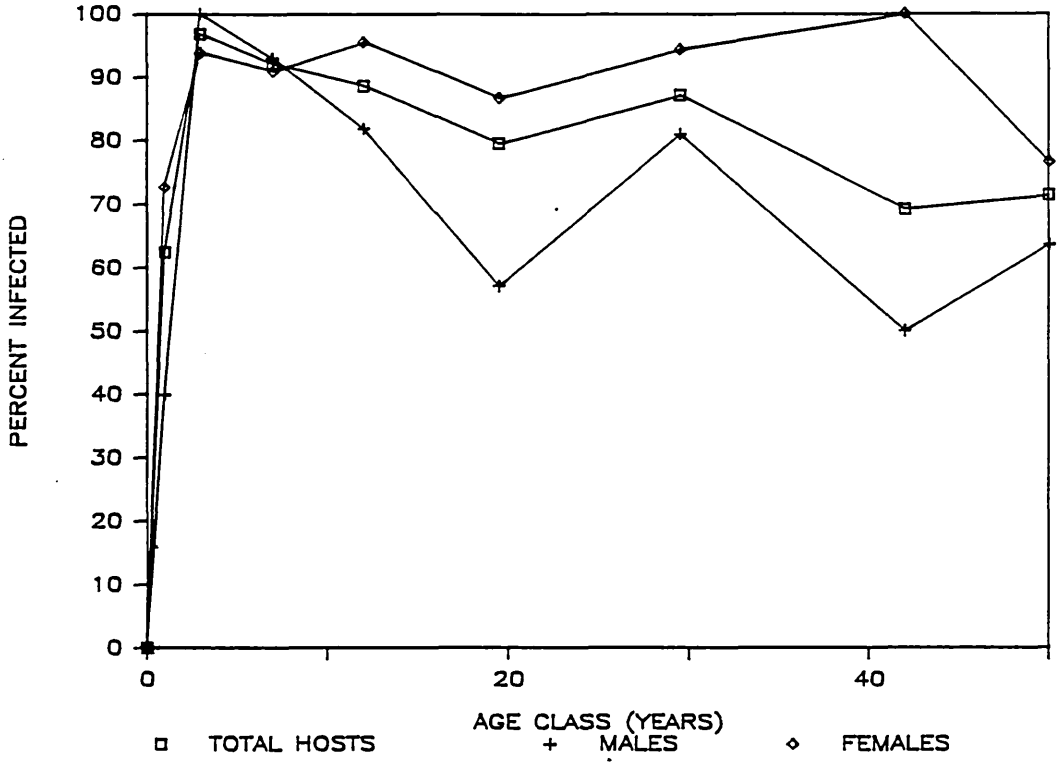


Figure 4.1

The age prevalence profile of Ascaris lumbricoides by host sex determined by worm recovery following initial anthelmintic treatment in January 1984. Sample sizes within each age group are shown in the legend of Figure 4.2(c).

Figure 4.2

Horizontal age intensity profiles of Ascaris infection in Vairavankuppam in January 1984, as measured by:

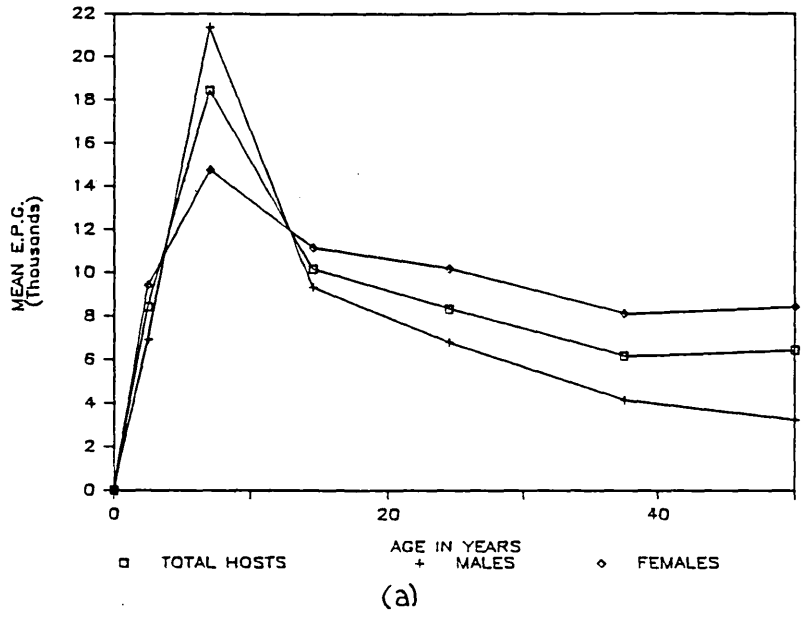
(a) Mean eggs per gram (EPG) of faeces collected immediately prior to mass anthelmintic treatment,

(b) Mean 24 hour egg output (EPD),

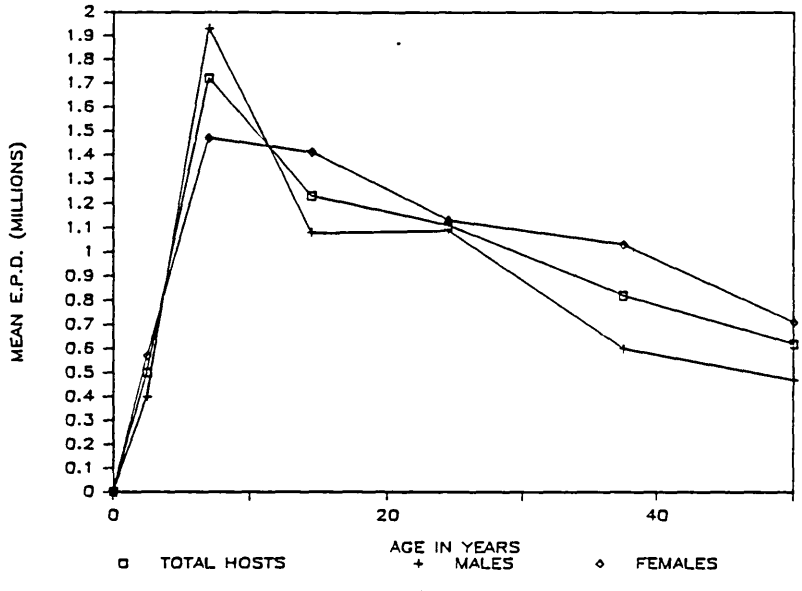
(c) Mean worm burden determined by expulsion chemotherapy.

Sample sizes within each sex/age group were:

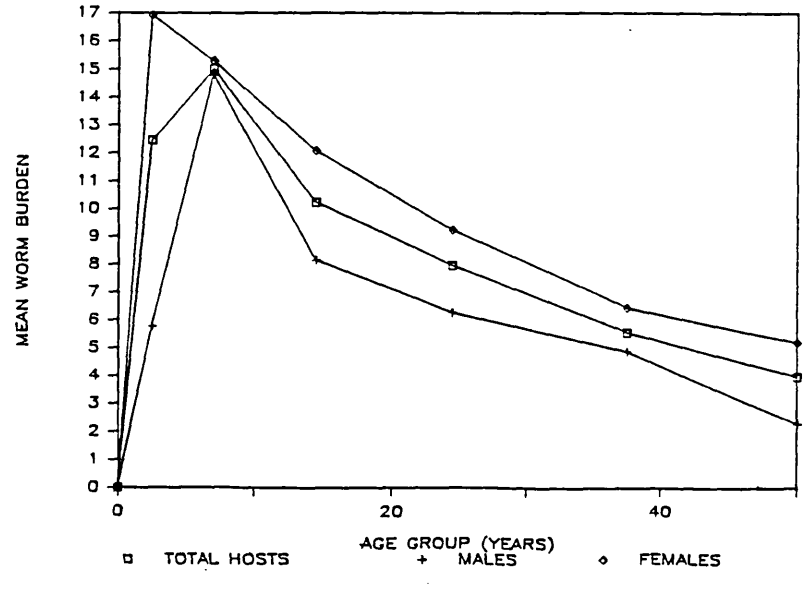
	EPG(a) and EPD(b)		Worm Burdens(c)	
Age Group	Males	Females	Males	Females
0-4	21	31	14	21
5-9	30	24	26	20
10-19	39	33	22	25
20-29	33	29	16	21
30-45	25	26	17	13
46+	13	21	10	14


MEAN E.P.G. (Thousands)
AGE IN YEARS
TOTAL HOSTS
MALES
FEMALES

(a)


MEAN E.P.D. (MILLIONS)
AGE IN YEARS
TOTAL HOSTS
MALES
FEMALES

(b)


MEAN WORM BURDEN
AGE GROUP (YEARS)
TOTAL HOSTS
MALES
FEMALES

4.2(ii) Distribution of Mature and Immature Ascaris in Host Age Groups

Over half of the total number of worms recovered by expulsion chemotherapy were found in children between the ages of 2 and 9 years (Figure 4.3). This age group constituted 34% of the total village population. A similar pattern arose for the distribution of immature worms (male worms under 10.5 cms in length and female worms under 12.5 cms in length; Seo & Chai, 1980) where 53% of all immature parasites were harboured by the 2 to 9 year age groups (Figure 4.3). Immature worms were recovered from all age classes, and 16% of the total of 2284 expelled worms were immature.

The percentage of worms that were immature can be used to estimate worm life expectancy, defined as $1/\mu$, where μ is the per capita parasite death rate (Anderson, 1986). If the death rate, μ, is constant and independent of host age, then the proportion of immature worms in the population, p, is given by:

$$p = [1-\exp^{-\mu T}] \qquad (E4.1)$$

where T is the prepatent period of Ascaris. Estimating T to be 50 to 60 days, equation 4.1 yields an estimate of $1/\mu$ of 9 to 11 months (where p = 0.16). This estimate is crude, however, and its validity is dependent on many assumptions which have not been experimentally confirmed. These assumptions include a stable age distribution of parasites, a parasite death rate that is independent of host age and the absence of density-dependent constraints on the growth rate and survival of worms.

4.3 Reinfection Following Mass Treatment

4.3(i) Reinfection on a Community Basis

The reinfection dynamics of Ascaris following mass chemotherapy in

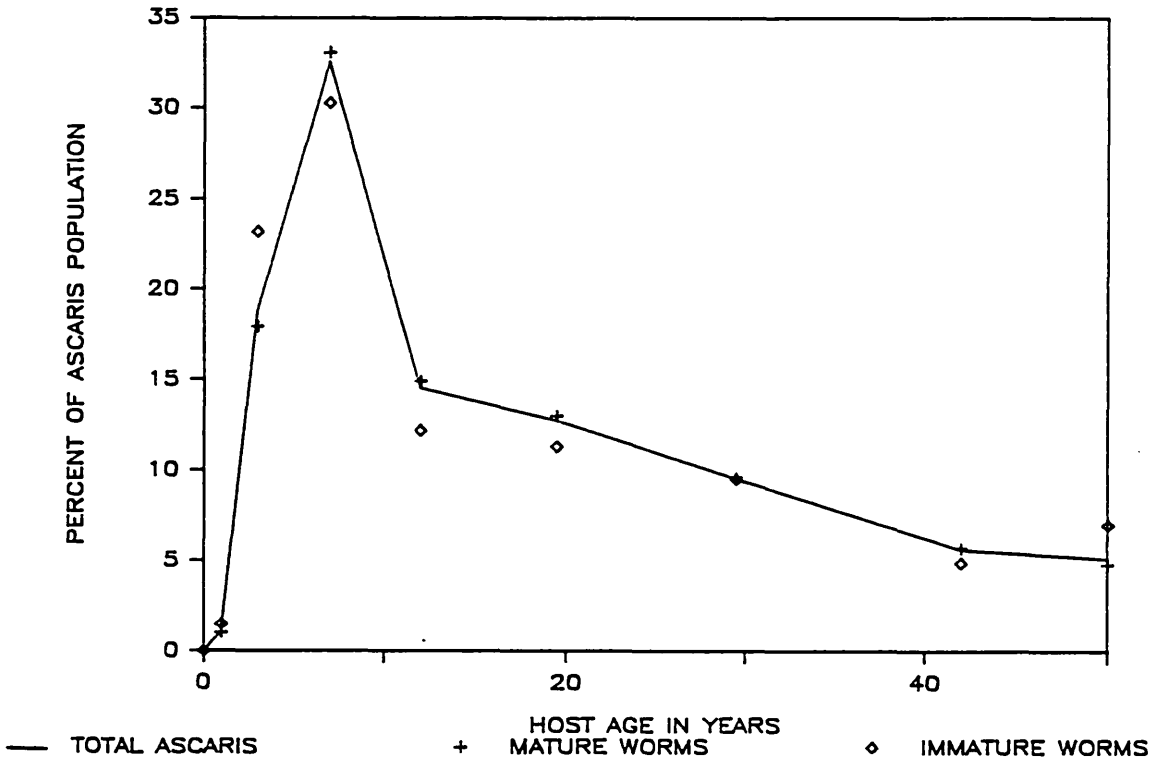


Figure 4.3

The percent distribution of the mature, immature and total Ascaris population within host age groups. The crosses represent the immature worms, the squares represent the mature group and the line denotes the total (immature plus mature) Ascaris population.

Vairavan Kuppam are described by qualitative changes in the proportion infected (Figure 4.4) and quantitatively by the increase in faecal egg output (Figure 4.5) or worm burden. Figures 4.4 and 4.5, illustrating the overall prevalence and mean egg output throughout the investigation period, are strikingly similar. The sharp drop in percent of egg positive individuals and in average egg output seen at month 2 were a result of pyrantel pamoate administered at month 0. Both parameters showed a steep rise approaching pre-chemotherapy levels following a 6 month period of reinfection. Over the final 5 months, prevalence remained constant at 75%, significantly lower than the initial prevalence of 85% (Student's t test using standard normal deviations of proportions (SND)=2.96, $p<0.01$). In contrast, egg output continued to rise, less steeply, to a value 10% above the initial mean.

The percent of individuals excreting only unfertilized eggs remained at a low level over time, while the proportion excreting fertilized eggs closely mimicked the overall egg positive curve at a lower magnitude (Figure 4.4). Mean EPG and EPD showed similar changes over time (Figure 4.5).

Based on egg intensity curves, it was expected that the mean worm burden had reached pre-chemotherapy levels following 11 months of reinfection. However, neither the prevalence nor the egg intensity curves accurately reflected the overall intensity of worm burdens. The overall mean was 9.89 worms per person at initial chemotherapy and was significantly lower (One way ANOVA on log(x+1) transformed counts, $F=24.59$, $df=1, 523$, $p<0.001$) at 5.41 worms at final treatment, only 54.7% of the initial intensity. Both male and female hosts harboured significantly lower mean burdens at reinfection (males, $F=11.93$, $df=1, 261$, $p<.001$; females, $F=11.92$, $df=1,260$, $p<.001$).

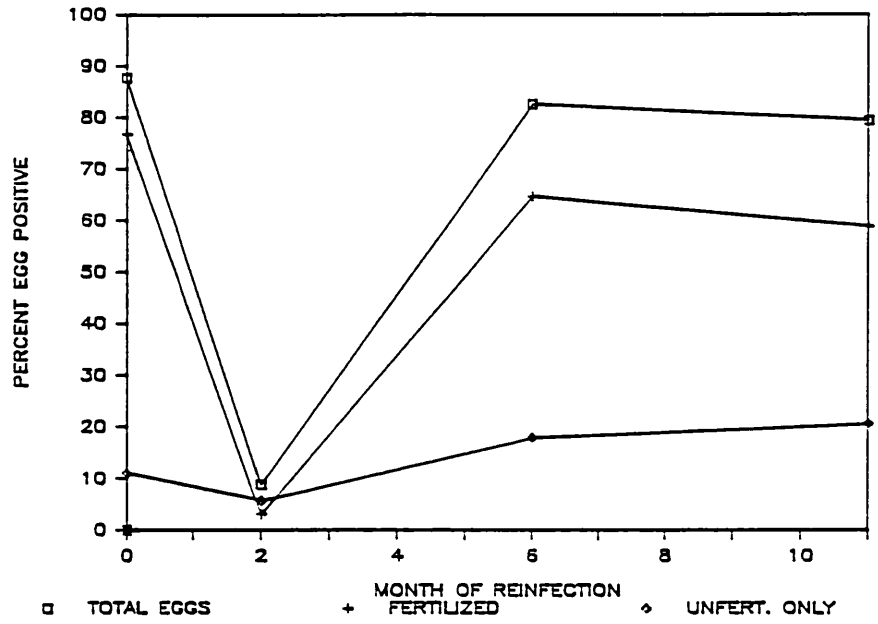


Figure 4.4

Ascaris prevalence during the 4 egg count sampling points: pre-treatment and following months 2, 6 and 11 of reinfection. Prevalence was determined by % egg positivity (also demonstrates unfertilized egg passers where no fertilized eggs were detected) within hosts who received pyrantel pamoate in January. Sample sizes as for Figure 4.5.

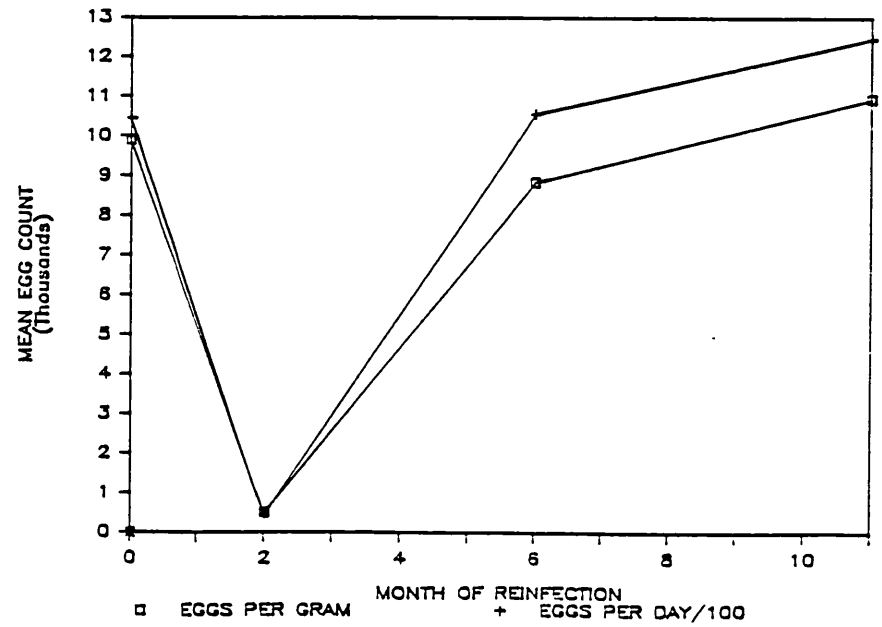


Figure 4.5

The rate of return of egg output (EPG and EPD) to pre-treatment levels (month 0) following mass treatment of the community. Sample sizes were 325 (month 0), 191 (month 2), 189 (month 6), 243 (month 11).

4.3(ii) Reinfection Described by Age Prevalence

The age prevalence profile of Ascaris by egg positivity showed little change with time, except at 2 months following treatment, when egg production by newly acquired Ascaris or previously migrating larvae had not yet begun (Figure 4.6). At the three other time points, similar patterns were seen with the peak prevalence found in the 5-9 age group and declining with age. The percent infected was lower following reinfection relative to initial levels in all groups above 4 years of age, but the 6 and 11 month profiles were nearly identical.

Although 85% of both males and females harboured Ascaris initially, the prevalence at 11 months reinfection differed significantly with the sex of host (females 80.2% and males 69.1%, SND =2.04, $p<0.05$). The decrease in the overall prevalence described in section 4.3(i) was caused by a lower proportion of males becoming reinfected compared to those initially infected (SND=3.28, $p<0.01$). Initial and reinfection prevalences in females did not differ significantly (SND=1.18, $p>0.05$). Figure 4.7 illustrates age prevalence according to the sex of host in November. Males and females showed a similar peak in the 5-9 age group at 95 to 100%, but the prevalence of Ascaris dropped sharply with age in males while remaining high in females.

There was little difference in the percent of negatives within age groups whether determined by absence of eggs in pre-chemotherapy stools or by absence of worms recovered following treatment in January and November (Figure 4.8). However there may have been a considerable number of false negative cases in March based on egg counts because of immature female and male only infections.

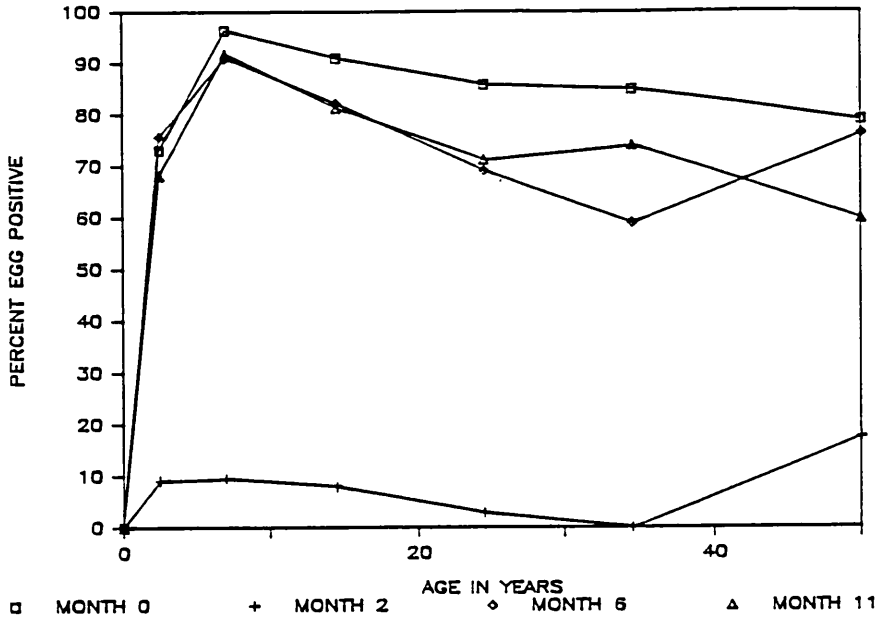


Figure 4.6

The age prevalence profiles of egg positive individuals (males and females) at the 4 egg count sampling points.

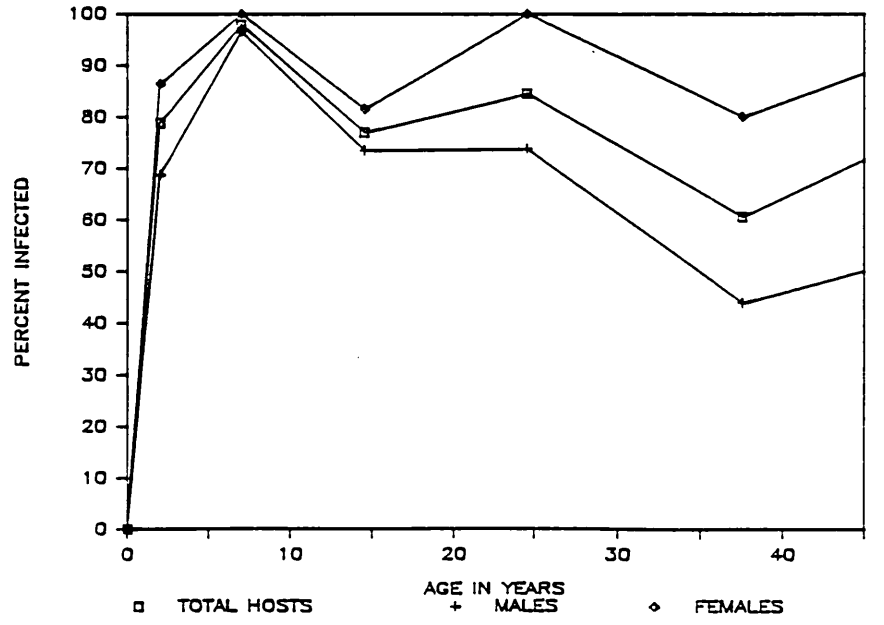


Figure 4.7

The age prevalence profile of Ascaris infection in male, female and total hosts according to worm recovery following expulsion chemotherapy in November.

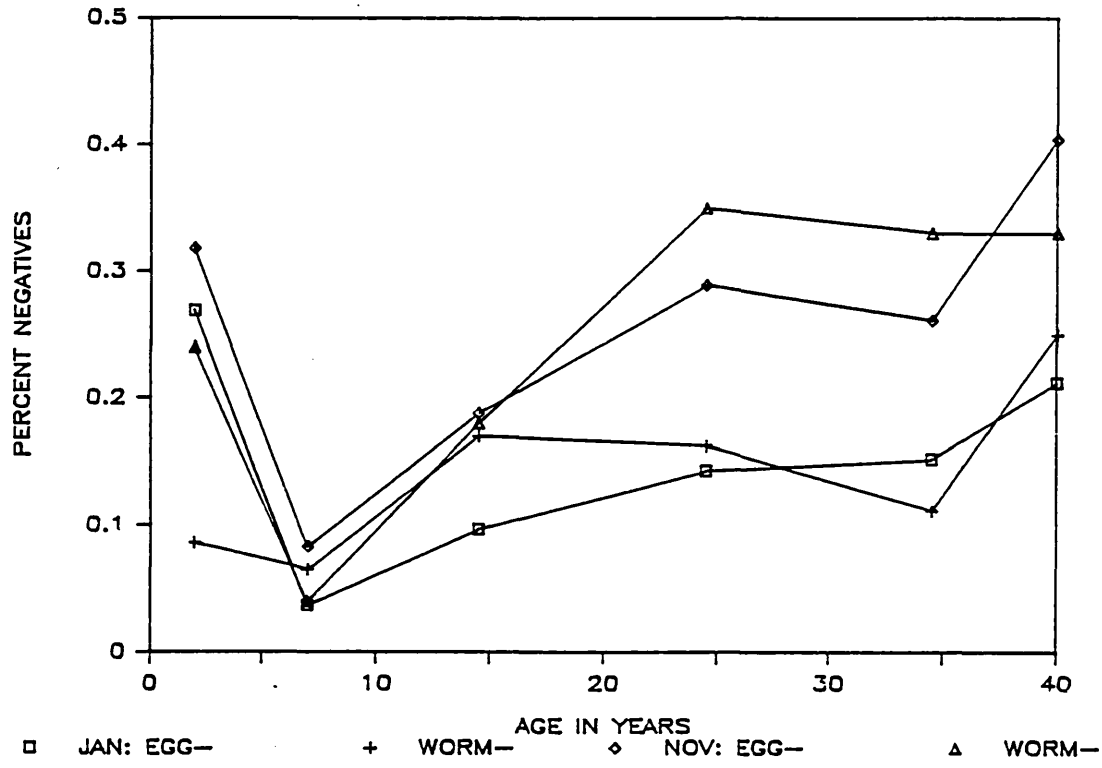


Figure 4.8

The close agreement of estimates of age prevalence determined according to egg positivity or worm recovery. Data of both treatments programmes are represented.

4.3(iii) Reinfection According to Egg Output

Figures 4.9(a,b,c) illustrate the age intensity profiles of Ascaris as estimated by mean EPG determined in January, March, July and November. Each graph demonstrates a consistent trend in egg intensity with host age. The peak egg output in each collection occurred in the 5 to 9 year age class, followed by a decline with age.

Mean EPG by age is demonstrated at the four sampling points in the total population (Figure 4.9(a)), in female hosts (Figure 4.9(b)) and in male hosts (Figure 4.9(c)). In the total population, the average egg output of most age groups was lower at 6 months reinfection than at months 0 and 11. By month 11, mean EPG scores were similar to the initial profile in the older ages (10 years and above), and the scores had surpassed the initial mean in the groups under 5 years and 5 to 9 years of age. None of these described differences in egg output with time are statistically significant on the basis of a factorial 2 way analysis of variance (ANOVA).

When the data are analyzed according to sex of the host, interesting patterns arise. In males (Figure 4.9(c)), egg output at month 6 was significantly lower than the initial levels at month 0 (Two way ANOVA on log(x+1) transformed counts by age and date, $F=6.33$, $df=1, 237$, $p<0.05$). This difference was consistent through most age groups, except in children under 5 years, where the average for all 3 time points (excluding month 2) was unchanged. In females under 10 years of age, the initial EPG values were significantly exceeded at 6 months of reinfection ($F=4.89$, $df=1,96$, $p<0.05$), but no significant difference was detected in females over 10 years of age ($F=2.39$, $df=1, 178$, $p>0.05$).

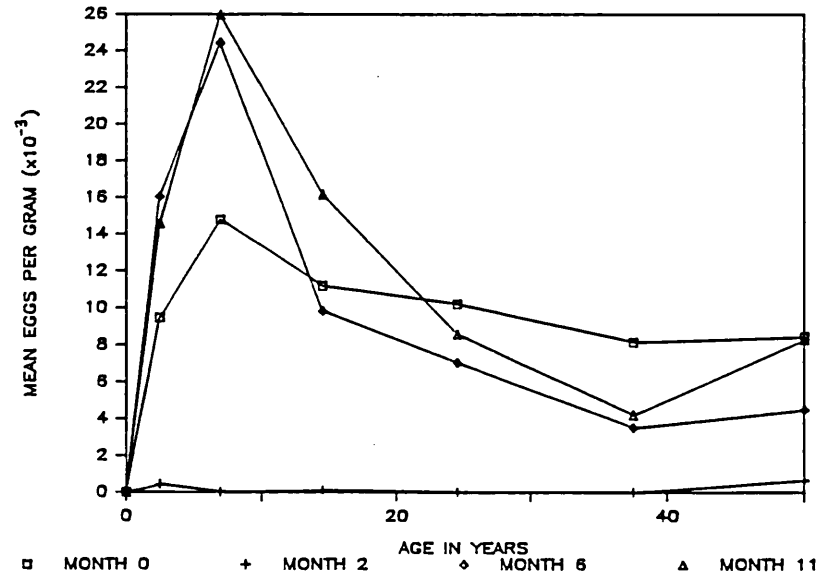
The differential rate of reinfection by host sex is also shown in Figures 4.10 (a,b), which illustrate the mean egg output of males and

Figure 4.9

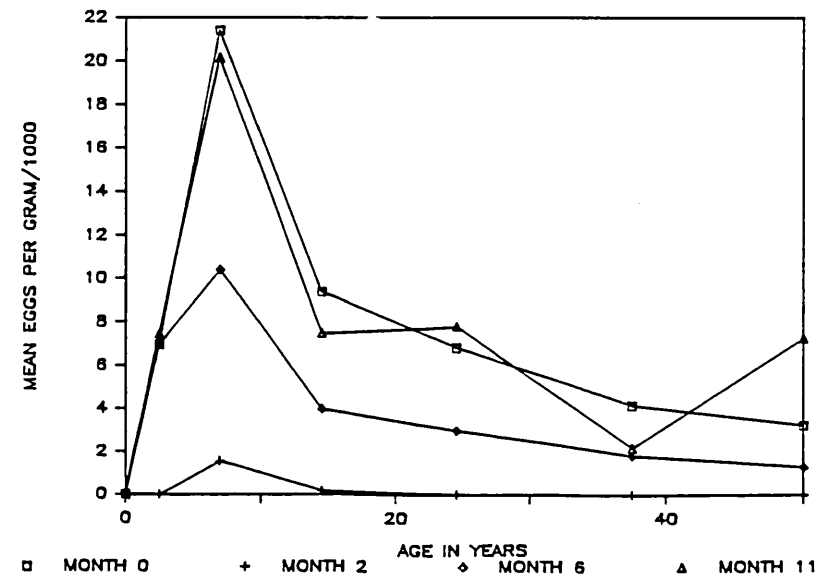
The age intensity profiles of Ascaris EPG determined in January, March, July and November in (a) the total population, (b) in female hosts and (c) in male hosts.


MEAN EGGS PER GRAM (x10^{-3})
AGE IN YEARS
MONTH 0
MONTH 2
MONTH 6
MONTH 11

(a)


MEAN EGGS PER GRAM (x10^{-3})
AGE IN YEARS
MONTH 0
MONTH 2
MONTH 6
MONTH 11

(b)


MEAN EGGS PER GRAM/1000
AGE IN YEARS
MONTH 0
MONTH 2
MONTH 6
MONTH 11

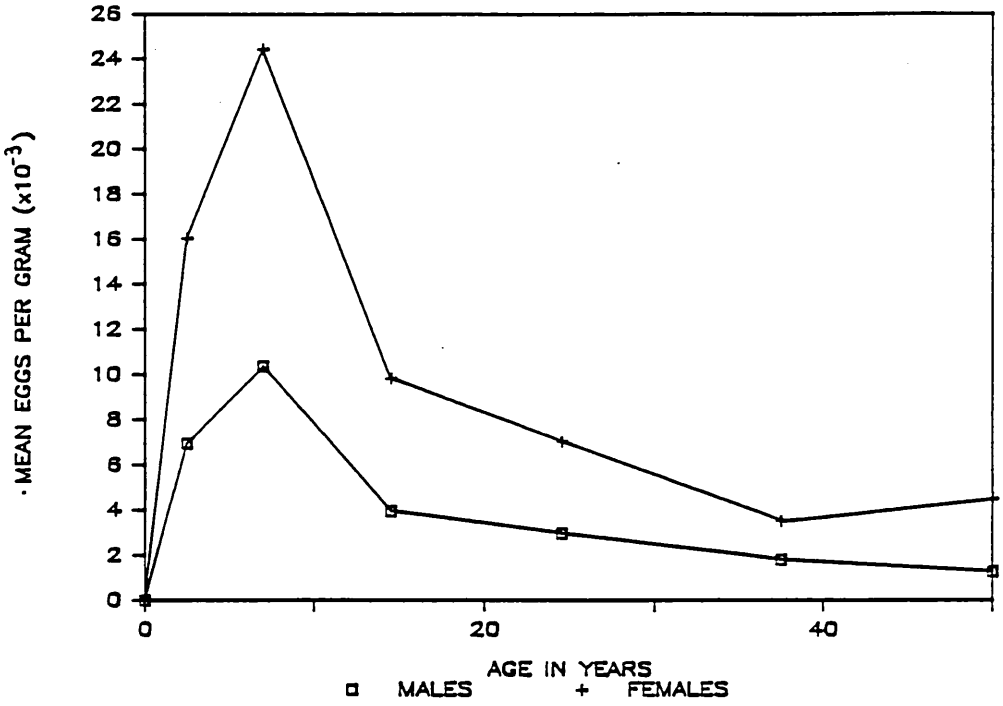
(c)

females at month 6 (Figure 4.10(a)) and month 11 (Figure 4.10(b)). Prior to initial treatment in January, there was no significant difference in the egg output of males and females (Figure 4.2(a), $t=0.92$, $df=324$, $p>0.05$). However, mean EPG in females exceeded twice the value for males in all age groups at the July sampling date, and the difference was highly significant (Student's $t=2.90$, $df=199$, $p<0.005$). In November the difference was less striking ($t=1.96$, $df=253$, $p=0.05$). During the final 4 months of observation, there was little change in female egg output except in the 10-19 year age group, while EPG values increased in males of all ages. Thus females appeared to reacquire Ascaris more quickly than males following chemotherapy, and egg output seemed to stabilize, and possibly decrease, after a certain level of infection was reached.

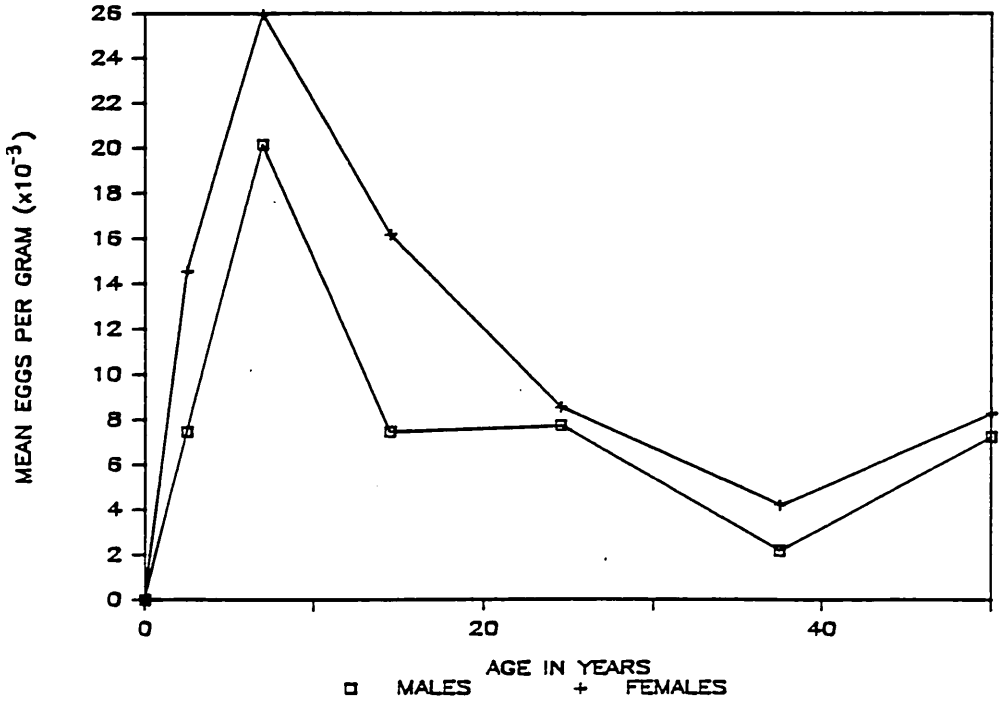
4.3(iv) Reinfection Described by Worm Counts

Although the overall intensity of Ascaris recorded in November following 11 months reinfection had reached only half the initial level, the trends in the sex and age intensity profiles were consistent and similar to the egg output curves. At both time points (Figures 4.11 (a,b)) intensity peaked in the 5 to 9 year age group and declined with age. Females harboured a significantly higher overall mean burden than males (Initial treatment, $F=8.75$, $df=1, 217$, $p<0.005$); reinfection, $F=17.39$, $df=1, 304$, $p<0.001$). This trend was consistent through all age groups, but the least difference in intensity between sexes was seen in the 5 to 9 age cohort in both sampling points.

Differences between the initial and reinfection profiles are evident however. In the initial collection, there was a distinct peak in females under 5 years of age, which was 3 times the average burden



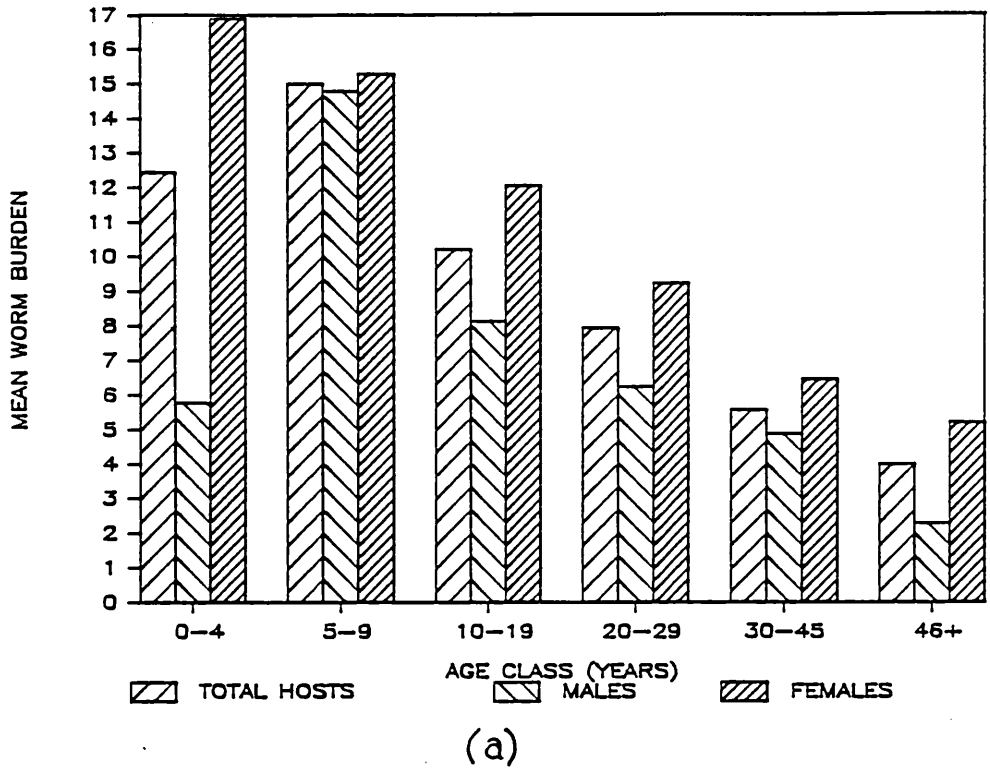
(a)



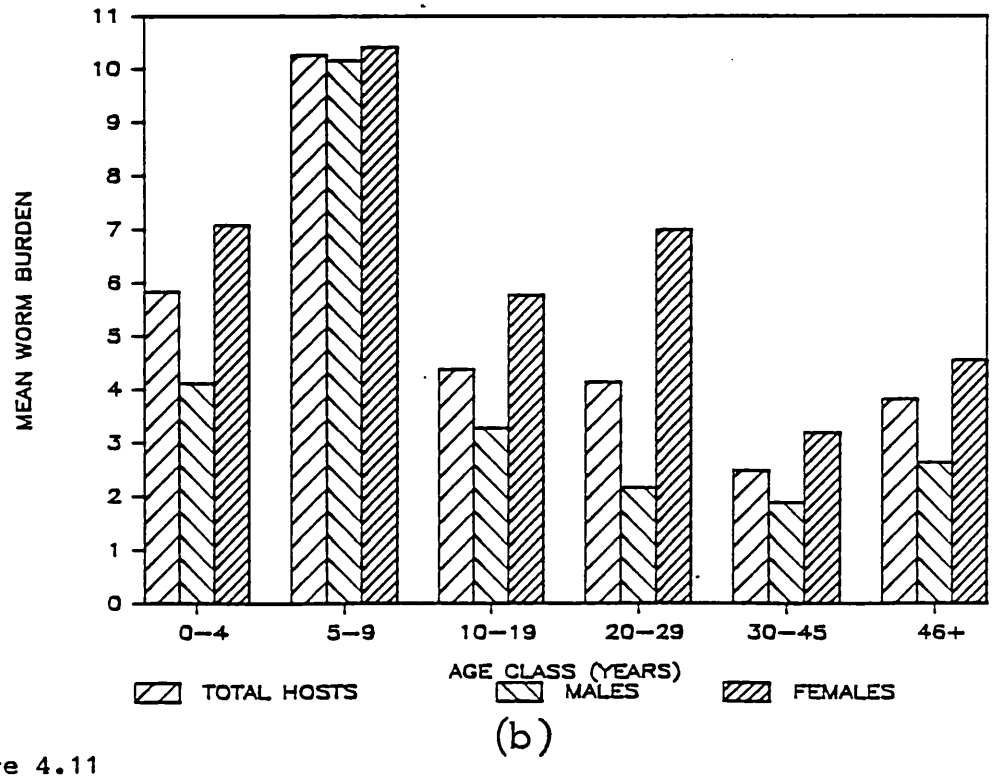
(b)

Figure 4.10

Comparison of the age intensity profiles of Ascaris EPG in male and female hosts, sampled (a) in July following a six month period of reinfection and (b) following 11 months of reinfection in November.



(a)



(b)

Figure 4.11

Age intensity profile of Ascaris worm burdens in male, female and total hosts (a) at initial treatment in January and (b) at final treatment following an 11 month period of reinfection.

of their male counterparts (Figure 4.11(a)). Following reinfection, this peak was not reestablished, and the maximum mean burden in females shifted to the 5 to 9 age group (Figure 4.11(b)). In addition, the decline in worm intensity in age groups over 9 years followed a more consistent pattern in January compared to the uneven age intensity pattern of reinfection detected in November.

The proportions of the total and the immature (see section 4.2(i)) Ascaris populations harboured by age groups initially and following reinfection are shown in Figure 4.12. These patterns were remarkably similar regardless of time of treatment and Ascaris maturity. At both time points, the largest proportion of both the juvenile and adult worm population was harboured by 5 to 9 year olds. The relative proportions of immature to mature worms recovered from age classes did not vary consistently with age at either time point (Figure 4.13; One way ANOVA, at initial treatment: $F= 0.64$, df=5, 182; at reinfection: $F=0.30$, df=5, 236). In addition, the percent of Ascaris that were immature at initial treatment did not differ with sex of host; 16.9% of worms recovered from males and 15.7% recovered from females were immature. Following reinfection, however, males expelled a slightly higher proportion of immature worms, 19.6%, while the percentage dropped in female hosts to 13.2%. This difference was not statistically significant (One way ANOVA, $f=3.65$, $.05<p<.10$, df=1, 240) but it may reflect a slower rate of reinfection in males when compared with females. Differences in the relative proportion of immature worms in groups of hosts could arise from a differential life span of adult worms, changes in the growth rate and maturation time and/or variation in the time of exposure to viable eggs.

According to month 6 and month 11 egg count estimations, the rate of return to initial levels of infection following treatment was

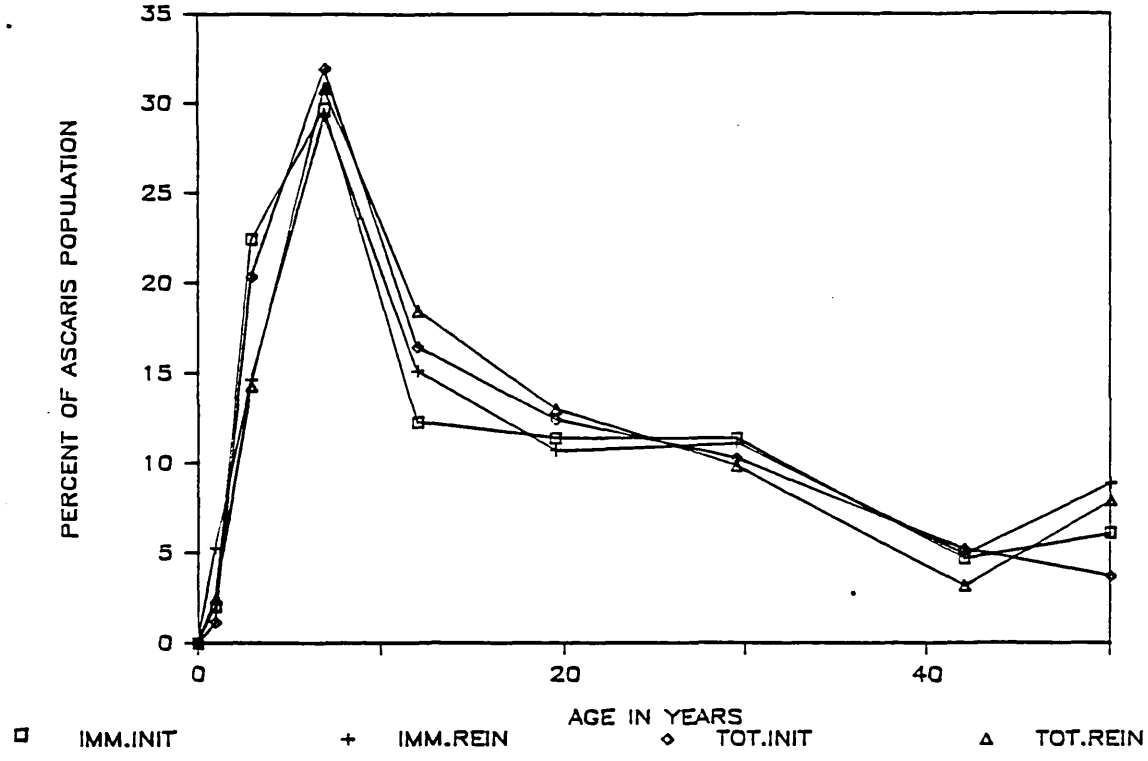


Figure 4.12

The proportions of the immature and total population (juveniles plus adults) harboured by age groups initially and following reinfection.

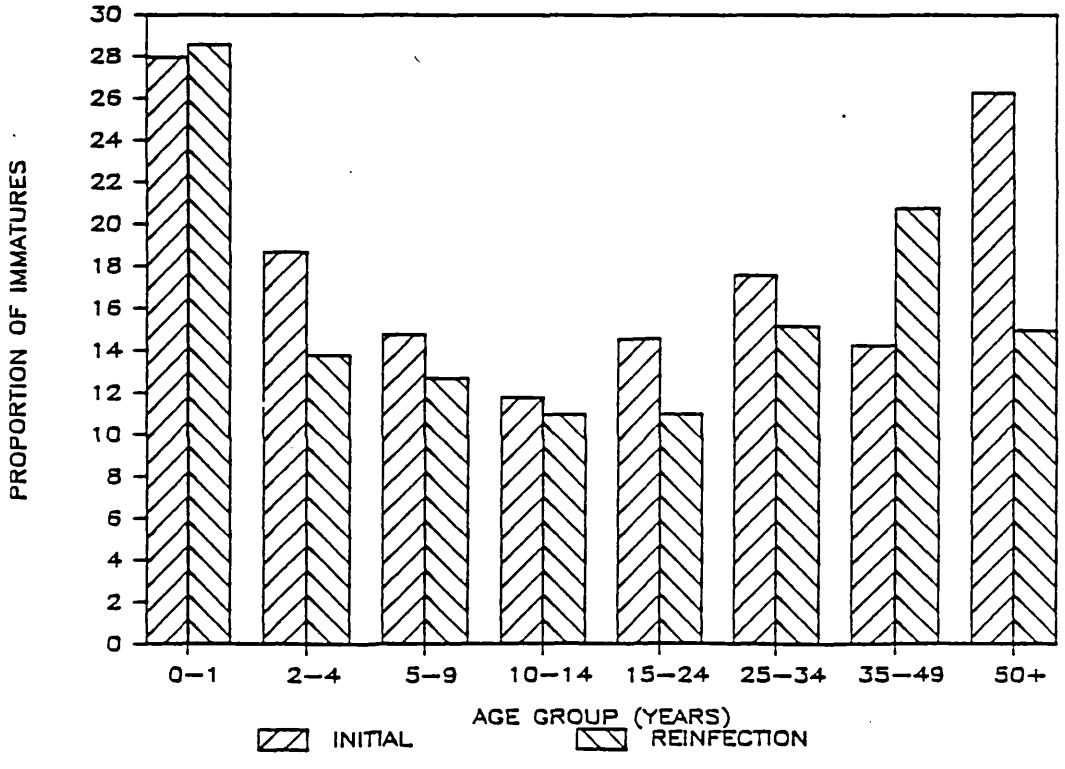
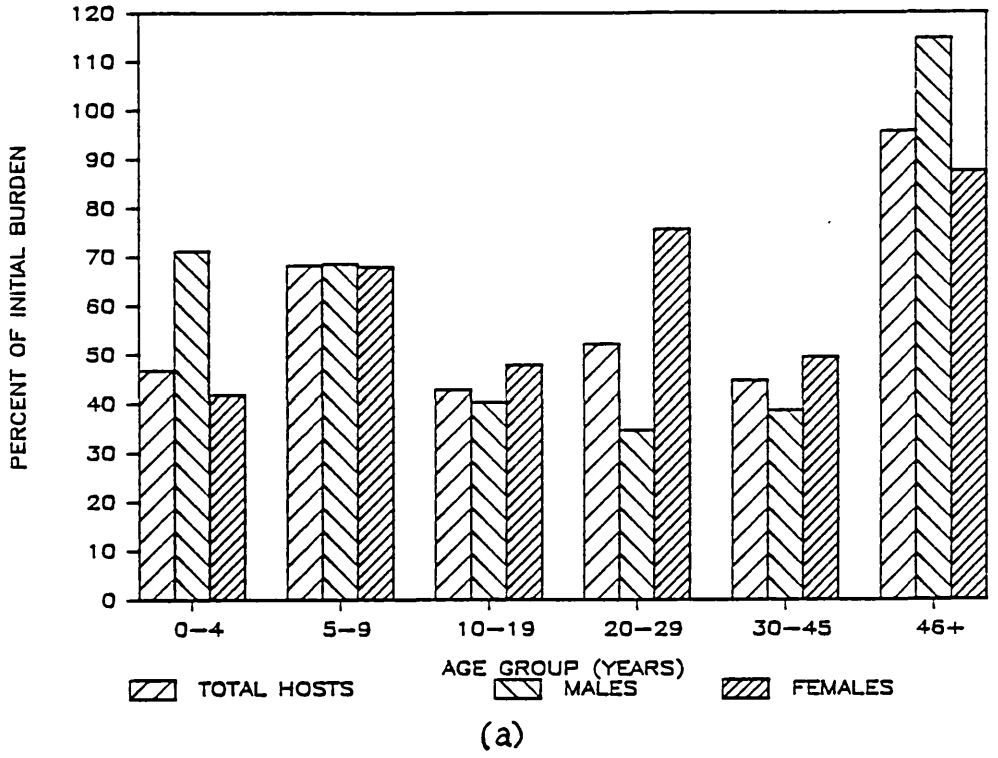
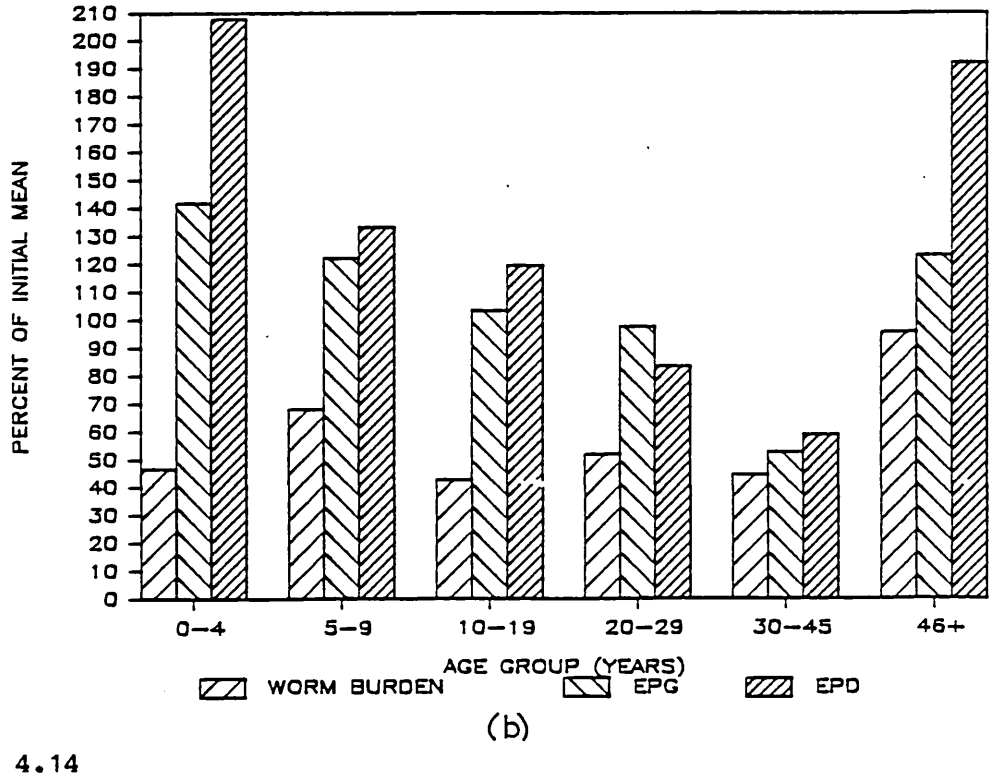


Figure 4.13

The proportion of the Ascaris recovered within age classes following initial and final treatments that were immature (male worms< 10.5 cm, females <12.5 cm).



(a)



(b)

Figure 4.14

The rate of return to initial levels of Ascaris infection (expressed as the mean following reinfection divided by the initial mean) following mass treatment within groups stratified by host age. (a) Comparing initial and reinfection worm burdens in male, female and total hosts. (b) Comparing initial and reinfection worm burden, EPG and EPD scores within total hosts.

strongly age-dependent (Figure 4.14(b)). The youngest age groups and the group 50 years and over returned to or surpassed their original EPG and EPD levels more rapidly following chemotherapy than young adult groups. This trend in egg output, however, was not found in the analysis of worm burdens (Figure 4.14(a) and 4.14(b)). There was no consistent trend with age or sex in the proportion of worms recovered at reinfection compared to the initial intervention (2 way ANOVA, for age: $F=.32$, $df=1,142$, $p>.05$; for sex: $F=.91$, $df=5, 142$, $p>0.05$). Mean reinfection burdens in females were closer to initial levels than those of males at age groups 20-29 and 30-45 years, but males reached a higher proportion in the youngest and oldest age groups.

4.4 Discussion

The horizontal trends of *Ascaris* prevalence and intensity of infection with age recorded in this study are typical for intestinal helminths in communities with stable endemic infection (Figures 4.1 and 4.2). The prevalence increased rapidly with age and declined gradually in the teenage and adult classes. By contrast, following a rapid rise in intensity of infection up to 9 years of age, mean worm burdens fell sharply in adult age classes. A comparison of the age-related trends in these two epidemiological statistics (prevalence and intensity) demonstrates the limitations of a qualitative measurement to describe the abundance of intestinal helminths. The relationship between the two statistics is determined by the frequency distribution of parasite numbers per host (Anderson & May, 1985; Anderson & Medley, 1986). Large changes in intensity may have little impact on prevalence where the parasites are highly aggregated within their host population, as *Ascaris* was in this community (see Chapter

5). This observation implies that any assessment of the efficacy of control measures within a community must be based on intensity as opposed to prevalence statistics (Anderson, 1982; Anderson & May, 1985). This point will be further illustrated later in this discussion.

The reasons for the decline in intensity in older age classes are likely to involve age-related changes in contact with infective stages (due to diet, activity, hygiene, etc.), resistance to helminth invasion and establishment acquired during exposure in the child and teenage years, or a combination of both mechanisms. These reinfection studies revealed a difference in the rate of reacquisition of worms in the different age classes (Figure 4.11(b)), with high rates in children and low rates in adults. These differences may have also been due to age-dependent transmission and/or acquired resistance to infection.

Information concerning age-dependent regulation of intensity can be obtained by analyzing the distribution of immature worms recovered following treatment. Juvenile Ascaris become sensitive to pyrantel pamoate upon arrival in the intestine, as demonstrated by the minimum size recovered of 0.4 cm. It is interesting to note from Figure 4.13 that the proportion of immature worms in the parasite populations recovered in each age class of the community did not appear to vary with host age. Parasite recruitment clearly took place in all age classes, and there was no evidence that acquired resistance mechanisms influence the proportion of young worms (recent recruits) in older people with long periods of past experience of Ascaris infection. More likely age-dependent regulation occurred at an earlier stage in infection, i.e. adults were exposed less frequently to infectious eggs and/or migrating larval stages were destroyed more efficiently in

older age groups. The distribution pattern shown in Figure 4.12 supports this hypothesis by demonstrating that the distribution of immature worms followed closely with total Ascaris by host age. Alternatively, the proportion of immature worms in different age classes of people could remain approximately constant, and independent of age, if a decline in parasite establishment (due to acquired resistance to infection) in older people acted concomitantly with a decrease in worm life expectancy and/or an increase in parasite maturation time. These questions are readdressed in Chapter 7, where very specific data concerning worm size are presented.

The ratios of immature to mature worms within the different age classes of the population also provides information on the life expectancy of Ascaris in man (Anderson, 1986). The estimation procedure adopted is crude, since it is based on the assumption that survival is independent of parasite density and host age. These assumptions require further investigation, but it is interesting to note that this calculation suggests a worm life expectancy of 9 to 11 months, agreeing closely with an estimate by Chai et al (1983) of 7 to 8 months, also based on worm size at reinfection. These estimates are lower than figures of 12 to 18 months widely quoted in past studies (Anderson, 1982b; Croll et al, 1982), but these previous estimates are not based on quantitative information concerning endemic infections.

Three measurements of the rate of Ascaris reinfection following community-wide anthelmintic administration provided conflicting estimates of worm abundance relative to preintervention levels. The overall mean egg output (EPG and EPD) reattained prechemotherapy levels by 6 months reinfection and continued to rise in the final 5 months. Prevalence according to egg positivity approached initial

levels by month 6, but stabilized at this significantly lower level. Intensity, according to worm burden, was reduced by nearly half of the initial levels following 11 months of reinfection. These discrepancies demonstrate the danger of using prevalence (discussed previously) as well as intensity of egg output to predict the level of reestablishment of the worm population following mass treatment.

A likely cause for the rapid return of egg output to initial levels, in spite of a lower intensity of worms, was the absence of density-dependent constraints acting on the worm population during reinfection. Prior to initial treatment when the parasite population was essentially stable, these regulatory constraints may have been operating to reduce fecundity, growth rates and average life span of the worms. The effects of these density-dependent mechanisms were interrupted by elimination of the established worm population through anthelmintic treatment, allowing maximum growth and per capita egg production to occur. Evidence for density-dependent regulation and possible mechanisms contributing to it are presented in Chapter 6.

Although estimation of worm burdens by expulsion/collection techniques yielded the most accurate measure of Ascaris abundance, examination of egg output provided useful information concerning the relative rates of reinfection and the degree of environmental contamination. The surprising speed of recovery of egg output despite considerably fewer worms of reproductive age demonstrates the remarkable ability of Ascaris to reestablish following severe perturbation. This analysis may aid in interpreting results from previous studies that have measured only faecal egg output following treatment. Most importantly, the sharp increase in faecal egg output of female hosts under 10 years of age by 6 months following initial treatment demonstrates the necessity for treatment, once initiated, to

be applied at frequent intervals to prevent an increase in the transmission potential of the parasite within the community.

Although overall prevalence and intensity of egg output were relatively stable compared to worm intensity, analysis according to sex of host revealed interesting differences over time. The data indicate that females became reinfected immediately, since egg production exceeding initial levels was detected by month 6 following treatment. In males however, there appeared to be a delay in either reinfection or maturation of worms to egg production since both prevalence and intensity of egg output remained significantly reduced at month 6. Further evidence of a slower rate of reinfection was the higher proportion of immature worms in the Ascaris population recovered from male hosts compared to female at the second intervention. Initially this proportion was nearly identical between sexes. The reasons for these differences in the rate of Ascaris reinfection between sexes are not clear since they occurred in both children and adults. There may be a relationship between the slower rate of reinfection and the lower mean worm burdens initially found in males in most age groups. The trends could result from sex-associated rates of exposure to infectious eggs, resistance mechanisms delaying establishment or an increased maturation time in male hosts. Older females may be more frequently exposed to Ascaris eggs due to their use of preferred defecation sites in shady areas and their close contact with young children. Alternatively, they may be more susceptible to heavier worm burdens due to an inferior nutritional status, often aggrevated by the additional strain of child-bearing.

There is no obvious explanation for the heavy Ascaris burdens in the 2 to 4 year old females observed in January, but not in November. The initial peak may have been a chance occurrence or anthelmintic

chemotherapy may have increased resistance to infection in this initially highly susceptible group. It would be interesting to know if a greater length of reinfection time would have allowed Ascaris infection to become reestablished at the levels recorded at initial treatment.

On the basis of worm counts determined from the initial intervention and at the second treatment 11 months later, it is clear that there was a fairly stable age and sex-determined pattern of infection. The age and sex intensity relationship was not greatly affected by one application of mass chemotherapy, with the notable exception of the initial peak in the 2 to 4 year old females. These data indicate that anthelmintic treatment repeatedly applied to age groups selected for their high intensity of Ascaris may achieve continual suppression of Ascaris populations. If treatment had been targetted toward infections in children aged 2 to 9 years, over 50% of the total Ascaris (Figure 4.12) and the maximum source of egg production (Figure 4.9(a)) would have been eliminated. It is clear, however, that treatment must be applied at intervals of 6 months or less because of the rapid recovery of egg output, particularly in females of this age group. Treatment at longer intervals can actually increase egg output by altering the age distribution of the parasites and reducing the density-dependent regulation of egg fecundity (discussed in Chapter 6 and 7). In practice, this group is easily accessible to treatment programmes coordinated through schools and primary health centres.

CHAPTER 5

DISTRIBUTION OF ASCARIS WITHIN INDIVIDUAL HOSTS

5.1 Introduction

Intestinal helminth populations have consistently been found to be highly overdispersed within their host community in experimental systems and in naturally infected animal and human populations (Li & Hsu, 1951; Fushimi, 1959; Crofton, 1971a; 1971b; Anderson, 1980, Croll *et al*, 1983). This implies that the majority of hosts are lightly infected with the parasite, and a relatively small proportion will be heavily infected and harbouring a majority of the total worms present in the host community. The negative binomial probability model (Bliss & Fisher, 1953) often provides a good description of the frequency distribution of the observed worm counts (Crofton, 1971a; 1971b; Seo *et al*, 1979; Croll *et al*, 1983, Thein-Hlaing et al, 1984; Anderson & Medley, 1985; Elkins *et al*, 1986). Because most helminth species do not multiply directly within the infected host, overdispersion is probably caused by a combination of factors generating heterogeneity in exposure to infectious eggs or larvae and in host resistance to parasitic larval and adult stages (Crofton, 1971a; 1971b; Anderson & May, 1985; Anderson & Medley, 1985). Aggregation is advantageous to the reproductive success of the helminth population by increasing the probability of mating, whether the parasite is monogamous or polygamous (May, 1977; Anderson, 1980).

In the previous chapter, data describing the infection dynamics of *Ascaris lumbricoides* by sex and age class of the host were presented.

Age and sex are important factors determining parasite distribution in the community. Both pretreatment and after a period of reinfection, children aged 5 to 9 years are the most heavily infected, and females of all ages harbour heavier burdens than their male counterparts. Repeated age-targetted treatment is an attractive option for control of Ascaris in this community due to the aggregation of the worm population in young age groups and the reestablishment of this aggregation after reinfection post-treatment (Figure 4.3, 4.12). These stable trends are thought to be caused by group-shared factors affecting exposure and susceptibility to Ascaris infection (Anderson & Medley, 1985).

Interestingly, a number of studies have found the degree of overdispersion of Ascaris is also high within age groups (Croll et al, 1983; Thein-Hlaing et al, 1984; Elkins et al, 1986). Each age class has its own relatively heavily burdened subgroup carrying the majority of the worms of the group. This suggests that factors affecting exposure and susceptibility differ within as well as between age groups. Such factors may include behavioural, spatial, genetic, social or nutritional status of the individual and/or the family.

The distribution of the parasite within individuals of the community may have an important influence on the effectiveness of population-based strategies of chemotherapy. Extensive field studies, coupled with theoretical research employing mathematical models of parasite transmission, have demonstrated problems in the use of mass chemotherapy in effectively controlling helminths in endemic communities (Anderson & May, 1985; Anderson & Medley, 1985). Because reinfection is rapid and persistent, mass treatment covering a majority of the population must be applied at frequent intervals for many years before long term control is achieved (Arfaa & Ghadirian,

1977; Seo, et al, 1980; Anderson, 1982; Anderson & Medley, 1985; Anderson & May, 1985). Alternative methods of applying anthelmintics more economically on a community basis are needed. One such strategy, based on selective treatment of heavily burdened individuals, has recently generated considerable interest (Walsh & Warren, 1979; Anderson, 1982b; Pawlowski, 1984). Theoretical studies have indicated that the benefits of selective treatment (as estimated by reduction in the parasite population relative to quantity of medication used) of heavily infected individuals is dependent upon the degree of overdispersion of parasites (Anderson & May, 1985). Field studies in Iran and Burma have demonstrated that a majority of the Ascaris population (up to 70%) is harboured by 20 to 25% of the human population (Croll et al, 1982; Thein-Hlaing et al, 1984). Selective treatment of this heavily burdened group can potentially result in the death of a substantial proportion of the parasites using a minimum of chemotherapy.

The aims of both selective and targetted treatment are the elimination of morbidity associated with heavy parasite loads (see Chapter 1) and a reduction in the net egg output into the environment, rather than eradication of the parasite. Eradication is probably a more difficult and expensive undertaking in the absence of improvement in hygiene. As in all other control strategies, selective treatment would have to be applied at frequent intervals due to the rapidity of reinfection. The problems associated with a strategy of selective control involve the economic and organizational costs of repeatedly identifying individuals for chemotherapy, particularly in light of the necessarily short treatment intervals. At present faecal egg counting is the only technique available for quantitatively estimating worm burden prior to treatment. This technique is time-consuming, prone to

technical error and presents many logistical difficulties. Reliable, and inexpensive quantitative assays, such as ELISA, specific for Ascaris would be very useful. The cost of identification could also be reduced if certain individuals are more likely to acquire heavy infections by recurring non-random factors affecting the degree of their exposure and susceptibility. Identification of these individuals could be performed once, and treatment could then be continually focused on this subgroup.

This chapter examines the distribution of Ascaris within individual hosts at both interventions, and the degree of overdispersion (described by the negative binomial parameter k) is determined for the total population and within sex and age-stratified groups. Subgroups of the most heavily infected individuals, who would be analogous to a target group in a treatment programme, from both treatments are described in terms of age, sex, and worm burden. Evidence for predisposition of individual hosts to heavy and light Ascaris infections is described.

5.2 Distribution of Ascaris at Initial Treatment and Following Reinfection

5.2(i) Initial Frequency Distribution

At initial treatment, over 50% of the community were found to harbour less than 6 Ascaris per person. Over 60% of the parasites were aggregated within the most heavily infected 23% of the population, who passed 17 or more worms (hereafter referred to as 'wormy people'). The negative binomial probability model provides a good description of the observed pattern (Chi square test for goodness

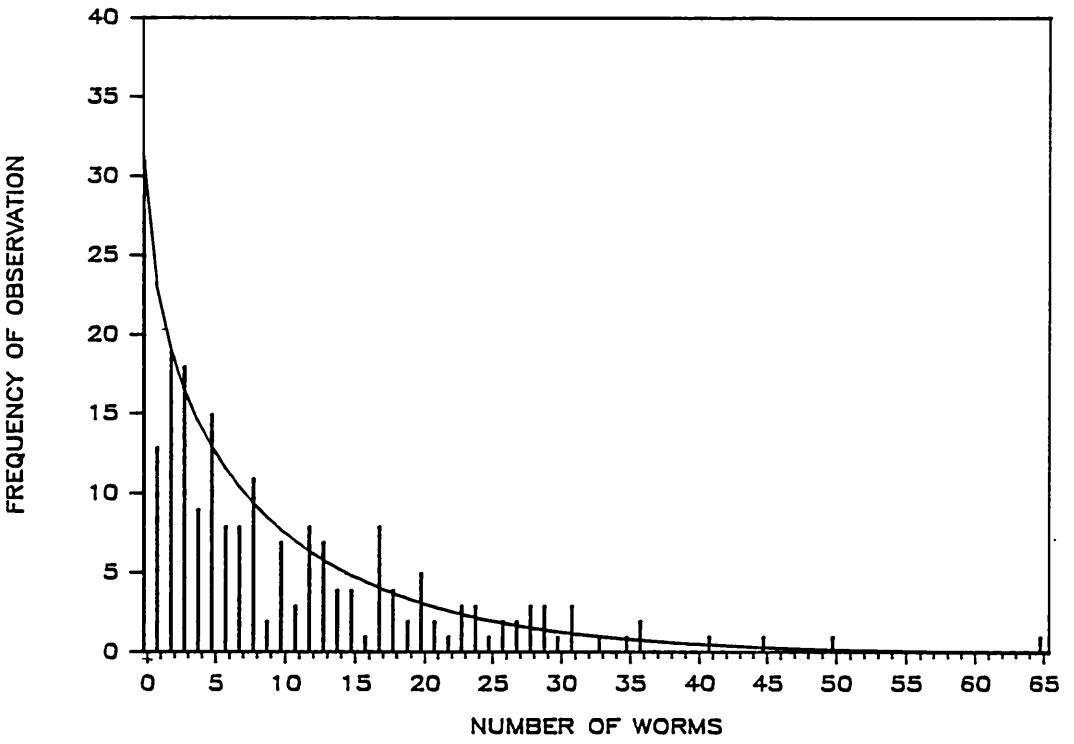


Figure 5.1

The frequency distribution of Ascaris worm burdens expelled following initial treatment in January from the total sample population (n=219). The bars represent observed worm counts and the line represents the predicted curve of the negative binomial probability model using the mean (9.9 worms), variance (113.3) and aggregation parameter k (0.81) of the observed counts. The predicted curve does not differ significantly from the frequency data (χ^2=18.8, df=22, p>0.05).

Table 5.1

The mean, variance, and aggregation parameter, k, of *Ascaris* infections grouped by host age and sex as determined in the initial treatment programme in Vairavan Kuppam. Results of the Chisquare Distribution test describe the goodness-of-fit of the observed data from each age group to the negative binomial model.

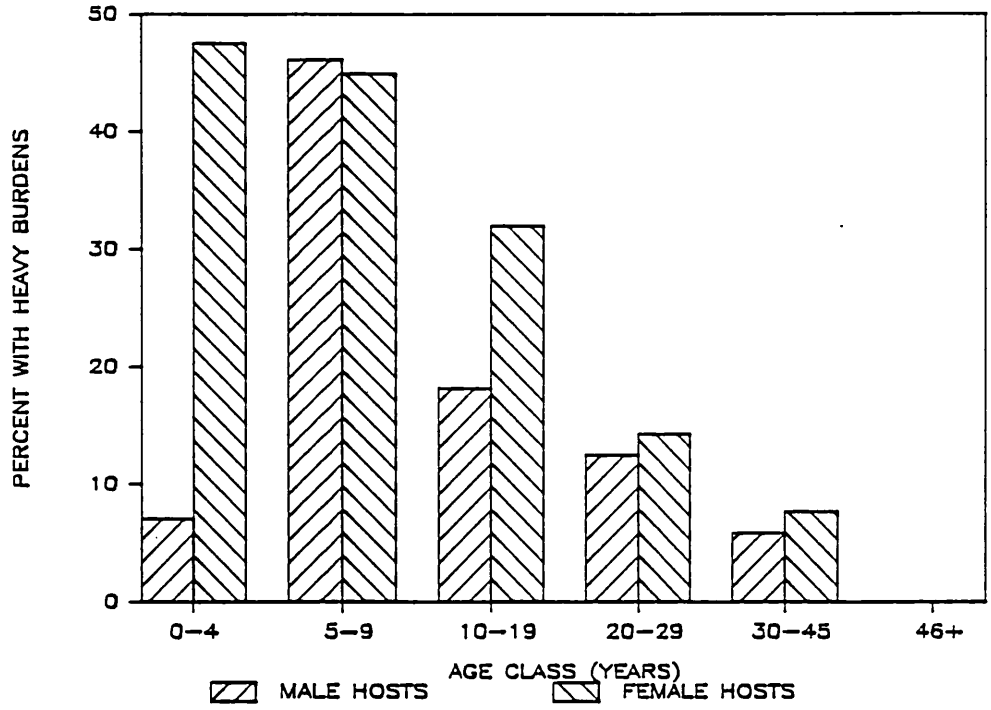
Age	Sex	Samples	Mean	Variance	k Value	Chisquare(df)
0-4	All	35	12.5	195.3	0.75	4.16(5)
	Males	14	5.8	60.6	1.13	0.00(2)
	Females	21	16.9	240.7	0.82	1.01(3)
5-9	All	46	15.0	170.1	1.20	7.74(7)
	Males	26	14.8	218.5	1.13	5.55(4)
	Females	20	15.3	115.2	1.35	2.68(3)
10-19	All	47	10.2	70.5	0.98	6.60(7)
	Males	22	8.1	69.8	0.61	1.36(3)
	Females	25	12.1	66.4	1.75	1.09(4)
20-29	All	37	8.0	71.3	0.91	0.44(5)
	Males	16	6.3	40.3	0.86	0.14(2)
	Females	21	9.2	94.0	1.01	0.49(3)
30-45	All	30	5.6	39.9	0.90	1.44(5)
	Males	17	4.9	51.2	0.51	0.90(3)
	Females	13	6.5	26.6	2.39	1.86(2)
46+	All	24	4.0	19.6	0.86	0.14(4)
	Males	10	2.3	6.5	1.27	0.02(1)
	Females	14	5.2	26.5	0.95	0.07(2)
Overall		219	9.9	113.3	0.81	18.8(22)
	Males	105	8.1	105.9	0.68	5.93(13)
	Females	114	11.5	115.4	1.02	9.58(17)

of fit, χ^2=18.8, n=219, df=22, p>0.05) (Figure 5.1). The aggregation parameter of the distribution, k, (which varies inversely with the degree of parasite clumping) had an overall value of 0.81, as derived by the maximum likelihood method of Bliss and Fisher (1953) (Table 5.1). The subpopulation of people who expelled 17 or more Ascaris ('wormy persons') were not evenly distributed within age groups (Figure 5.2(a)). Over 40% of females under 10 years of age, 40% of the 5 to 9 year old males, and less than 15% of individuals over 20 years of age were found in this 'wormy group'.

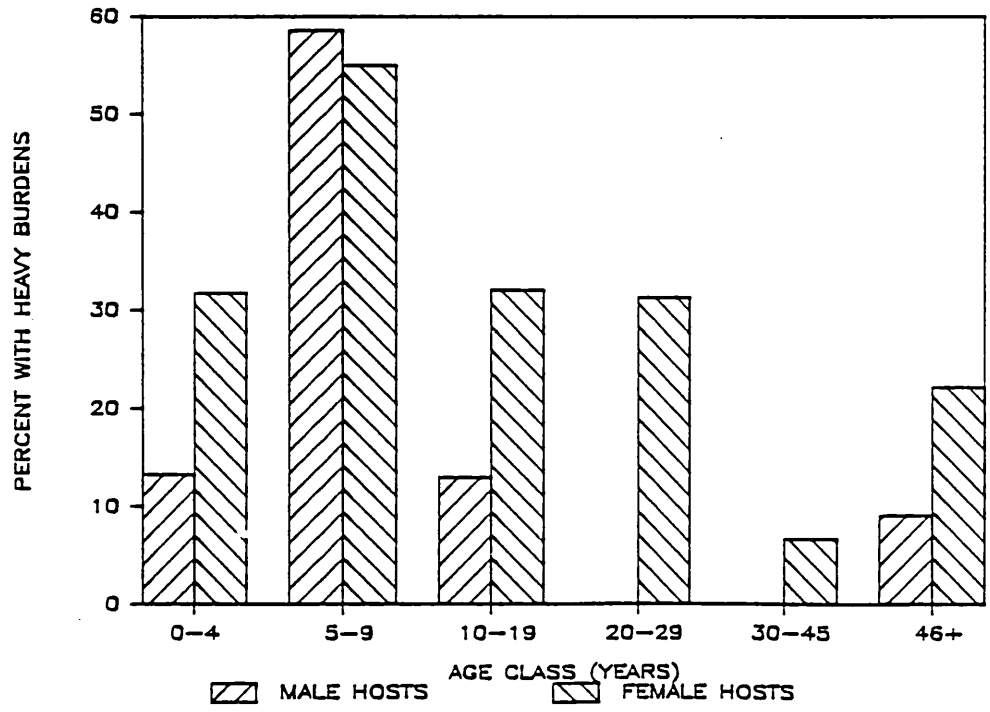
The within age group frequency distributions were also aggregated in form (see Table 5.1), and the negative binomial model is an acceptable model of observed trends within all age groups (Chi-square test, p>0.05). The within age group values for the aggregation parameter k ranged from 0.75 to a peak in the 5 to 9 year age group of 1.20. The degree of aggregation was higher among males when compared with females, both overall and within age classes. This difference between sexes was particularly striking in groups over 19 years of age, where the values for the parameter k ranged from 0.51 to 1.27 in males and 0.95 to 2.39 in females.

5.2(ii) Frequency Distribution of Ascaris Following Reinfection

The frequency distribution pattern of worm burdens following the 11 month period of reinfection appears similiar to the pretreatment curve (Figure 5.3). Once again the data are well described by the negative binomial probability model (χ^2=12.3, n=248, df=17, p>.05). Although worm abundance differed considerably at the two different sampling times, the overall degree of overdispersion was remarkably consistent. Following reinfection, the most heavily infected 24% of the sample population (reinfection 'wormy people') expelled 9 or more



(a)



(b)

Figure 5.2

The proportion of individuals within age groups who expelled heavy Ascaris burdens during the treatment programmes. (a) The proportion who passed 17 or more worms in January. (b) The proportion who received treatment in January and expelled 10 or more Ascaris in November.

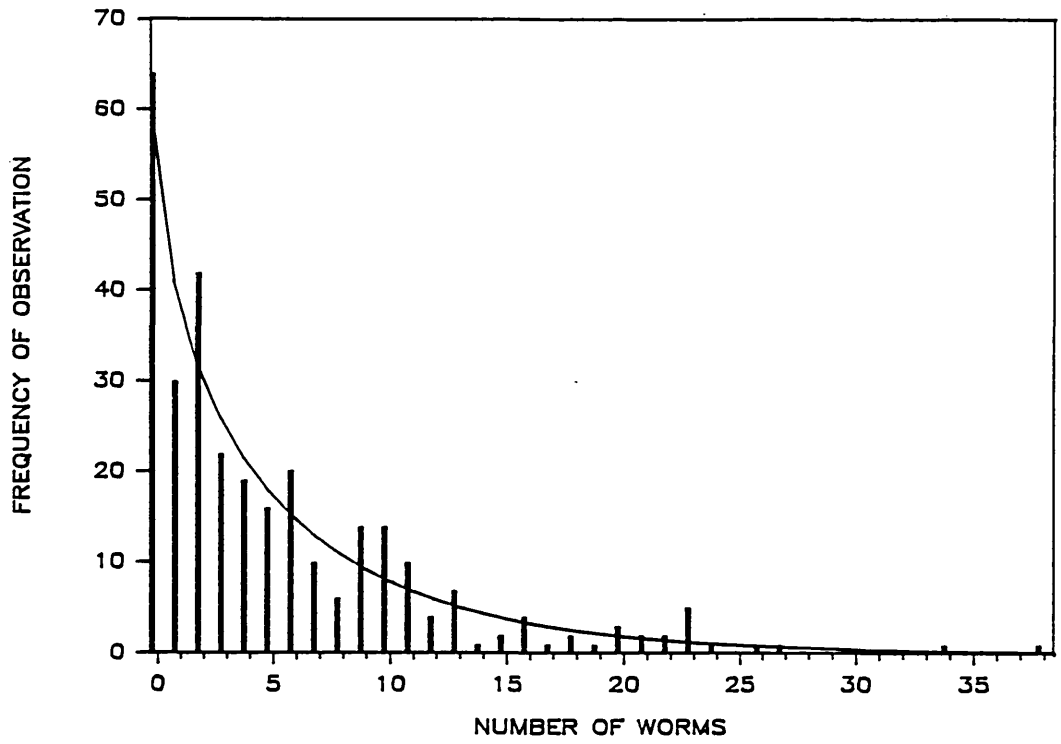


Figure 5.3

The frequency distribution of Ascaris burdens (n=248) expelled by individuals during the second treatment programme following 11 months reinfection. The bars represent observed worm burdens and the curve is predicted by the negative binomial probability model using the mean (5.4 worms), variance (37.2) and k value (0.86) of the observed counts. The predicted curve does not differ significantly from the frequency data (χ^2=12.3, df=17, p>0.05).

Table 5.2

The mean, variance and aggregation parameter k of *Ascaris* infections grouped by host age and sex as determined in the final treatment programme. Results of the Chisquare distribution test describe the goodness-of fit of the observed data to the negative binomial model.

Age	Sex	Samples	Mean	Variance	k Value	Chisquare(df)
0-4	All	38	5.8	33.3	0.99	2.17(5)
	Males	16	4.1	23.5	0.66	0.09(2)
	Females	22	7.1	38.2	1.39	1.09(3)
5-9	All	48	10.3	64.5	1.71	2.96(7)
	Males	29	10.2	64.8	1.82	3.34(5)
	Females	19	10.4	67.7	1.57	3.34(5)
10-19	All	61	4.4	28.5	0.81	7.07(8)
	Males	34	3.3	18.0	0.87	2.30(5)
	Females	27	5.8	39.4	0.87	3.59(4)
20-29	All	39	4.2	22.7	1.14	1.04(6)
	Males	23	2.2	4.2	1.98	0.56(3)
	Females	16	7.0	36.7	1.99	0.58(2)
30-45	All	33	2.5	9.6	0.68	2.35(4)
	Males	18	1.9	6.6	0.42	0.10(2)
	Females	15	3.2	12.9	1.32	0.19(2)
46+	All	29	3.8	14.4	1.00	1.76(4)
	Males	11	2.6	12.7	0.45	0.06(1)
	Females	18	4.6	14.7	1.70	0.23(2)
Overall		248	5.4	37.2	0.85	12.3(17)
	Males	131	4.5	33.7	0.67	13.7(11)
	Females	117	6.4	39.4	1.16	6.7(13)

Ascaris and harboured 64% of the total worms.

As displayed in Figure 5.2(b), the proportion of reinfection 'wormy people' was once again uneven across age groups. Following the second treatment intervention, 55 to 59% of all 5 to 9 year olds of both sexes expelled 9 or more worms. All other age groups of males contained less than 14% 'wormy people'. However, within the 0 to 4, 10 to 19 and 20 to 29 year age groups of female hosts, nearly one third of the members passed 9 or more Ascaris. Compared to the initial treatment, there was a reduction in the proportion of heavy infections within females under 5 years of age and males between the ages of 20 and 45. There was an increase in the proportion of 'wormy people' within the 20 to 29 year old females and in individuals of both sexes over 46 years of age.

The values for the aggregation parameter, k, of 0.81, 0.68 and 1.02 at initial treatment were remarkably similar to the reinfection values of 0.85, 0.67 and 1.16 for the total population, male and female hosts respectively (Table 5.1 and 5.2). The higher degree of aggregation, as described by the lower values for k, in males relative to females was consistent and not affected by mass treatment.

The values for k by age and sex of host are presented graphically in Figure 5.4. The lines representing the initial and reinfection values for k in combined male and female hosts varied little with age. The peak in the 5 to 9 age group indicates a lower degree of overdispersion in this group. Between sexes, however, there was considerable variation. Worm burdens within groups of females over 29 years of age consistently showed k values markedly higher (= lower degree of aggregation) than those of males. Interestingly the aggregation parameter in the 20 to 29 year age group decreased when sexes were combined compared to values derived for males and females

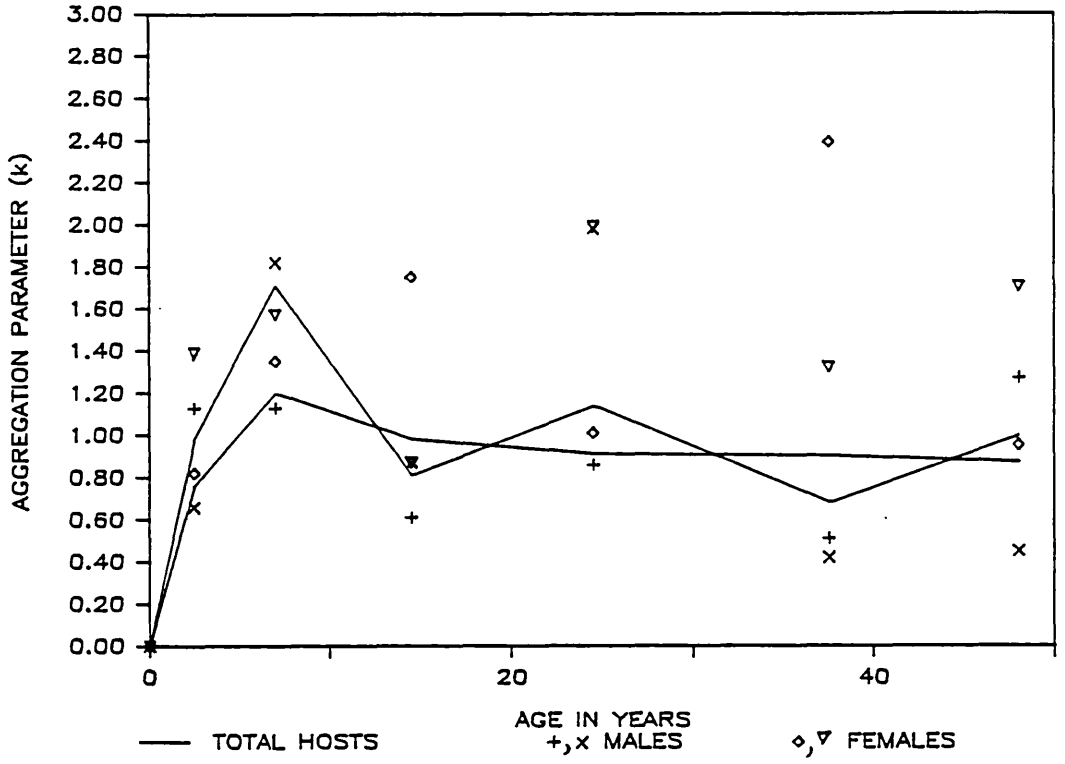


Figure 5.4

The aggregation parameter, k, of Ascaris burdens within sex and age groups at both treatment programmes. The symbols represent k values of male (+ , Jan and × , Nov) and female (◇ , Jan and ▽ , Nov) hosts calculated seperately. The lines represent k values at the two sampling dates within age groups unstratified by sex.

seperately. In age groups over 29 years, however, the combined k values fell between the low values for males and the high values recorded in female groups.

5.2(iii) Egg Output of the Heavily Infected Group

Egg counts (EPG and EPD) were assessed in the population prior to anthelmintic treatment to determine the reliability of these measurements in the identification of 'wormy people'. For this analysis, a subgroup of individuals who donated both pre-treatment 24 hour stools and full 48 hour post-treatment faecal output were considered. This sample provided egg and worm count data for 201 and 213 individuals from the initial and second treatments, respectively. In the initial treatment, 49 of the individuals who were identified as 'wormy' by worm counts donated stools for egg count assessment. Of these 49, 33 had EPG scores within the top 25% of the egg counts recorded of 15,550 or more. Sixteen 'wormy individuals' would not have been identified on the basis of faecal egg counts. Following reinfection, 36 of the 55 'wormy individuals' who donated pre-treatment stools had EPG scores within the highest 25% for the group (over 17,000). Expansion of the egg count group to 30% did not selectively improve detection of 'wormy people' compared to false negatives. In both treatments, 65% of the heavily burdened individuals determined by worm counts were also identifiable by their relatively high eggs per gram scores.

Following treatment in January, the initial 'wormy people' reaccumulated Ascaris at a faster rate than lightly infected individuals according to faecal egg count scores (Figure 5.5). The average EPG scores for the initial heavily burdened group were significantly higher following a 6 month (Kruskall-Wallis

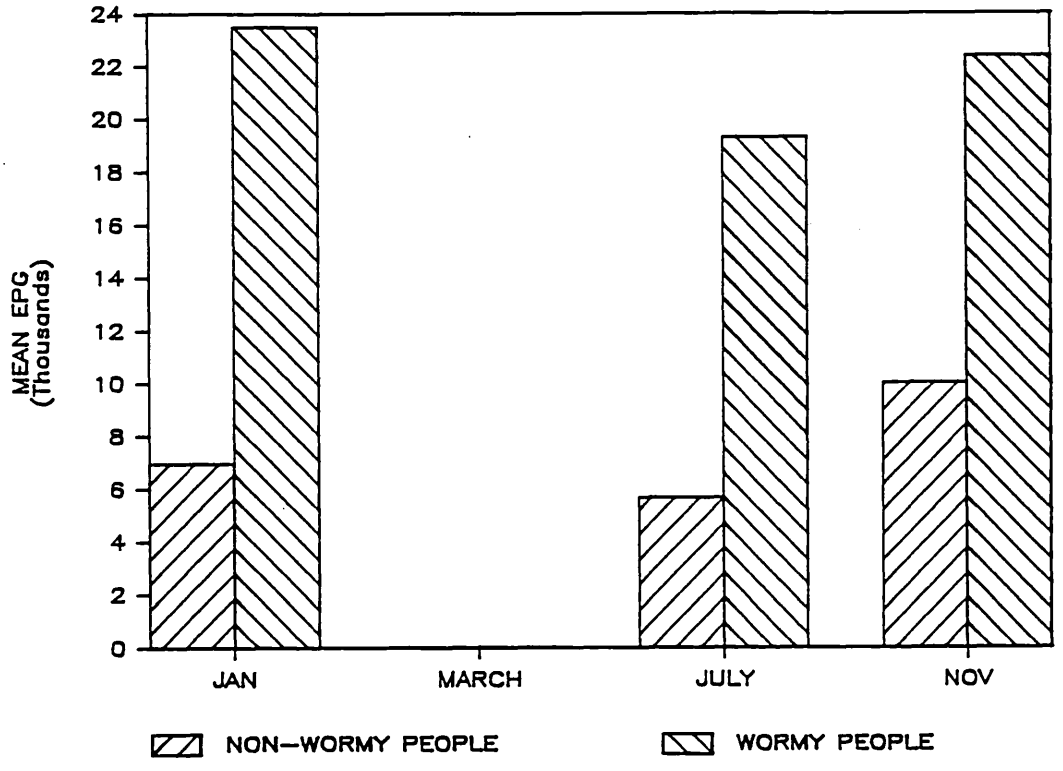


Figure 5.5

Comparing the mean EPG from all four 24 hour stool collections of the group who passed 17 or more Ascaris in January with the mean of the January 'non-wormy' group. The initial 'wormy group' had significantly higher mean egg output in July and November than the 'non-wormy' group (Kruskall-Wallis test, H=24.77, 18.95, p<0.000, respectively).

Non-Parametric Test, H=24.77, p=.0000) and 11 month (H=18.95, p=.0000) reinfection period. The percent egg reduction rate induced by treatment with pyrantel pamoate did not differ between the two groups.

5.3 Analysis of Worm Counts within a Fully Cooperative Subgroup

In the next two sections of this chapter (5.3 (i,ii)), analyses were performed on a subsample of 174 individuals who donated complete 48 hour post-treatment faecal output during both the initial and second treatment programmes. Individuals who cooperated during only one of the 2 worm collections have been excluded. This allowed for a comparative examination of the initial and reinfection worm burdens within the same individual hosts.

Within this subsample, 44 individuals (25%) passed 17 or more Ascaris following initial treatment and 49 (28%) expelled 9 or more following the second treatment. These 'wormy groups' harboured 63 and 68% of the total Ascaris recovered from the 174 individuals following the first and second treatments, respectively.

5.3(i) The 'Wormy Group'

Figure 5.6 demonstrates the number of individuals from each age class who harboured 17 or more worms following initial treatment, 9 or more worms following the second treatment and those who were repeatedly 'wormy' in both sampling times. As expected from results shown in Figures 5.2(a) and 5.2(b), the majority of initial and reinfection 'wormy people' were found in the young age groups. If the age group stratification was divided between the younger 50% of the village (groups under 15 years of age) and the older half (ages 15 plus), only 6 and 10 individuals from the older 50% of the village expelled heavy burdens at the initial and second treatments,

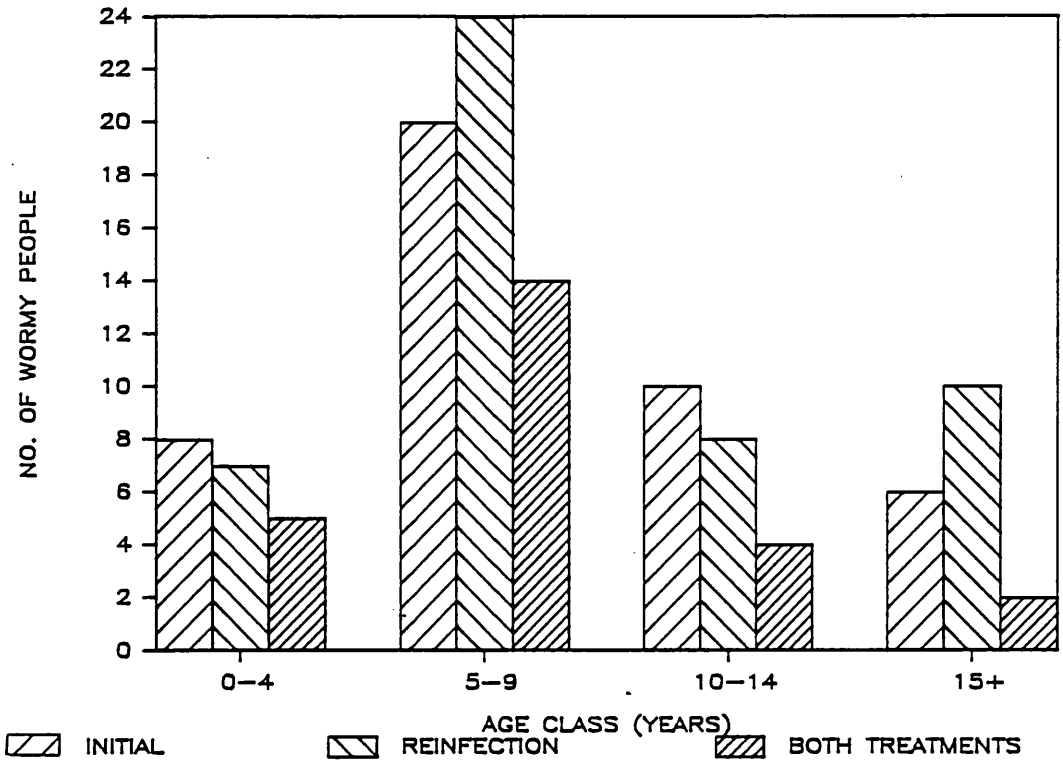


Figure 5.6

The number of individuals grouped by age who were 'wormy' in January, November and at both sampling points.

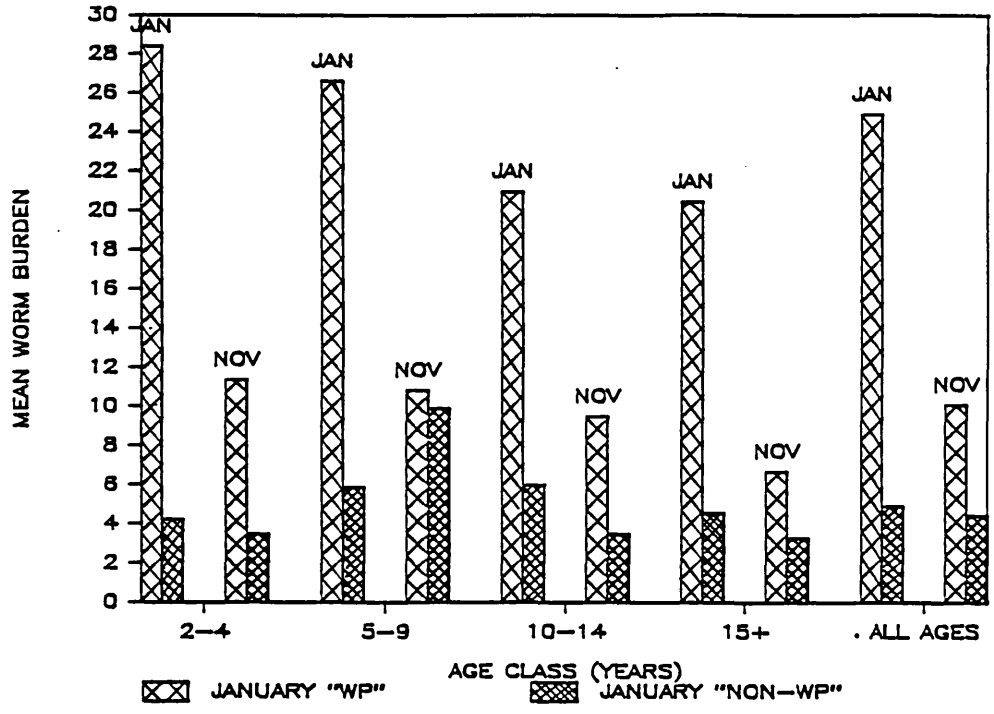
respectively. It is important to note, however, that every group contained some individuals who were heavily burdened relative to other individuals in his/her age group. Only two individuals over 15 years of age were 'wormy' at both treatments.

The 5 to 9 age class contained half of the 'wormy people' in both treatments. Of these individuals, 16 were 'wormy' during one programme only, while 14 harboured heavy burdens at both the initial and reinfection treatments. In January, the age group 0 to 4 years included only one male who harboured a heavy infection, while 10 females expelled 17 or more Ascaris. This male and four of these females were 'wormy' at both treatments. The 10 to 14 year age group contained 10 individuals with heavy burdens at one treatment and 4 who were within the 'wormy group' of both treatments.

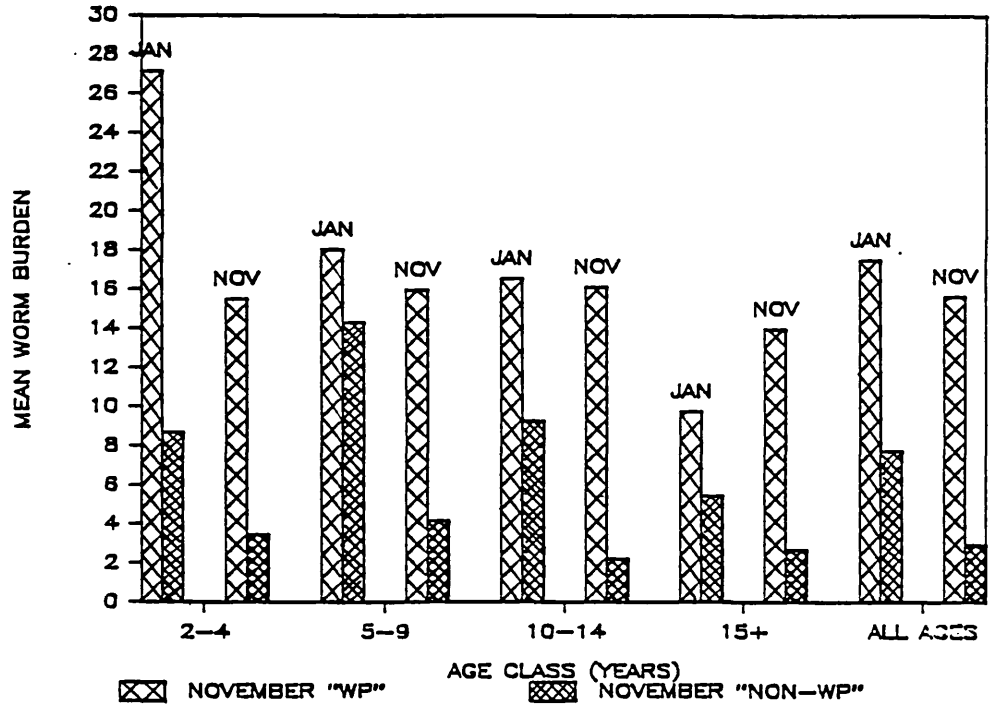
The initial and reinfection mean worm burdens of the two wormy groups are shown in Figures 5.7(a) and (b). Individuals who passed 17 or more worms in the first treatment reacquired significantly higher burdens during the following 11 months than initially lightly burdened individuals (Student's T test on log(x+1) transformed counts, $t=3.57$, $df=97$, $p<0.001$, Figure 5.7(a)). The initial mean worm burden of individuals who passed 9 or more Ascaris following reinfection were significantly higher than the initial burdens of the lightly reinfected group ($t=3.46$, $df=97$, $p<0.001$, Figure 5.7(b)).

5.3(ii) Predisposition of Individuals to Heavy or Light Infection

Analysis can be refined by considering reinfection within each individual in the sample population by using non-parametric statistical methods such as the Kendall's rank correlation test. Because it is based on the relative rank order of the data, this test assumes no particular parent distribution and does not require that



(a)



(b)

Figure 5.7

Age-stratified mean worm burdens of once-identified 'wormy people' compared to 'non-wormy' people recorded from the two treatment programmes. (a) Initial and reinfection burdens of those who passed 17 or more Ascaris in January relative to January 'non-WP'. (b) Initial and reinfection burdens of November 'WP' compared to those who became reinfected with less than 10 Ascaris.

the variables have similiar means and variances. Kendall's rank test revealed a highly significant positive correlation between the initial and reinfection burdens from individuals in the sample population (Table 5.3, Figure 5.8(a)). This positive association in the unstandardized worm counts was probably influenced by the differences in the intensity of infection due to group factors such as age and sex. To remove the influence of age and sex on the worm counts, the burdens were standardized by expressing each individual count as a positive or negative deviation from the mean of the age or age/sex group. The standardization procedure is defined by the following equation:

$$Y_{i,j,k(t)} = (X_{i,j,k(t)} - x_{j,k(t)}) / s_{j,k(t)} \qquad E5.1$$

where $X_{j,k(t)}$ is the mean worm burden of individuals of sex k and age class j at time t, $X_{i,j,k(t)}$ recalls the data count for individual i and $s_{j,k}(t)$ is the standard deviation of the mean for that age/sex group. This procedure reduced the value of tau (Table 5.3), but it did not effect the significance of the positive correlation.

Strong correlations were also seen between initial and reinfection worm burdens of individuals within the age groups 0-5, 6-11 and 26 years and over, but not in the group 12-25 years. The relationships between the unstandardized (log transformed) scores of these age groups are shown in Figure 5.8(b,c,d,e).

Table 5.3

Kendall's Non-Parametric Rank Test using actual worm counts and standardized data to test for significant associations between the initial and reinfection worm burdens within individual hosts.

GROUP ANALYZED	SAMPLE SIZE	KENDALL'S TAU	SIGNIFICANCE
A. Complete Data, All Ages			
Raw Counts	174	0.302	p<0.001
Age Standardized	174	0.247	p<0.001
Sex/Age Stand.	174	0.218	p<0.001
B. Raw Data, Grouped by Age			
0-5	37	0.313	p<0.01
6-11	41	0.254	p<0.01
12-25	43	0.097	n.s.
26 Years Plus	53	0.358	p<0.001

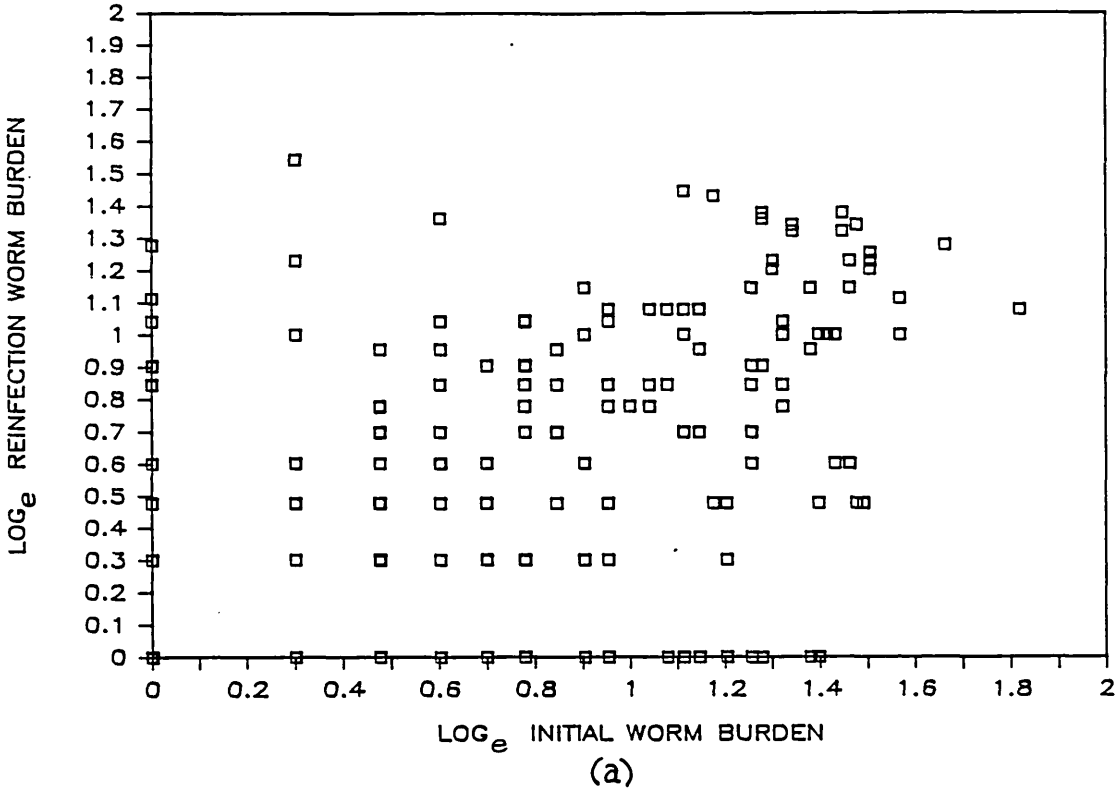
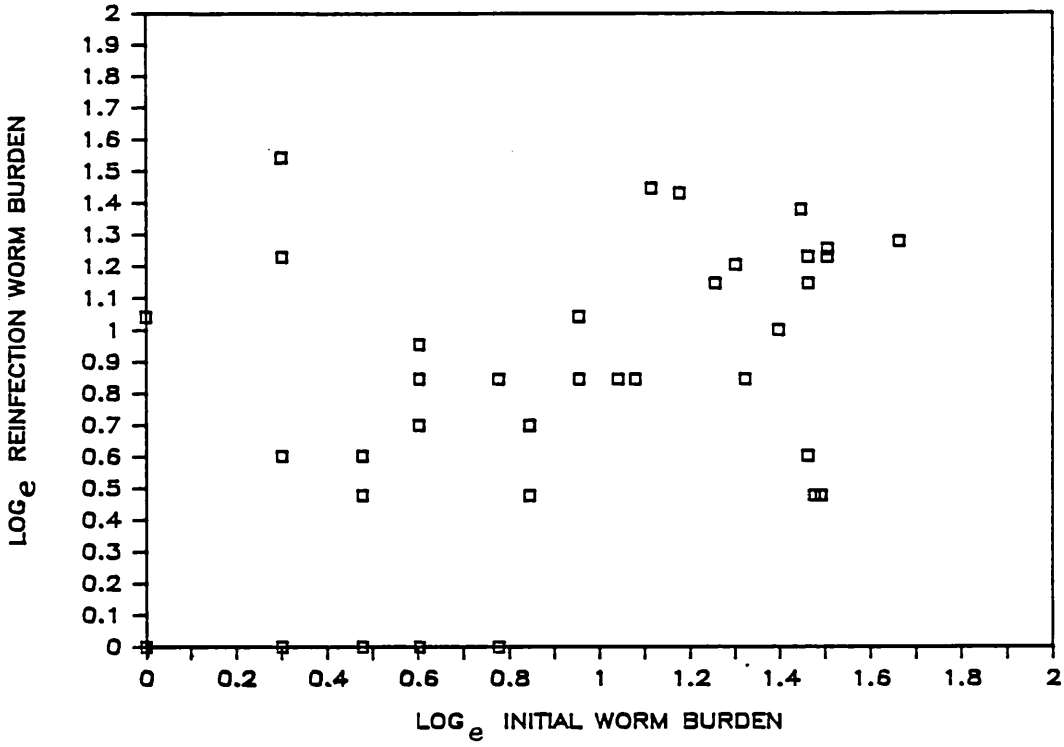
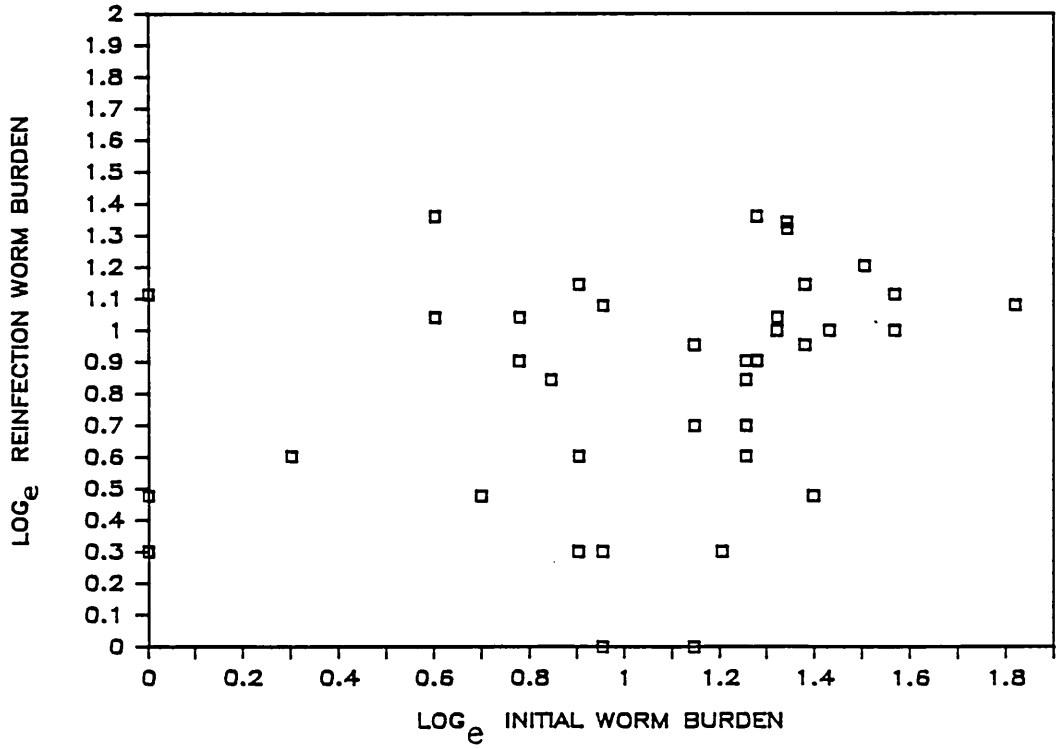


Figure 5.8

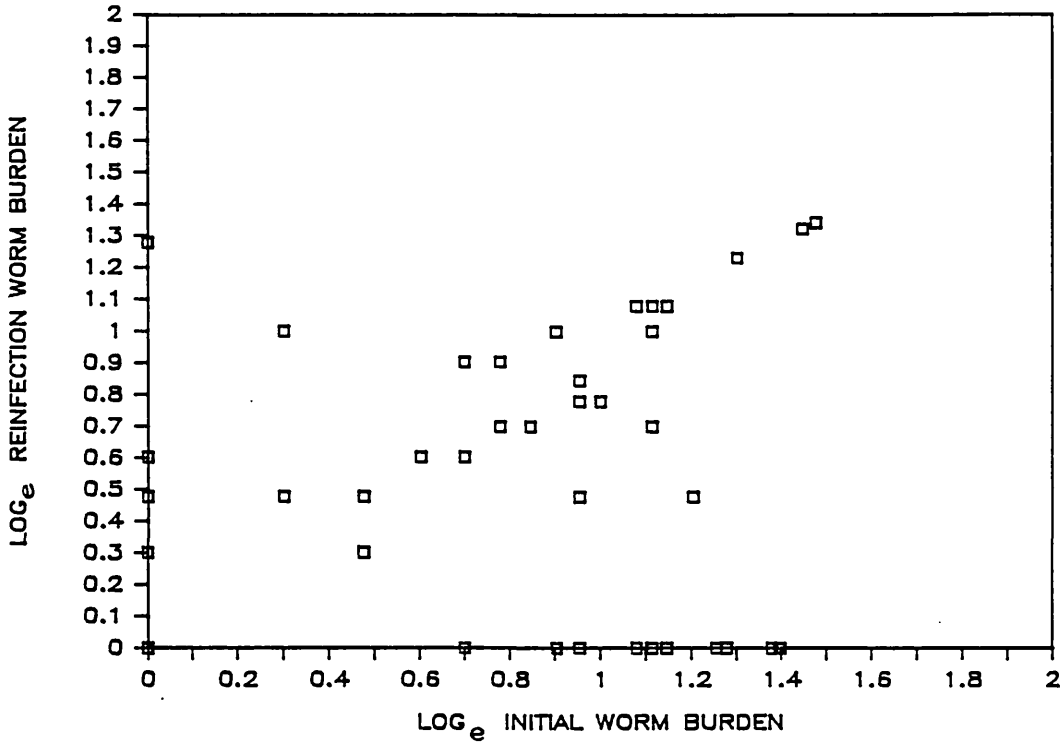
Initial worm counts (x axis) of individuals plotted against their reinfection burdens (y axis) within the (a) Total Group (n=174) and in groups stratified by age: (b) 0 to 5 years (n=37), (c) 6 to 11 years (n=41), (d) 12 to 25 years (n=43) and (e) 26 years plus (n=53).


LOG$_e$ REINFECTION WORM BURDEN
LOG$_e$ INITIAL WORM BURDEN
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
2

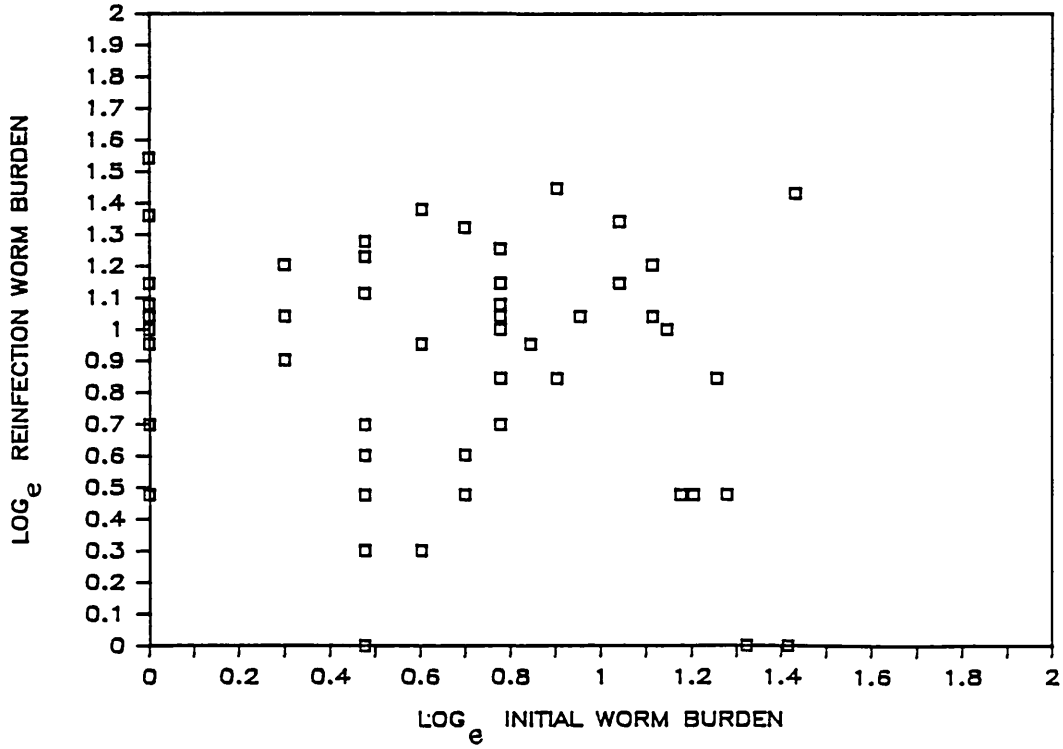
(b)


LOG$_e$ REINFECTION WORM BURDEN
LOG$_e$ INITIAL WORM BURDEN
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
2

(c)


LOG$_e$ REINFECTION WORM BURDEN
LOG$_e$ INITIAL WORM BURDEN
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
2
0.2
0.4
0.6
0.8
1.2
1.4
1.6
1.8

(d)


LOG$_e$ REINFECTION WORM BURDEN
LOG$_e$ INITIAL WORM BURDEN
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
2
0.2
0.4
0.6
0.8
1.2
1.4
1.6
1.8

(e)

5.4 Discussion

The distribution of Ascaris within individuals in this community was highly aggregated, such that most people were lightly infected, and relatively few harboured the majority of the worm population in heavy infections. The worms were highly aggregated (as described by the negative binomial parameter k) within all age classes of the population and within both sexes. In combination with density-dependent fecundity (described in the next chapter), parasite aggregation acts to create a regulatory constraint on parasite transmission via the production of transmission stages within the community.

The generative mechanisms of helminth parasite aggregation within human populations are probably many and varied (Anderson, 1982; Anderson & May, 1985). For Ascaris, these are likely to include heterogeneities in social, behavioural, spatial factors affecting the degree of exposure to infectious eggs, and genetic, nutritional and parasitological factors determining the host's ability to resist invasion and acquire resistance to infection. Variability in these factors occurred within age groups of this community, although the degree of aggregation varied considerably between sexes. The aggregation of Ascaris was less severe within groups of females compared to males, particularly in adult age classes. Experiments in inbred animal models have indicated that acquired immunity tends to decrease the degree of parasite aggregation in the host population, but the significance of these findings to Ascaris infections in humans is unclear (Anderson & Medley, 1985).

The similarity in the degree of parasite aggregation at initial treatment and following reinfection, as described by parameter k for the total sampling population and for male and female hosts, was striking. At both sampling programmes, 20-24% of the human population harboured 60-64% of the total worms of the community. The relative degree of aggregation (as determined by k) within male and female hosts did not change. Males, who had a significantly lower mean intensity than females, also exhibited a lower overall k value consistently within age groups at both the initial and reinfection treatments.

It should be noted that the 'cutoffs' used in analysis of the 'wormy people' were arbitrarily chosen as the likely target group for a selective treatment programme, rather than a precise level of infection where morbidity is likely to occur. Quantitative information is needed from studies of the incidence of ascariasis complications, including acute surgical emergencies and its contribution to malnutrition, to define the relationship between intensity of Ascaris and the likelihood of morbidity. In the initial treatment, individuals passing 17 or more Ascaris were designated as 'wormy people'. Since the intensity of Ascaris was lower in the second treatment, the cutoff of 9 worms or more was chosen to include the most heavily infected quarter of the population. Because the intensity of Ascaris remained lower within both the wormy group and the lightly infected group following an 11 month period of reinfection, control of morbidity may not necessarily require multiple annual mass treatment of the community. Examination of Figure 5.5 and the data presented in Chapters 4 and 7, however, indicate that treatment applied at wide intervals can actually increase the level of egg output into the environment, and may ultimately raise the

transmission potential of the parasite. The heavily infected population showed an substantial increase in egg output by 6 months following treatment, despite the presence of fewer egg-producing female worms. This reflects a decrease in the density-dependent constraints acting on egg fecundity. These are discussed in more detail in Chapter 6.

Analysis of individuals ranked by worm burden at both sampling points revealed a highly significant positive correlation between the initial and reinfection burdens of each individual in the sample population. This positive correlation was also found when the data were standardized by age and sex and examined within most age groups. Thus there was a strong tendency for individual hosts to reacquire worm burdens of a similar magnitude following treatment and reinfection relative to others in the sample population. Factors influencing predisposition may be similar to those that generate aggregated parasite distributions and the age and sex intensity profiles. These include social, spatial and behavioural factors influencing the frequency of exposure to infectious eggs and nutritional, immunological and physiological factors determining the degree of natural and acquired resistance to the migrating larval and adult parasitic stages. The strength of the correlation between initial and reinfection burdens within individuals indicates that selective treatment repeatedly applied to once-identified 'wormy people' may be an effective means of controlling morbidity and reducing the transmission of Ascaris in endemic communities.

Figures 5.5, 5.6 and 5.7(a,b) demonstrate the relationship of these non-parametric statistical correlations to the practical issues of selective treatment targetted against once-identified 'wormy individuals'. Nearly one half of all children under age 15 who

expelled heavy burdens in the initial treatment became heavily reinfected. The mean egg output of the initial 'wormy group' was significantly higher prior to treatment than the means following periods of 6 and 11 months reinfection (Figure 5.5). This indicates that the degree of environmental contamination with Ascaris eggs may also be efficiently reduced by selective treatment. The average reinfection burdens of initially wormy people were significantly higher than the mean reinfection burdens of the initial lightly burdened group (Figure 5.7(a)). In addition, by retrospective examination, the initial mean burdens of the 'reinfection wormy group' were significantly higher than the average initial burden of the lightly reinfected group (Figure 5.7(b)). This pattern was consistent within age groups, however the least difference between the mean burdens of initially 'wormy' and 'non-wormy' groups at reinfection was seen in the 5 to 9 year olds who also had a relatively low degree of overdispersion (k=1.2). Age-targetted treatment of this group would probably be a more appropriate control strategy than selective treatment since there appeared to be a high degree of susceptibility to heavy burdens within the majority of the members of this age range (Figure 5.2(a,b) and 5.6). This group is also easily accessible because most attend school. Another highly susceptible group at initial treatment was females aged 0 to 4 years. Within this age group 48% were found to harbour more than 16 worms, compared with only 7% of their male counterparts. Special attention should be focussed upon these susceptible groups in the design of control strategies.

At present, identification of 'wormy people' can be carried out using two techniques. Heavy worm burdens can be determined through treatment and worm recovery from 48 hour stools as used in the above analysis, or by quantification of faecal egg output. There are

considerable difficulties involved in both techniques with respect to the implementation of large-scale programmes. Recovery of expelled worms following treatment is the ideal choice for high accuracy, largely because of the high efficacy of the drug used and the fact that the majority of worms are passed in the first 48 hours following treatment (see Chapter 3). Inaccuracies can arise, however, when individuals do not collect every stool passed within the designated period. Collections are time-consuming because every individual must be carefully questioned to determine whether stools were passed outside of the container provided. The field team must be continually present during the 2 days to distribute fresh containers in exchange for those used and to encourage full cooperation. The isolation of worms is quick if quantification of large worms only is acceptable. Collection of faecal samples for egg counting is an easier option, but the degree of sensitivity in identifying 'wormy people' will vary according to the techniques used. The technique employed for this study, formalin/ethyl acetate sedimentation of samples from mixed, whole stools, yielded a 65% success rate in identifying 'wormy people'. Sensitivity might be improved by determining egg counts in multiple pre-treatment stools.

CHAPTER 6

THE EFFECT OF HOST FACTORS AND PARASITE DENSITY ON FECUNDITY

6.1 Introduction

The abundance of parasites within their host population is often influenced by density-dependent factors (Anderson & May, 1978; Keymer, 1982; Anderson & Medley, 1985). These may act on the parasites' ability to establish, survive and reproduce within individual hosts. In the case of intestinal worms, these effects are thought to be the result of limited resources within the host gut, such as nutrients and space (Nesheim, 1985), and/or host-mediated responses, both non-specific and immunological in nature (Lloyd & Soulsby, 1985). The net effect of these density-dependent constraints on the larvae and adult stages of the parasite life cycle is dependent on the degree of worm aggregation found within the host population. The more skewed the distribution (ie, the lower the k value of the negative binomial probability model, see Chapter 5), the greater the role density-dependence will play in regulating parasite population growth (Anderson, 1985). The effects of density-dependence are most marked within the relatively few individuals who harbour the majority of the total parasite population.

Literature on the mechanisms responsible for density-dependent regulation of nematode populations is scarce (Keymer, 1982; Anderson, 1982b). This is largely due to the difficulties of quantifying the nutrient intake of helminths within the host and measuring the relevant host response to increases in the degree of antigenic

stimulation resulting from a larger worm population. Recent work by Keymer and Hiorns (1986) on Heligmosomoides polygyrus in mice indicates that per capita EPD may be functionally related to host faecal production. Heavily burdened mice were observed to pass fewer eggs per day per female worm (but not lower eggs per gram) and significantly lighter stools per 24 hour period. The authors suggested that the reduction in fecundity, measured by EPD per female worm, is a result of a decrease in faecal production, possibly related to parasite-induced undernutrition.

Within human communities infected with Ascaris, evidence for density-dependent relationships has been obtained between per capita egg fecundity (both EPG and EPD) and total worm burden. A non-linear relationship between Ascaris burden and egg production was observed in a number of epidemiological surveys measuring egg output immediately prior to determining worm burden by expulsion chemotherapy (Croll et al, 1982; Martin et al, 1983; Thein-Hlaing et al, 1984; Elkins et al, 1986). In these studies the inverse relationships between the egg output (eggs per gram) per female worm and total worm burden harboured by the host were described empirically by power functions. When egg output per unit time (eggs per day (EPD)) was examined by Hlaing et al (1984), exponential models, $y = a \exp(-bx)$, provided the best description for the observed data.

The density-dependent relationship between egg fecundity and worm burden, as discussed in section 1.2(iv), may influence the accuracy of determining worm burdens based solely on egg counts. Doubts of the validity of Ascaris egg counts as an accurate measure of worm burden have been raised by many authors. The unreliability of measuring egg production is not only due to the decrease in per capita egg output as worm burden rises, but also due to the increase in the degree of

variability as the average egg output rises (Croll et al, 1982) and the considerable degree of daily variation within individual hosts (Sinniah, 1982). Keymer and Hiorns (1986) concluded that EPG was a more accurate measure of worm burden than EPD since egg concentration was independent of the daily fluctuations of stool weight in H. polygyrus in mice.

This chapter examines the effects of host and parasite factors on per capita egg fecundity in Vairavan Kuppam recorded at the initial treatment programme and following an 11 month period of reinfection. Included in this analysis are the influences of host age, sex, stool weight and worm intensity on the egg production of mature female Ascaris.

6.2 Results

6.2(i) The Validity of Egg Counts to Estimate Worm Burden

The determination of worm burden by egg counts is used for two different purposes. One is to estimate the number of worms in a particular person for the purpose of individual treatment. The second is to determine the ranking of individuals in a population relative to others for the purpose of treatment at the population level. To examine the usefulness of egg counts as an index of relative worm burden in the community, Kendall's Non-Parametric Rank Test was conducted to compare the rank order of individual scores of EPG and EPD, EPG and worm burden, and EPD and worm burden in both January and November. A highly significant positive correlation was found between all of the ranking comparisons at both sampling dates (Table 6.1). If only the mature females in each burden are examined with EPG and EPD, both indeces of egg output were also found to be highly correlated

Table 6.1

Correlations between EPG, EPD and worm count scores of individual hosts in January and November using Kendall's non-parametric rank test.

Collection	Variables Tested	Sample Size	Kendall's Tau
January	EPG, Total Count	151	.5352 ***
	EPD, Total Count	151	.4385 ***
	EPG and EPD	169	.6770 ***
November	EPG, Total Count	176	.5155 ***
	EPD, Total Count	176	.5058 ***
	EPG and EPD	205	.7253 ***

*** = p<.001

Table 6.2

Product-Moment correlations between the number of mature female worms (length > 12.5 cm) harboured by individual hosts and their EPG and EPD counts (All scores transformed using log(x+1)).

	MALES			FEMALES		
Age Gp.	EPG	EPD	(n)	EPG	EPD	(n)
0-4	.544 *	.412 *	17	.550 ***	.347 **	36
5-9	.563 ***	.435 **	42	.737 ***	.707 ***	35
10-19	.624 ***	.558 ***	35	.610 ***	.589 ***	40
20-29	.580 **	.665 ***	24	.391 *	.370 *	30
30 +	.611 **	.709 ***	22	.632 ***	.594 ***	44

* = p<.05

** = p<.01

*** = p<.001

when tested within host age groups (Table 6.2). There were no obvious differences in the correlation coefficient between the two egg count scores and either total burden or mature females. In addition, no trends in the degree of correlation were apparent according to host age or sex.

6.2(ii) The Effect of Host Age and Sex on Parasite Fecundity

As discussed in Chapter 4, the distribution of Ascaris within the host population was strongly influenced by age and sex, and it is important to determine whether these host factors also effect the fecundity of mature female worms. Two way analysis of variance (ANOVA) was performed to examine the degree of variation in per capita EPG and EPD grouped according to age class or sex of the host at the initial treatment and following reinfection. The mean per capita EPG showed no significant variation with either host factor (Two way ANOVA on log(x+1) transformed data, Table 6.3, Figure 6.1(a,b)). Mean per capita EPD varied significantly ($p<.05$) with age at initial chemotherapy as shown in Figure 6.2 (a), though the large peak seen in the 20 to 29 year olds may be due to the heavier stool weights in the older age groups. No significant variation with EPD per female worm was detected when examined according host age and sex following reinfection (2 way ANOVA, Table 6.3, Figure 6.2(b)). The average fecundity of the initial Ascaris population did not differ significantly from that of the Ascaris population harboured in November (One way ANOVA, for EPG: $F=1.05$, for EPD: $f=2.04$, $df=1,227$, $p>.05$).

6.2(iii) The Relationship between Faecal Output and Fecundity

To test the hypothesis that large stool weights are associated

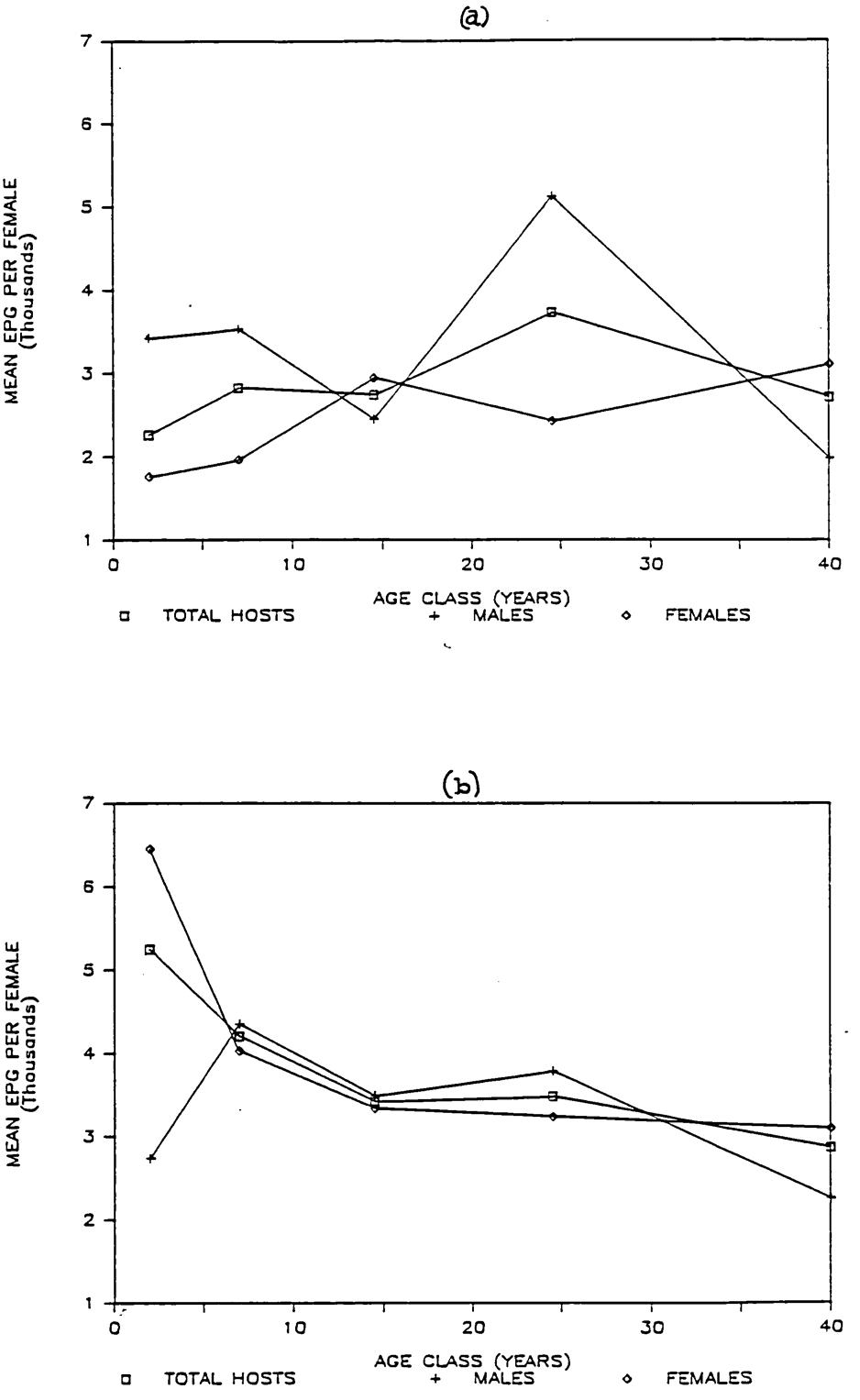


Figure 6.1

Mean eggs per gram of faeces per mature female Ascaris according to host age and sex in January (Graph(a)) and in November (Graph(b)). Sample sizes are:

	January			November		
Age Class	Total	Males	Females	Total	Males	Females
0-4	23	7	16	34	11	23
5-9	40	22	18	42	23	19
10-19	37	15	22	45	24	21
20-29	31	15	16	29	13	16
30+	34	12	22	37	10	27

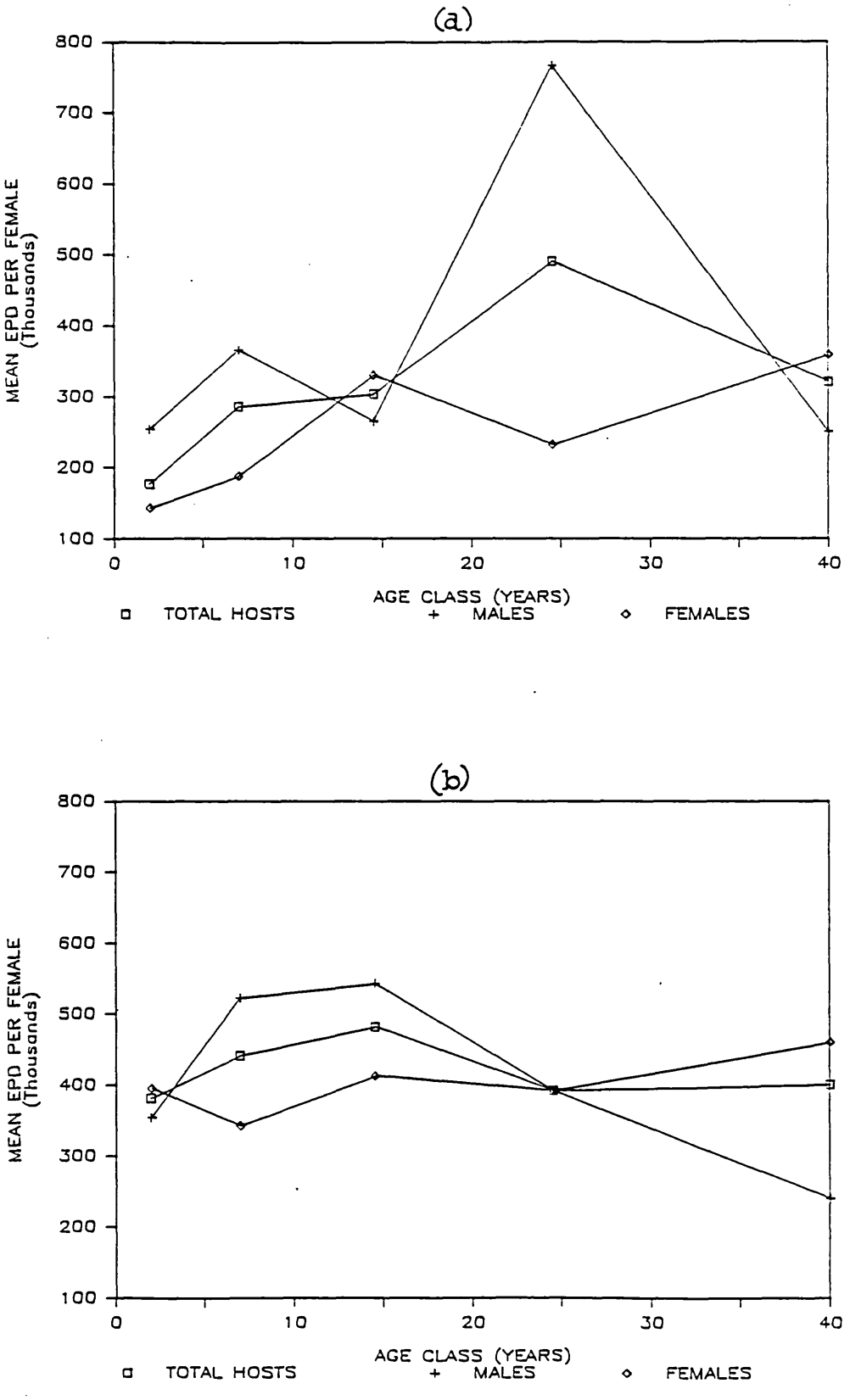


Figure 6.2

Mean per capita 24 hour egg output of mature female Ascaris according to host age and sex in January (Graph(a)) and in November (Graph(b)). Sample sizes as shown in the legend of Figure 6.1.

Table 6.3

Results of two-way ANOVA of *per capita* EPG and EPD according to the age class and sex of the host by sampling date.

Collection	Meas.	Variable	F value	d.f.	p value
January	EPG	age	0.89	5,139	.4922
		sex	0.12	1,139	.7341
	EPD	age	2.85	5,139	.0174 *
		sex	0.08	1,139	.7736
November	EPG	age	0.91	5,164	.4745
		sex	0.00	1,164	.9633
	EPD	age	0.74	5,164	.5979
		sex	0.39	1,164	.5326

* = $p<.05$

with increased worm fecundity (as suggested by Keymer and Hiorns (1986)), product-moment correlations between the log transformed values of host 24 hour faecal weight and EPD per mature female worm were calculated. As shown in Table 6.4, highly significant ($p<.001$) positive correlations were found in the majority of the age groups and in the total population. This relationship between daily egg fecundity and stool weight was plotted in Figure 6.3 as a linear function with a gradient of approximately 3.5 ($b=3.46 \pm .08$; $t=11.49$, $df=321$, $p<.001$). Interestingly, the lack of a significant correlation between stool weight and *per capita* fecundity in the 20 to 29 and 30+ age groups of male hosts (Table 6.4) does not support the suggestion provided in Section 6.2(i) to explain the significant variation found with *per capita* EPD in January by age.

Keymer and Hiorns (1986) suggested that the strong positive association observed between stool weight and fecundity is a result of reduced faecal weight in individuals with higher worm burdens. This hypothesis was tested in the Vairavan Kuppam population in January and November by comparing the mean stool weights of the 'wormy' population (17+ *Ascaris* in January, 10+ *Ascaris* in November) and the 'non-wormy' group (see Chapter 5). There was no significant difference between the average stool weight of these two groups at either time point.

6.2(iv) The Relationship between Intensity of Infection and Fecundity

The rate of *per capita* parasite egg production has recently been examined at the population level based on the average egg fecundity within burden classes at a range of intensities (Croll *et al*, 1982; Martin *et al*, 1983; Thein-Hlaing *et al*, 1984; Elkins *et al*., 1986). Negative density-dependent relationships have been reported by these authors. In the present analysis, the relationship between intensity

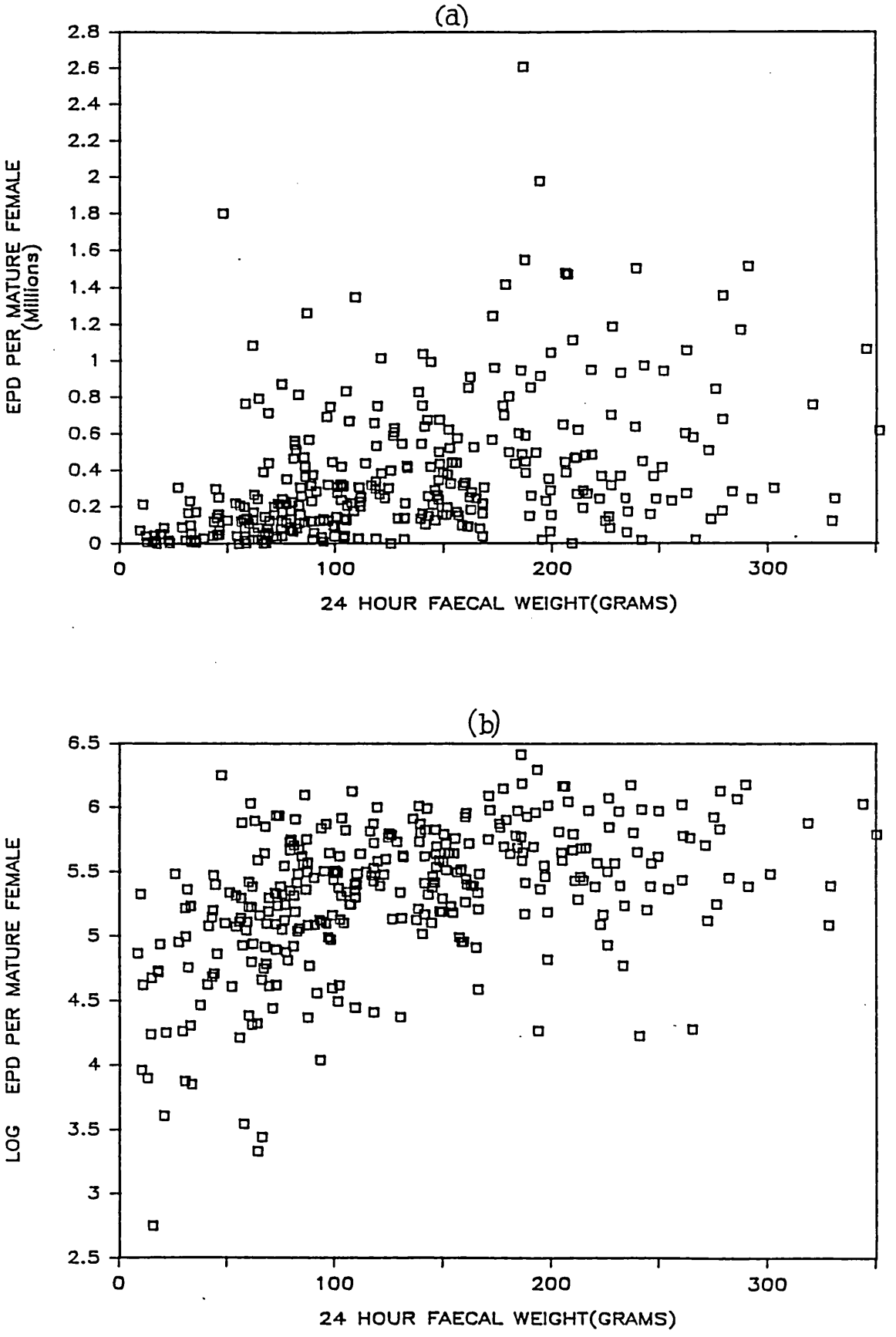


Figure 6.3

The relationship between egg output per day per mature female worm and 24 hour stool weight. Data from the January and November treatment programmes are combined, providing a total of 325 samples. Graph(a) shows untransformed data, while Graph(b) demonstrates log-transformed per capita EPD scores against faecal weight.

Table 6.4

Correlations between stool weight and per capita EPD within age groups (both parameters transformed using Log(x+1)). Data from both initial and reinfection collections are combined in this analysis.

Age Group	Males	(n)	Females	(n)
0-4	.444 *	17	.603 ***	36
5-9	.486 ***	42	.614 ***	35
10-19	.651 ***	35	.391 ***	40
20-29	-.219	24	.654 **	30
30 +	.332	22	.559 ***	44

* = $p<.05$

** = $p<.01$

*** = $p<.001$

and fecundity is described differently, on the basis of correlations among egg and worm counts of individual hosts. When the data from initial treatment of the total sample population were examined, no significant correlation was found between egg production (EPG and EPD per mature female worm) and the number of worms harboured by the host.

An examination of the individual burdens of less than five worms, however, revealed that many people produced small quantities of eggs, of which the majority were infertile. To examine the effect these unfertilized burdens had on the measurement of fecundity at the population level, product-moment correlations were calculated between *per capita* EPG and EPD stratified with respect to host age and sex. These tests were performed first using all individuals passing eggs, whether or not fertilized eggs were present. The analysis was then repeated following the removal of the unfertilized burdens from the data. The correlations of the log transformed egg and worm count data are shown in Table 6.5. At initial chemotherapy there appeared to be no density-dependent relationship (negative correlation) between total worm counts and *per capita* EPG. This result changed significantly when the 12 individuals passing only unfertilized eggs (UAEs) were removed from the analysis. When these individuals were removed, *per capita* fecundity was negatively correlated with total worm burden within the total host population (n=139), within males (n=57) and within children (0-14 yrs; n=79)). In these groups, but not in female hosts or in adults, the significant correlations indicated that egg output per female decreased with increasing worm burden. A similar 'masking' effect of unfertilized worm burdens was seen with *per capita* EPD, particularly in children, where the absence of a correlation between combined (fertilized and unfertilized) counts and worm burden became significantly ($p<.001$) negative following removal of just

Table 6.5

Relationship between *per capita* EPG and EPD and the number of worms harboured within various host age groups with and without unfertilized burdens from the two sampling dates.

Initial Treatment

	EPG/female worm				EPD/female worm			
	fert + unfert	(n)	w/out unfert	(n)	fert + unfert	(n)	w/out unfert	(n)
Total	.088	151	-.201 **	139	.034	151	-.247 **	139
Males	.176	65	-.270 *	57	.166	65	-.282 *	57
Females	.128	86	-.150	82	.165	86	-.217 *	82
Children	.152	85	-.207 *	79	.275 **	85	-.353 ***	79
Adults	.088	66	-.191	60	.064	66	-.082	60

Reinfection

	EPG/female worm				EPD/female worm			
	fert + unfert	(n)	w/out unfert	(n)	fert + unfert	(n)	w/out unfert	(n)
Total	.158 *	150	-.128	119	.135	150	-.083	119
Males	.088	68	-.106	55	.100	68	-.060	55
Females	.202 *	82	-.163	64	.160	82	-.102	64
Children	.202 *	84	-.135	71	.193 *	84	-.075	71
Adults	.074	66	-.139	48	.110	66	.030	48

six individuals who excreted only unfertilized eggs.

Following 11 months reinfection, 20% of the population passed only infertile eggs. This large number of low burdens, combined with fewer individuals harbouring heavy Ascaris infections, resulted in an absence of detectable density-dependent constraints between fecundity and burden (Table 6.5). When per capita EPG was considered in November with combined fertile and infertile egg carriers (n=150), a significant positive correlation ($p<.05$) was found in samples from the total sample population, and from females and children (0-14 years). When the 31 UAE only producers were removed no correlation was found between these groups and per capita EPG. Per capita EPD was positively correlated ($p<.05$) in children only when the fertile and infertile egg passers were combined.

A non-linear regression analysis of per capita EPD (y) versus worm burden (x) in January from children (0-14 years) is shown in Figure 6.4(a). The solid lines denote the best fit power function of the form:

$$y = ax^b$$

where a and b are constants and x is total worm burden. The squares in Figure 6.4(a) denotes the per capita fecundities for the samples which contained fertilized eggs (best fit power function has parameters, $a = 39.68$, $b = -.155$). A logarithmic plot of this same data is shown in Figure 6.4(b). Regression analysis gave a correlation coefficient of $r = 0.397$.

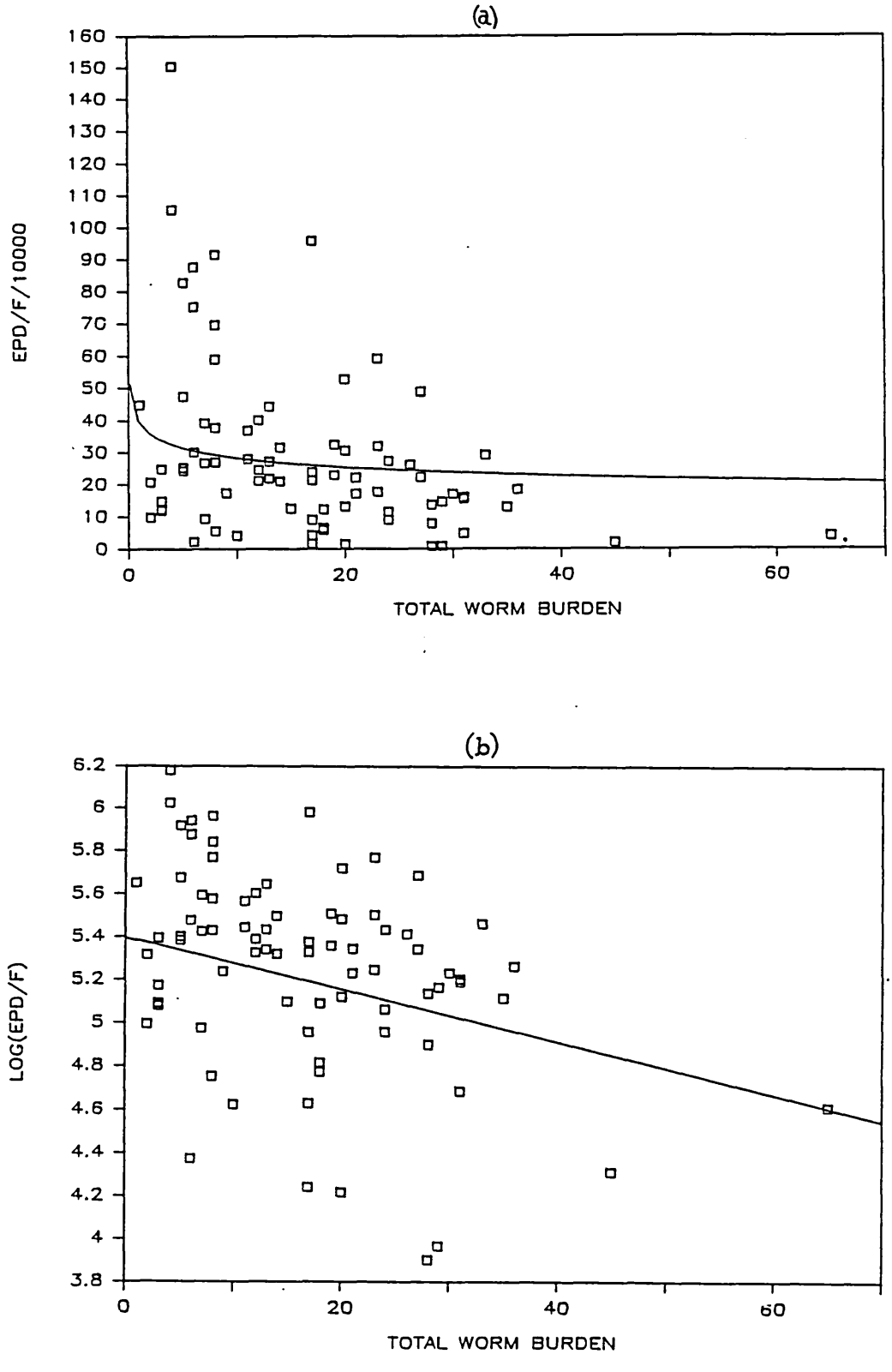


Figure 6.4

The density-dependent relationship between EPD per female worm (y) and total worm burden (x). Data are pretreatment egg counts divided by number of mature females (>12.5 cm in length) plotted against total Ascaris burden of 79 patients, 0 to 14 years of age, in January 1984. Graph(a) displays untransformed data described by a power curve, $y= 39.68x^{-.155}$. In Graph(b), per capita EPD scores are transformed (log(x+1)) and described by a linear equation, y= -.0162x+5.53 (ANOVA, F=14.38, p<0.000).

6.3 Discussion

Although estimates of the fecundity of *Ascaris* have been reported in a number of previous studies, none have based the calculation of *per capita* female worm egg production on the number of mature female worms (Sinniah, 1982; Croll *et al*, 1982; Martin *et al*, 1983; Thein-Hlaing *et al*, 1984; Anderson, 1985). A special feature of this study was the distinction between immature (non-egg producing) and mature female worms (collected from 48 hours post-treatment stool) for the basis of estimating *per capita* fecundity. This approach provides more precise information on the accuracy of faecal egg counts (both EPG and EPD) as measures of worm burden and the relationship between parasite fecundity (with the number of mature females recovered used as the divisor) and number of parasites harboured.

Highly significant correlations between the scores for EPG, EPD and worm burdens of individuals were observed at both sampling points (Kendall's Non-Parametric Rank Test, $p<.001$; Table 6.1). A similiar result was found when both egg count measures were correlated with mature female burdens. This result does not provide information on the specific relationship between egg counts and worm burden, but it does support the use of egg counts as a relative method of identifying the most heavily infected people in the community for population-based chemotherapeutic treatment. In this study, 65% of the 'wormy group', discussed in Chapter 5, could have been identified on the basis of their high pretreatment egg count scores, being among the highest 25% of those recorded.

In an effort to examine the host parameters which may effect the fecundity of *Ascaris*, an analysis of variance was performed with EPG and EPD per female worm by age and sex of the host at both treatment

dates (Table 6.3; Figure 6.1(a,b); Figure 6.2(a,b)). Although EPG per female worm did not vary with host age, significant variability was detected in *per capita* EPD between age groups in January. This variation occurred predominantly in the 20-29 year old males, where a sharp peak was observed.

The finding of a highly significant correlation between the weight of 24 hr faecal output and daily egg fecundity has also been reported in experimental *H. polygyrus* infections in mice (Keymer and Hiorns (1986)) (Table 6.4; Figure 6.3). Mice that were heavily infected with the parasite suffered a reduction in faecal output. The reason for this association in humans is unclear, however, since a comparison of mean stool weights of groups with relatively light and heavy *Ascaris* burdens revealed no difference between the groups.

With respect to the regulation of *Ascaris* transmission and abundance, it is of particular interest to note from Figure 6.5 that the density of fertilized eggs was influenced adversely at low worm burdens (1 to 5 worms) as a consequence of the dioecious habits of the parasite. The probability of successful mating was lower in hosts with few worms. In addition, a high variability in the level of egg production was observed in these low worm burdens probably due to three different situations, including 1) false negative cases (all worms are immature or mature males with no mature females), 2) individuals passing only infertile eggs and 3) individuals positive for fertile eggs. In the former two groups, though few in number, their low egg production tends to generate a positive association with total burden, neutralizing the density-dependent effects of the heavily burdened individuals in the community. In November, the parasite population was likely still increasing following the treatment of the community in January. The greater number of low

burdens (20% of the population) resulted in a positive ($p<.05$) association between fecundity (*per capita* EPD) and total burden of the total population, in females and in children.

In conclusion, both positive and negative density-dependent relationships between parasite fecundity (the production of fertilized eggs) and parasite burden per host have been demonstrated for *Ascaris* infections in this community. Fecundity is reduced at low and high worm burdens as a consequence of mating probabilities and density-dependent restrictions, respectively. The influence of the latter process was greatest in children and in male hosts in January, but was not detectable in November, where *Ascaris* intensity averaged 50% lower. These observations combined with the strong association between *per capita* EPD and stool weight (as well as the variable age composition of the female worms in burdens examined in Chapter 7) suggests that fecundity is dependent on a variety of host and parasite mediated factors.

CHAPTER 7

THE SIZE AND GROWTH OF ASCARIS LUMBRICOIDES

7.1 Introduction

A great deal of experimental research in animal models has determined that host factors (including age, sex, genetic strain and immune status), parasite density and method of exposure to infection (including route, intensity, frequency, and duration) play a dominant role in determining the establishment and survival of intestinal nematodes (Kelly & Nayak, 1964; Cypess et al, 1973; Prowse et al, 1979; Mitchell, 1980; Keymer, 1982; Wakelin, 1985). However, much less is known about how these factors influence the growth of worms within their hosts, particularly in human infections. A brief review of the available information concerning the effects of density, immune responses and arrested development on the size of worm populations follows.

7.1(i) Density-Dependent Influences on Worm Growth

A clear density-dependent relationship between worm size and total worm burden has been well documented in infections with the cestode, Hymenolepis diminuta, in rats (Holmes, 1959; Read & Phifer, 1959; Roberts, 1961). Stunting in heavy infections is due to the availability and competition for carbohydrates in the host gut (Keymer, 1982). Chandler (1939) and Roberts and Mong (1968) reported that the average weight of secondary worms developing in a

superinfection is inversely proportional to the number of previously established primary worms. These data are consistent with the hypothesis that resource limitation or physiological factors secreted by the parasite causes growth inhibition since expulsion of the primary worms prior to secondary infection diminishes the observed growth inhibition. Evidence for similar constraints on worm growth in heavy infections has been demonstrated in Opisthorchis viverrini in hamsters (Flavell, 1982). These more recent experiments, however, did not determine whether immune mechanisms proportional to the degree of antigenic stimulus, space or resource limitation are primarily responsible for the observed reduction in size.

Dose-dependent host immunological responses have been shown to regulate establishment, growth and fecundity in heavy infections within other parasite systems. Using both single and trickle infections in cattle, it has been demonstrated that the establishment of Ostertagia ostertagi is proportional to the number of larvae penetrating the abomasal mucosa (Smith and Grenfell, 1985). These authors suggested that host responses are the major determinants of the degree of parasite survival. Krupp (1961) detected a decrease in the percentage of Ancylostoma duodenale larvae which developed into adult worms in infected dogs as the number of inoculating larvae was increased above 100. She also supported the hypothesis that a dose-dependent host-mediated response limited the success of parasite establishment. Kwong and Dobson (1982) found that the degree of establishment and the early growth rate of Angiostrongylus cantonensis in primary infections in rats is independent of the number of inoculating larvae. When the larvae reach the lung stage of migration, however, worm length is retarded in proportion to the initial dose, and maximum fecundity of female worms is delayed. It

was suggested that dose-dependent host responses within the lung act to reduce the growth rate of the parasite.

The search for density-dependent constraints on worm growth in other cestodes and trematodes and in nematodes have not been successful (Sarles, 1929; McCoy, 1931; Wykoff, 1958). Sarles (1929) and McCoy (1931), working with Ancylostoma caninum in experimentally infected dogs, found an inverse relationship between egg production and the number and age of worms, but found no correlation between the total worm burden and size of individual worms. Although Pawlowski and Arfaa (1984) suggest that the body size of Ascaris may depend on worm burden, the available data do not support this. Martin et al (1983) found no evidence for density-dependent influences in the weight of Ascaris of either sex in the burden range of one to 43 worms. This conclusion, however, was based on a 24 hour faecal output collection following treatment with pyrantel pamoate, an anthelmintic requiring 48 hours to allow clearance of worms from the host (Seo et al, 1972; see Chapter 3). No negative relationship between the total burden and weight or length of 134 female and 69 male Ascaris recovered from 20 children was detected by Mello (1974). Cho et al (1977) found no evidence for reduced weight or length of Ascaris in heavy infections within a burden range of one to 47 worms.

7.1(ii) Immune Responses Limiting Worm Growth

Immune mechanisms acquired during previous exposure may play a role in determining growth rate and size of nematodes (Chandler, 1936; Soulsby, 1961; Thorson, 1970; Keymer, 1982). Suggested molecular mechanisms of growth inhibition include antibodies which inactivate enzymes secreted by the parasite to obtain nutrients from the host, mechanical blockage of the digestive tract by specific antibody and

damage but not death of larval stages by cellular responses (Taliaferro & Sarles, 1939). Thorson (1956) demonstrated that immunizing doses of esophageal extracts of Ancylostoma caninum elicited a growth-inhibiting antibody response in subsequently challenged puppies. Nematospiroides dubius larvae which survive and develop to adult stages in hosts receiving passively transferred immune serum are significantly smaller than those recovered from control hosts (Jones & Rubin, 1974; Behnke & Parish, 1979a; Dobson & Cayzer, 1982). Similar stunting in Ascaris species due to acquired responses has been observed in the larval stages within animal hosts (Sprent & Chen, 1949; Soulsby, 1961; Crandall & Arean 1964). These responses may prolong the migratory phase of the larvae and increase larval mortality. In addition, acquired immune mechanisms may trigger non-specific inflammatory reactions resulting in the expulsion of adult worms from the intestine (Wakelin, 1978; Behnke & Parish, 1979b). The frequency of expulsion and other factors influencing the average life span of the adult stage worm is likely to affect the size of Ascaris harboured by the host.

7.1(iii) Arrested Development

The phenomona of arrested development is a well characterized survival tactic possessed by many parasitic nematodes (for review, see Michel, 1974) including Ancylostoma duodenale in man (Schad et al, 1973). This is a process by which the growth of some migrating larvae is retarded or temporarily halted while other larvae in the same host proceed normally through development (Michel, 1974). Arrested development is believed to be triggered by many different mechanisms in various host-parasite systems. Postulated mediators include immune

responses which increase and decrease according to the degree of antigen stimulation, hormones of the host, inhibitory signals elicited from established adult worms, the absence of growth requirements or signals and environmental conditions experienced by the free-living stages prior to entry into the host. Little research has been done to investigate the existence of arrested development in Ascaris infections.

In order to detect evidence for any of the above described phenomona acting on the size and growth of Ascaris lumbricoides in humans, all of the Ascaris recovered (over 4,000) in 48 hour post-treatment stools from both chemotherapy programmes were weighed, measured and sexed. The results of the analysis of these data are presented in this chapter in the following order with emphasis on comparing the measurement of worms recovered during the treatment programmes. First, the relationship between weight and length of male and female worms are shown to demonstrate the growth dynamics of Ascaris. Secondly, size is examined according to Ascaris density, i.e. total number and total weight of burden per host. Thirdly the influence of host factors such as age and sex on the average weight and the weight distribution of worms is examined. Possible explanations for the observed changes in worm size are discussed.

7.2 Results

7.2(i) Formalin Preservation of Worms

Due to the large numbers of worms recovered following mass treatment of the community, it was not possible to measure freshly passed worms. Whole stools were preserved by adding 10% formalin of

approximately equal volume as the faecal mass, resulting in a final dilution of formalin to 5%. This process may have affected the weight and/or length of the worms.

To assess this possibility, 200 adult female Ascaris collected from a different community (Nadu Kuppam) were measured immediately after recovery. The 46 individuals who provided these worms had not received anthelmintics prior to this treatment and collection. Because the preserved weights and lengths of these worms were not determined, a length-matched subgroup (n=225) and the total adult females recovered from the initial Vairavan Kuppam treatment (n=2111) are used for comparison. Figure 7.1 demonstrates a consistently higher weight for length ratio in preserved females compared to fresh worms grouped according to weight. Analysis of variance of the weight for length ratio grouped according to either worm weight or worm length confirmed this highly significant difference ($F=96.01$ by weight classes, and $F=63.06$ by length grouping, $p<.0000$, $df=1, 2095$ with total females and $F=50.75$, $p<.0000$, $df=1, 409$ by weight grouping with length-matched females). Importantly, the increase in the weight for length ratio apparently caused by preservation was consistent at 9-13% across the weight groups. When the weight for length value of each preserved worm was reduced by 10%, two way ANOVA of the adjusted scores against the unpreserved worm ratios demonstrated that the differences between the worm populations were no longer significant by length grouping ($F=.02$, $p=.892$, $df=2, 2095$). Thus formalin altered the weight for length ratio to a degree proportional to worm size, via decreasing length by shrinkage or increasing weight by absorption of formalin through the cutical.

Multiple regression analysis of worm weight against worm length confirmed these results. The apparently small but highly significant

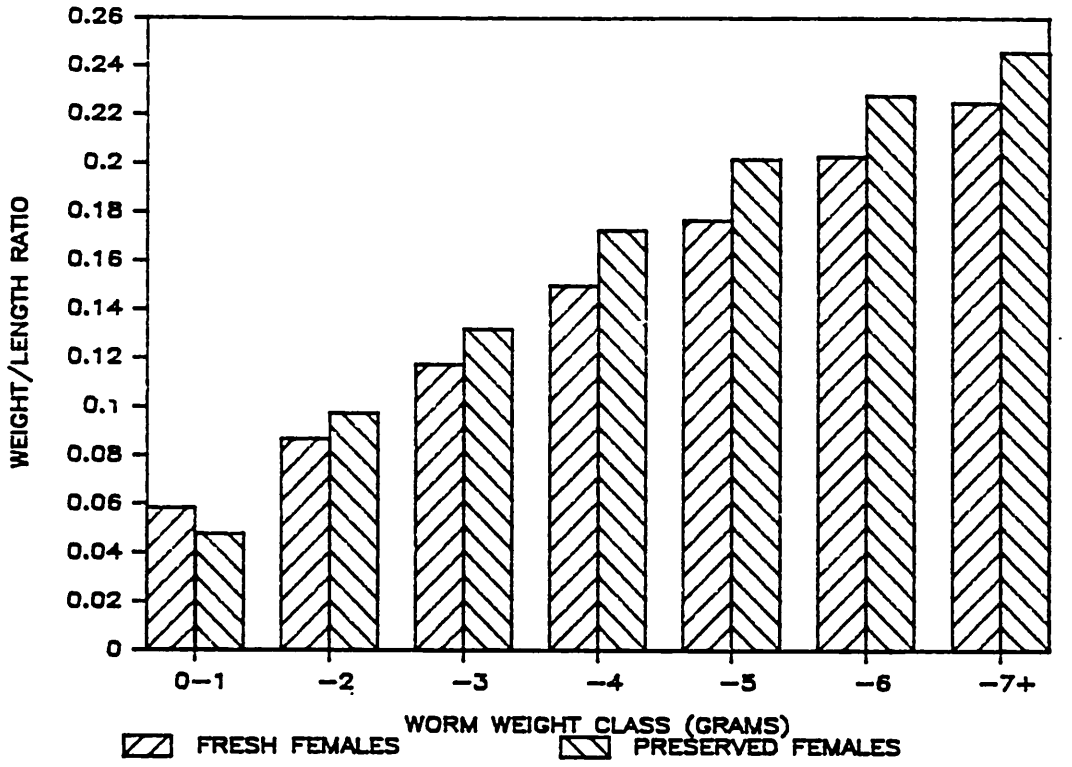


Figure 7.1

The mean weight/length ratios of freshly passed female worms and of worms preserved in 10% formalin grouped by worm weight.

Sample sizes are:

Weight Class	Fresh Females	Preserved Females
0-1	10	93
1-2	39	249
2-3	41	300
3-4	25	337
4-5	36	431
5-6	29	349
6-7+	18	154

difference ($F= 111.82$, $p<.0000$, $df= 2, 2106$) in the linear relationship between worm types are described by the following equations:

$$Y = .3789X - 4.3622 \quad (E7.1)$$

$$Y = .3518X - 4.5863 \quad (E7.2)$$

where Y = worm length and X = worm weight for preserved (E7.1, $r^2 = 82.4$) and fresh (E7.2, $r^2 = 74.9$) Ascaris respectively. Increasing the length measurement of each preserved worm by 10% removed the significant difference between the linear regressions of fresh and preserved worms ($F= 2.19$, $p>.05$, $df= 2, 2107$). All Ascaris used in the remaining analysis of this chapter were preserved according to the above described procedure, and fixation probably did not play a major role in the observations.

7.2(ii) Growth Curve by Weight and Length

The next set of graphs are shown to illustrate the precise pattern of growth, in terms of increase in length against weight gain, for both male and female Ascaris. The relationship between weight and length of individual Ascaris can be described by a power curve that varied little by sex of Ascaris or by treatment programme (Figures 7.2 (a,b)). In both sexes there was a rapid increase in length relative to weight gain up to 10 to 12 cm when maturation occurs. Thereafter an increase in weight became more prominent. The maximum length recorded for male worms was 23.1 cm and female worms, 35.7 cm. The heaviest male and female Ascaris recovered weighed 3.0 and 9.4 grams, respectively.

Non-linear regression of the weights and lengths of recovered

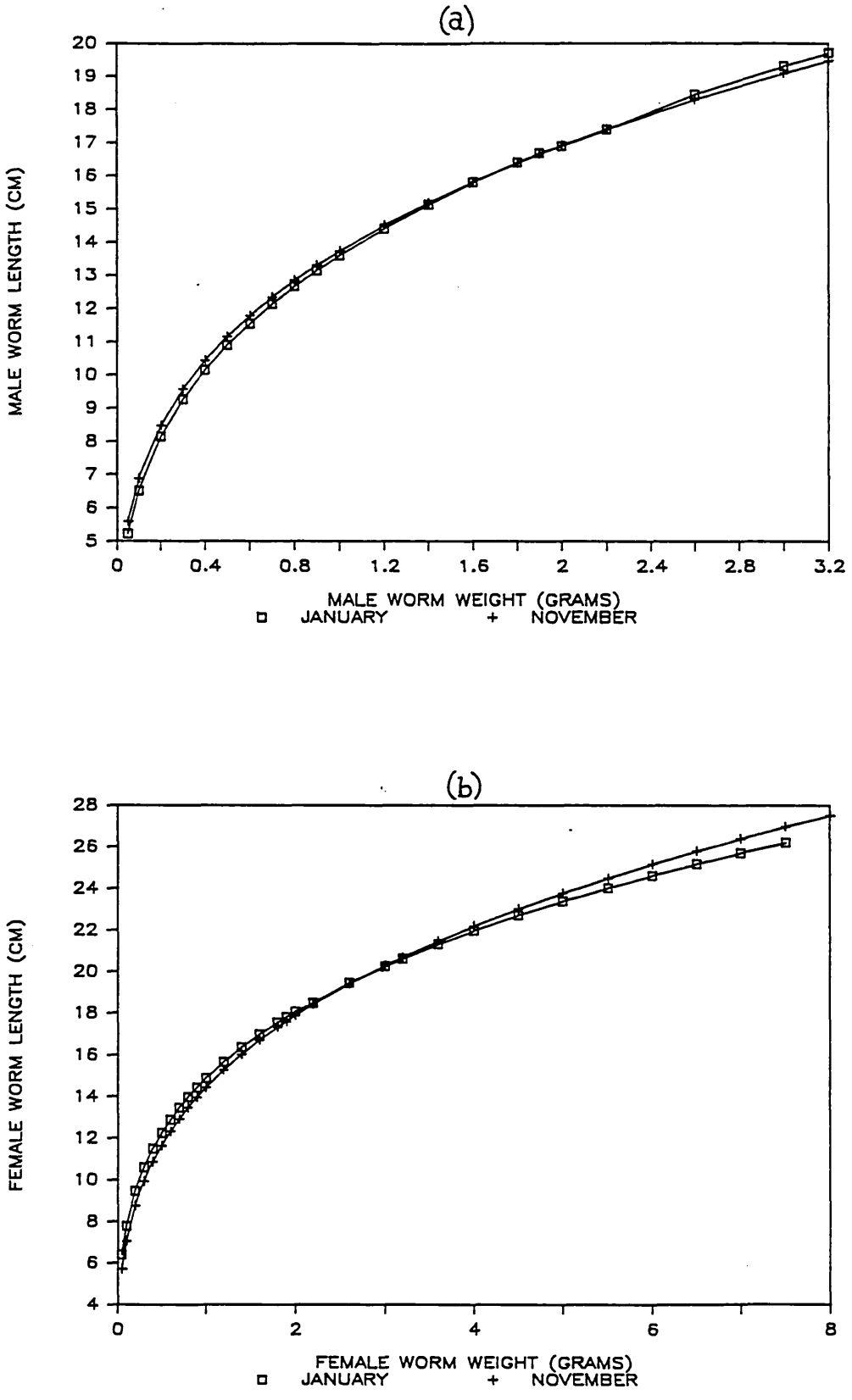


Figure 7.2

Power curves representing the relationship between weight and length of male (Graph(a)) and of female (Graph(b)) Ascaris recovered in January and in November. Sample sizes for derivation of the equations were: Male worms(a): January 978, November 749;

Female worms(b): January 1066, November 922.

Ascaris generated the following equations for male (Figure 7.2(a)) and female worms (Figure 7.2(b)), respectively:

Male Ascaris

$Y = 13.60\ X^{.319}$ Initial Treatment (E7.3)

$Y = 13.60\ X^{.300}$ Reinfection Treatment (E7.4)

Female Ascaris

$Y = 14.88\ X^{.281}$ Initial Treatment (E7.5)

$Y = 14.44\ X^{.310}$ Reinfection Treatment (E7.6)

where Y = worm length and X = worm weight. The fit of the observed data to these equations is highly significant for all 4 curves, with r^2 values ranging from 81.5 to 86.5 ($p<0.001$).

The consistent rise in the mean weight per centimeter of male and female Ascaris with increasing body weight is demonstrated in Figure 7.3. Male worms were consistently heavier per unit length than females (Two way ANOVA, F=41.74, p<.0001, df=1, 2429) in the range of <0.5 to 2.5 grams. Male Ascaris reach sexual maturation at approximately 10.5 cm, while females begin egg production at 12.5 cm (Seo & Chai, 1980). The higher weight for length ratio in males at these weight groups was probably due to development and activity of the reproductive organs. In weight classes above the maximum weights attained by male worms, females continued to increase consistently in weight per centimeter (Figure 7.4). When examined according to worm length, females in the longest worm classes (32 to 36 cm) experienced a decrease in weight per centimeter (Figure 7.5). This decrease must be cautiously interpreted because of the small sample size (n=6), but it may correlate with the decrease in egg output of female Ascaris over 27.5 cm detected by Chai et al (1981) and sterility in females over 30 cm reported by Seo et al (1979).

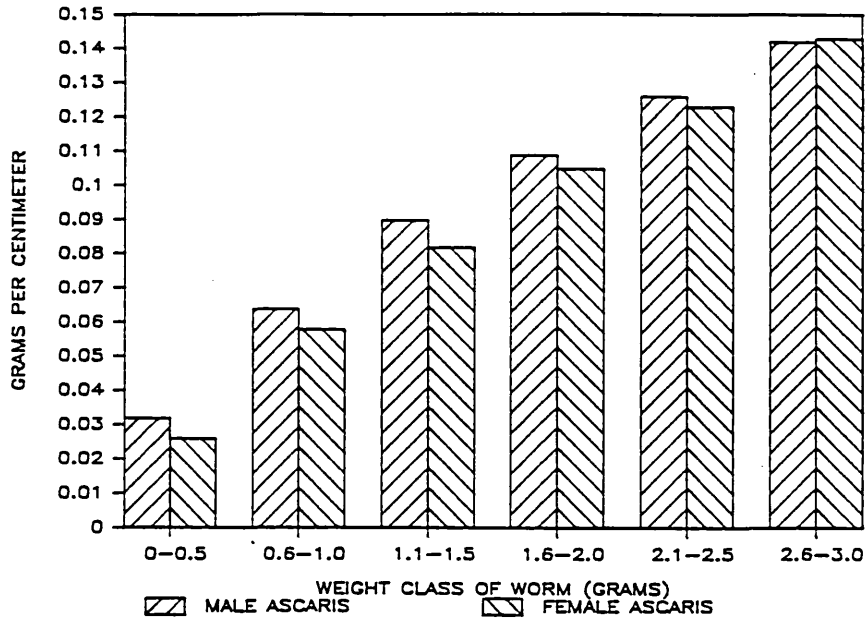


Figure 7.3

The mean weight/length ratios of male and of female worms within the weight classes achieved by male Ascaris. This analysis is based on the measurement of 1727 males and 714 females recovered during both treatment programmes.

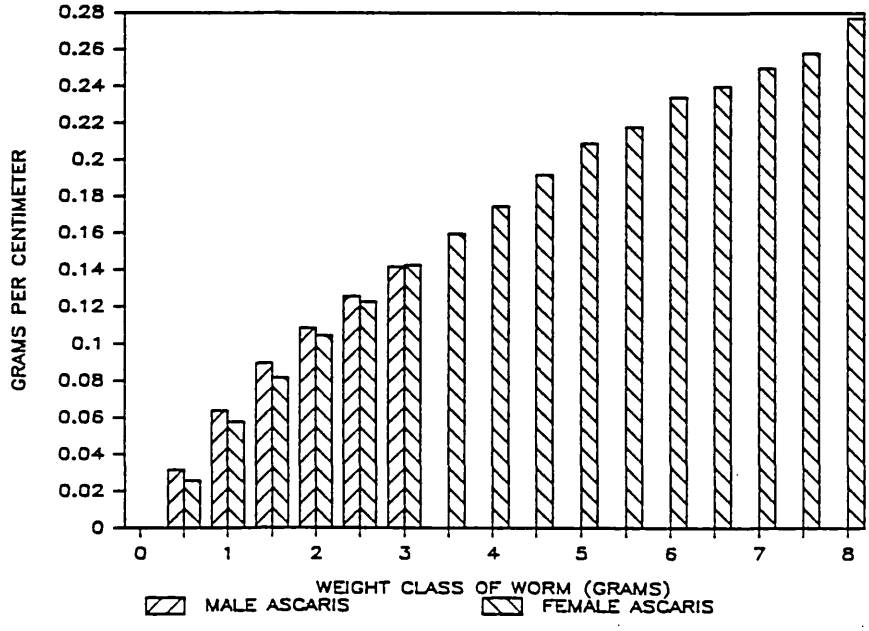


Figure 7.4

The mean weight/length ratios of male and of female worms within the weight classes achieved by female Ascaris. The analysis is based on measurements of 1727 males and 1988 females.

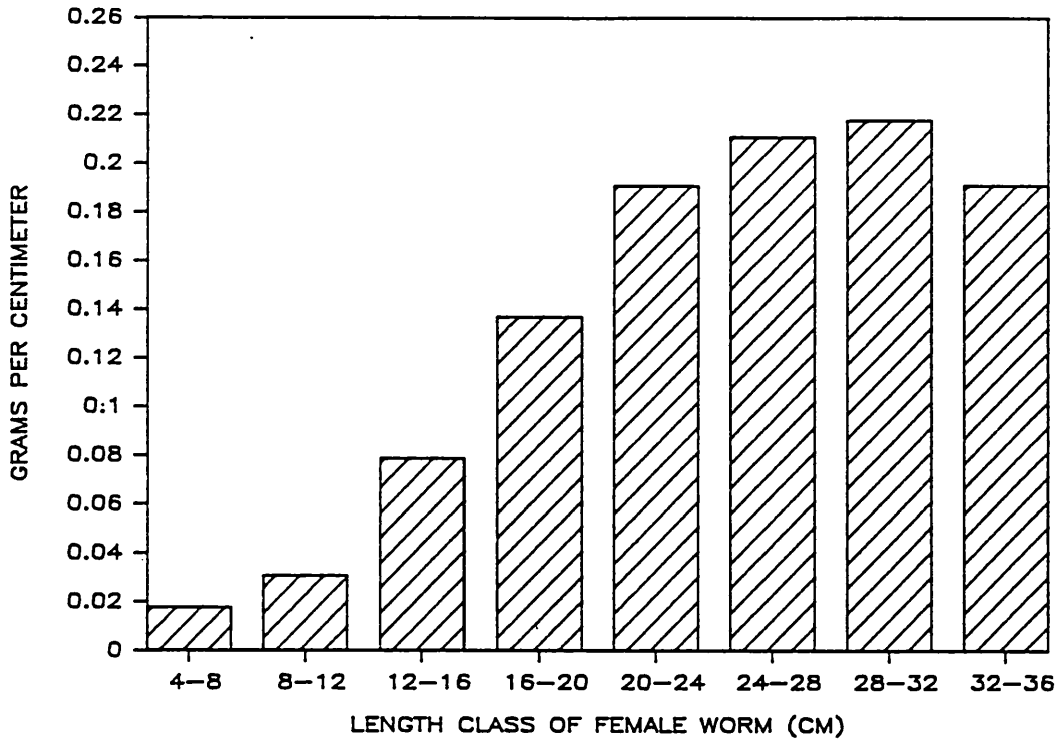


Figure 7.5

The mean weight/length ratios of female Ascaris grouped according to worm length. Sample sizes for the weight classes shown on the graph are: 14, 48, 180, 484, 757, 420, 75 and 6, respectively.

7.2(iii) Time-related Growth of Ascaris

The age distribution of worms collected following the initial and reinfection treatment programmes can be approximated by employing the growth equations for Ascaris lumbricoides derived by Seo & Chai (1980). These were generated using the maximum length and weight of worms expelled by chemotherapy allowing 2, 4, 6 and 12 month intervals of reinfection. The following equations were derived in the Korean study for male and female Ascaris respectively:

$$Y = 9.213 \ln(X+1)+0.025 \quad (E7.7)$$

$$Y = 11.953 \ln(X+1)+0.025 \quad (E7.8)$$

where X= age in months and Y= length of body in centimeters. The value '0.025' is added to account for the length of drug-resistant larval worms which may have been present in the host and beginning development prior to treatment.

When these formulas were applied to the present worm length data, male worms recovered in January were aggregated (72.9% of the total) in the two to four month age groups. Following reinfection, the estimated age of the majority of males (70.5%) was three to five months. At neither time point were more than 10% of the males calculated to be older than five months according to this formula. A similar shift in age distribution was seen between females recovered initially and following reinfection. In January 71.9% of the females were estimated to be two to five months old, while in November, females were clustered within the four and eight months age range. If the juvenile worms of unknown sex were divided equally between the sexes (the ratio in mature worms was approximately equal) and also taken into consideration, there was an approximately tenfold percentage increase in those estimated to be less than one month old in both sexes and at both time points.

7.2(iv) Size of *Ascaris* by Date

The initial analysis of the worm measurement data according to treatment date yielded evidence that a dramatic shift in worm size had occurred with reinfection. Although the mean burdens of *Ascaris* infected individuals (excluding negatives) had dropped 40% from 11.56 to 6.94 at reinfection, the average burden weight decreased just 21.1%, while the mean total length of burdens fell 34%. In contrast, the average weight of individual worms rose 21% from 2.32 to 2.93 grams per worm, while mean worm length per individual worm increased 7.5%. As a result of the greater increase in average weight relative to length, the overall weight for length ratio increased 12% at reinfection.

To explore this discrepancy, an initial analysis was done to determine if a decrease in the ratio of male to female and immature to mature *Ascaris* had occurred at reinfection. A smaller proportion of males and/or immature worms would result in an increase in both the overall mean size and egg output of the worm population. However, neither of these ratios differed with time of treatment. The male to female ratios were 1:1.06 and 1:1.04, and the percent of immature worms was 16.06% and 16.12% at the initial and final treatments, respectively. Neither of these factors were likely to contribute to the overall observed size differences.

When the worms were separated by sex, increases in mean weight, length and weight for length at reinfection were observed in both male and female *Ascaris* populations. Males increased 13.5% in mean weight and 4.7% in length, while the mean weight of female worms rose 20.07% and length increased 7.3%. All of these differences were highly significant by Student's t test ($p<.0000$).

For closer examination of worm size, the percent distribution of the worm populations by weight at the two interventions are shown in Figures 7.6, 7.7, 7.8 and 7.9. At initial treatment, both sexes of worm were relatively evenly distributed across a wide weight range (0.0 to 5.0 grams in females and 0.6 to 1.8 grams in males) containing approximately 70% of the total males and females recovered. Both figures suggest a bimodal distribution consisting of two peaks across the weight range. In November, there was a distinct reduction of intermediate sized worms and one sharp peak in heavier classes (4.5 to 6.0 gms in females and 1.4 to 2.0 gms in males) containing 50 to 60% of the total. The differences in these weight distributions were highly significant by chisquare analysis (χ^2=170.87, df=6, p<.000 in females and χ^2=77.47, df=9, p<.000 in males). The distribution of the Ascaris population by length also differed significantly with treatment date (χ^2=134.23, df=9, p<.000 in females and χ^2=51.23, df=7, p<.000 in males), and the observed patterns were very similar to those shown in Figures 7.6 through 7.9. These shifts in worm size will be examined in greater detail throughout this chapter.

7.2(v) Density-Dependent Influences on Worm Size

An alternate explanation for the smaller size of worms recovered during the initial programme is that the worms were not of younger age, but crowding of worms in the intestine exerted a negative influence on their growth. Following reinfection, fewer worms were harboured, and therefore the postulated density-dependent constraints may have been relaxed, allowing the worms present to realize their full growth potential. To test this hypothesis, several analyses of worm size against the total number, weight and length of worms in individual burdens were performed.

Figure 7.6

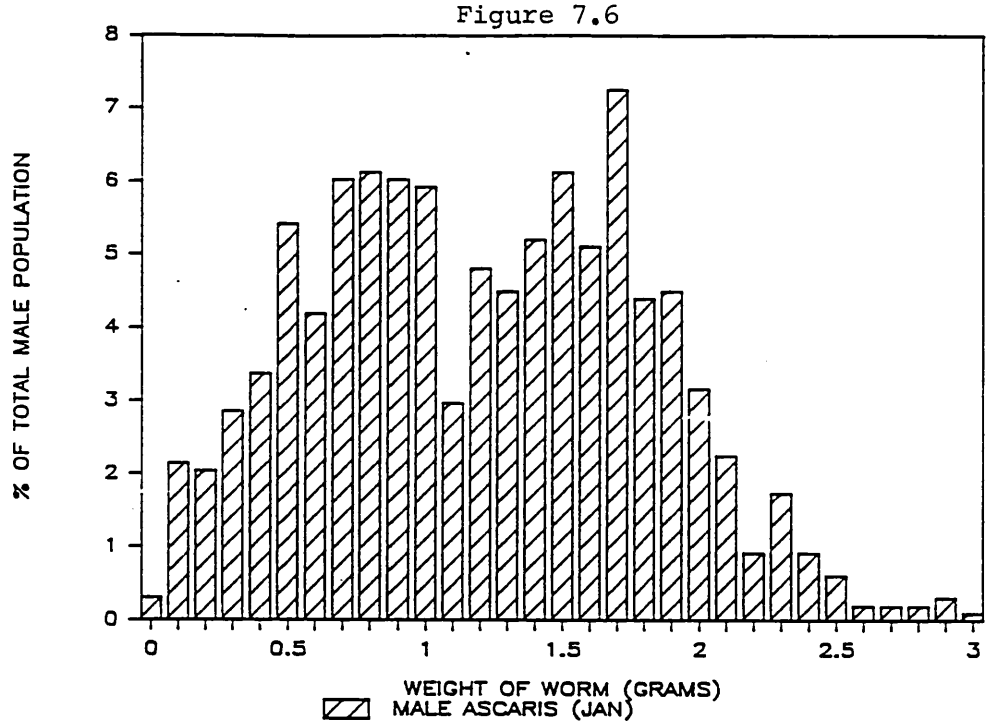


Figure 7.7

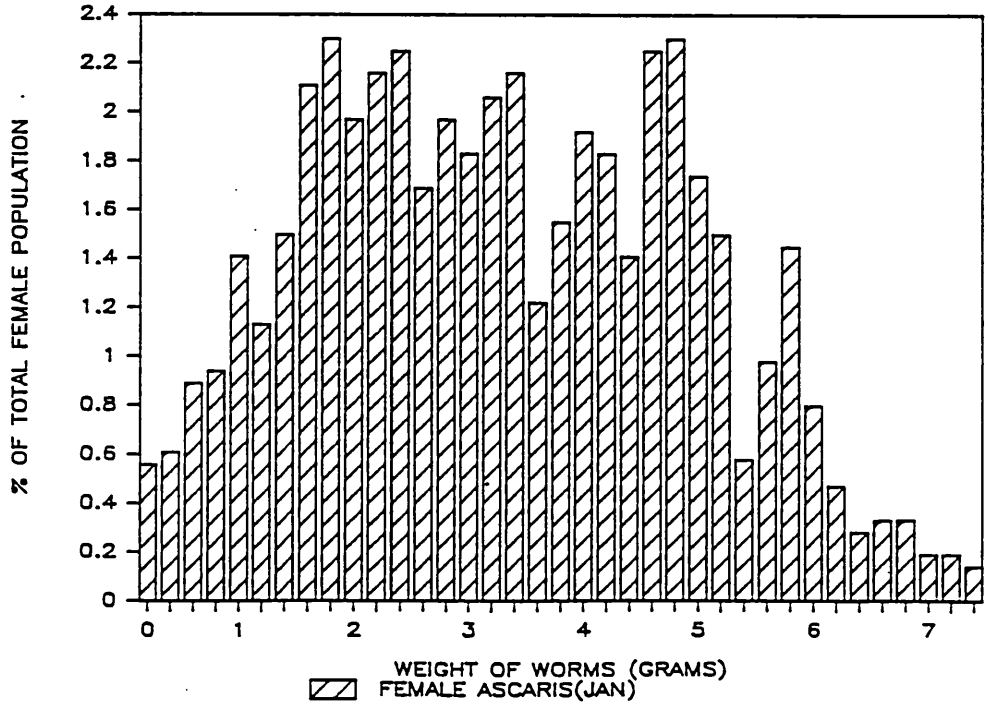


Figure 7.6 and Figure 7.7

The percent weight distribution of male (Figure 7.6) and of female (Figure 7.7) Ascaris recovered from all infected individuals during the initial treatment programme. The curves are based on measurement of 931 male and 1020 female worms.

Figure 7.8

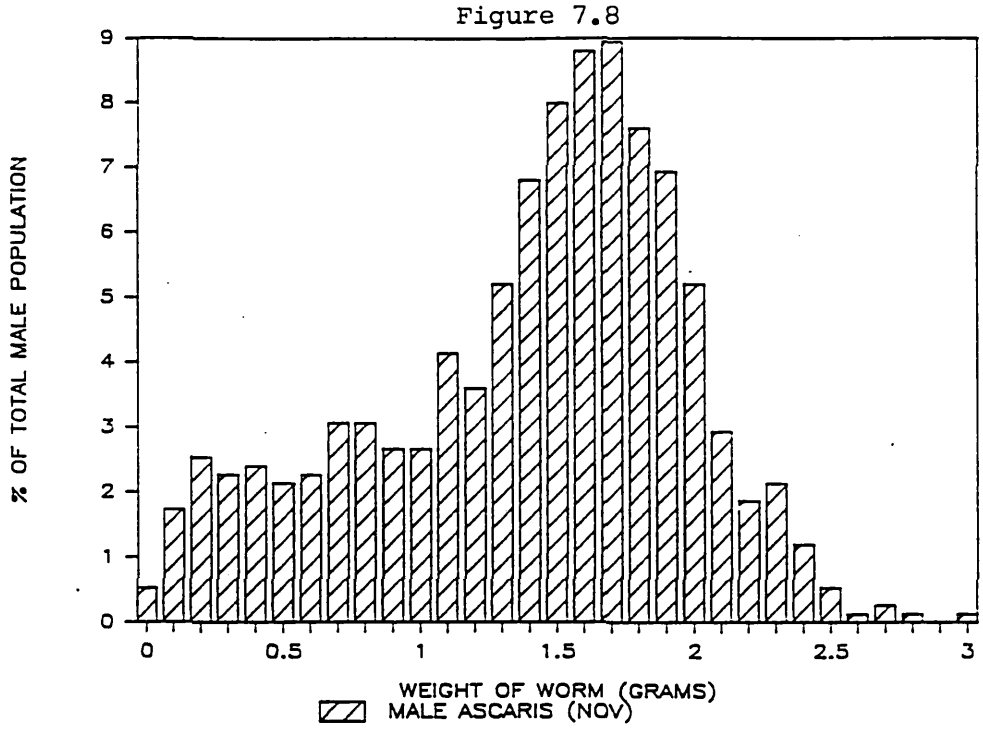


Figure 7.9

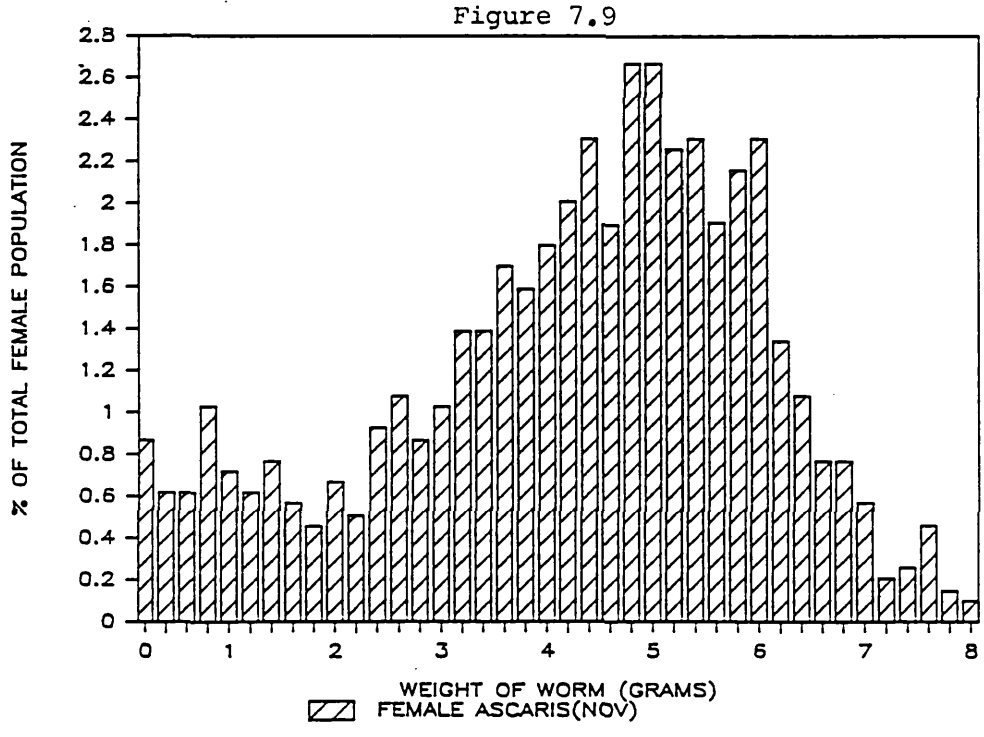


Figure 7.8 and Figure 7.9

The percent weight distribution of male (Figure 7.8) and of female (Figure 7.9) Ascaris recovered from all infected individuals during the second treatment programme in November. The curves are based on measurement of 692 male and 700 female worms.

At initial treatment, highly significant linear regressions were derived from plotting the total worm burden against the sums of the length and weight of the individual worms (Table 7.1 and Figures 7.10 (a,b)). Following reinfection, the relationship between worm number and total weight and length of the burdens was similarly well fitted to positive linear equations for all sizes of burdens between 1 and 25 worms (Table 7.1 and Figures 7.11 (a,b)). Little deviation from the regression line describing total length and worm number was observed, as confirmed by the r^2 value of 98% at both time points. The November regression equations for weight and length, however, differ significantly from those derived at initial treatment (F test for equality of regression lines, for length: $F=20.3$, $df=1,427$, $p<.000$, for weight: $F=36.38$, $df=1,427$, $p<.000$). The slope of the line describing the relationship between total burden and weight following reinfection was considerably steeper than at initial treatment, as expected since the worms were consistently larger. Although more variation in total weight was seen in burdens of over 15 worms, there was an equal distribution of points above and below the regression line even in the highest burden classes. There was no indication that the rate of increase in the total weight or length declined as worm number increased. The total weights and lengths of both the light and heavy reinfection burdens fell above the initial regression equation, suggesting that the Ascaris acquired during the period of reinfection were heavier and longer, regardless of burden size.

If the burdens are separated by worm sex, the data for both male and female worms again fit closely to positive linear regression equations with most r^2 values over 90% (see Table 7.1 for equations and statistics). When the measurement data of worms collected from both treatments were combined, the mean weight and length per worm

TABLE 7.1

Linear regression of total male and female worm weights and lengths vs. the total number of the respective sex calculated from measurements of Ascaris recovered at the initial and reinfection treatment programmes.

(a) Worm Weight

Worm Sex	Date	Regression Equation	d.f.	St.Dev.	r^2	Ftest
Males,	Jan.	Y = 0.50 + 1.1X	187	2.19	86.2%	***
	Nov.	Y = 0.15 + 1.4X	198	1.53	89.2%	***
Females,	Jan.	Y = 0.87 + 3.2X	185	6.72	85.9%	***
	Nov.	Y = 1.02 + 3.9X	220	5.72	83.8%	***
Combined,	Jan.	Y = 0.74 + 2.0X	186	9.14	83.0%	***
	Nov.	Y = 0.78 + 2.6X	241	11.18	84.7%	***

(b) Worm Length

Males,	Jan.	Y = 1.75 + 13.7X	187	2.49	98.1%	***
	Nov.	Y = 1.02 + 14.5X	198	7.91	97.2%	***
Females,	Jan.	Y = 0.27 + 20.3X	185	16.12	97.7%	***
	Nov.	Y = 1.40 + 21.6X	220	14.50	96.0%	***
Combined,	Jan.	Y =-1.99 + 16.2X	186	3.41	98.0%	***
	Nov.	Y =-0.29 + 17.7X	241	3.88	98.0%	***

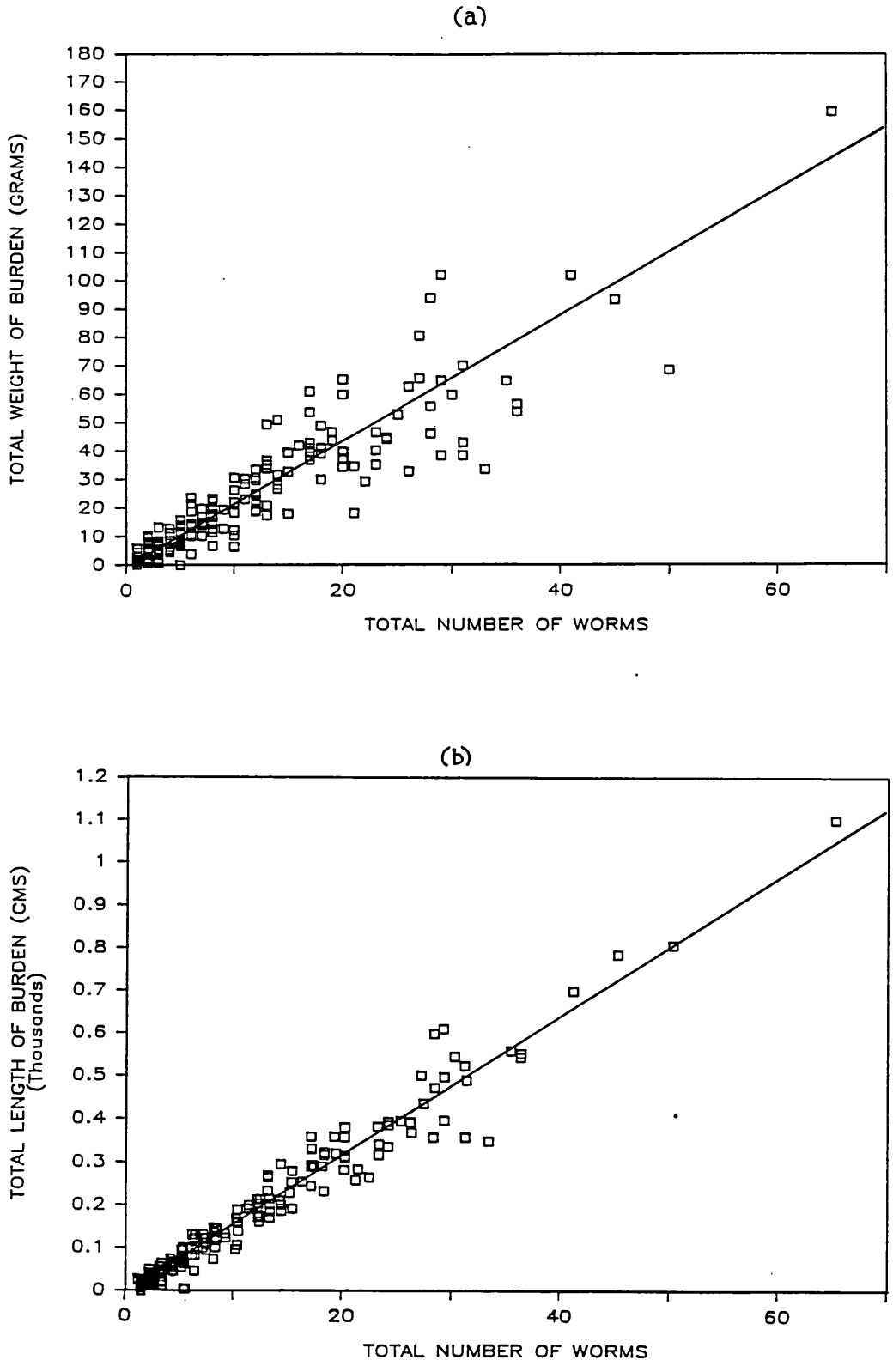


Figure 7.10

The total weight (Graph(a)) and total length (Graph(b)) of worm burdens plotted against the total number of worms harboured by individual hosts in January 1984. Linear regression of the 188 recorded worm burdens derived the equations: y= 2.03x+.746, where y= total weight (a) (F=4185, p<.000) and y= 16.16x-1.99, where y= total length (b) (F=908, p<.000).

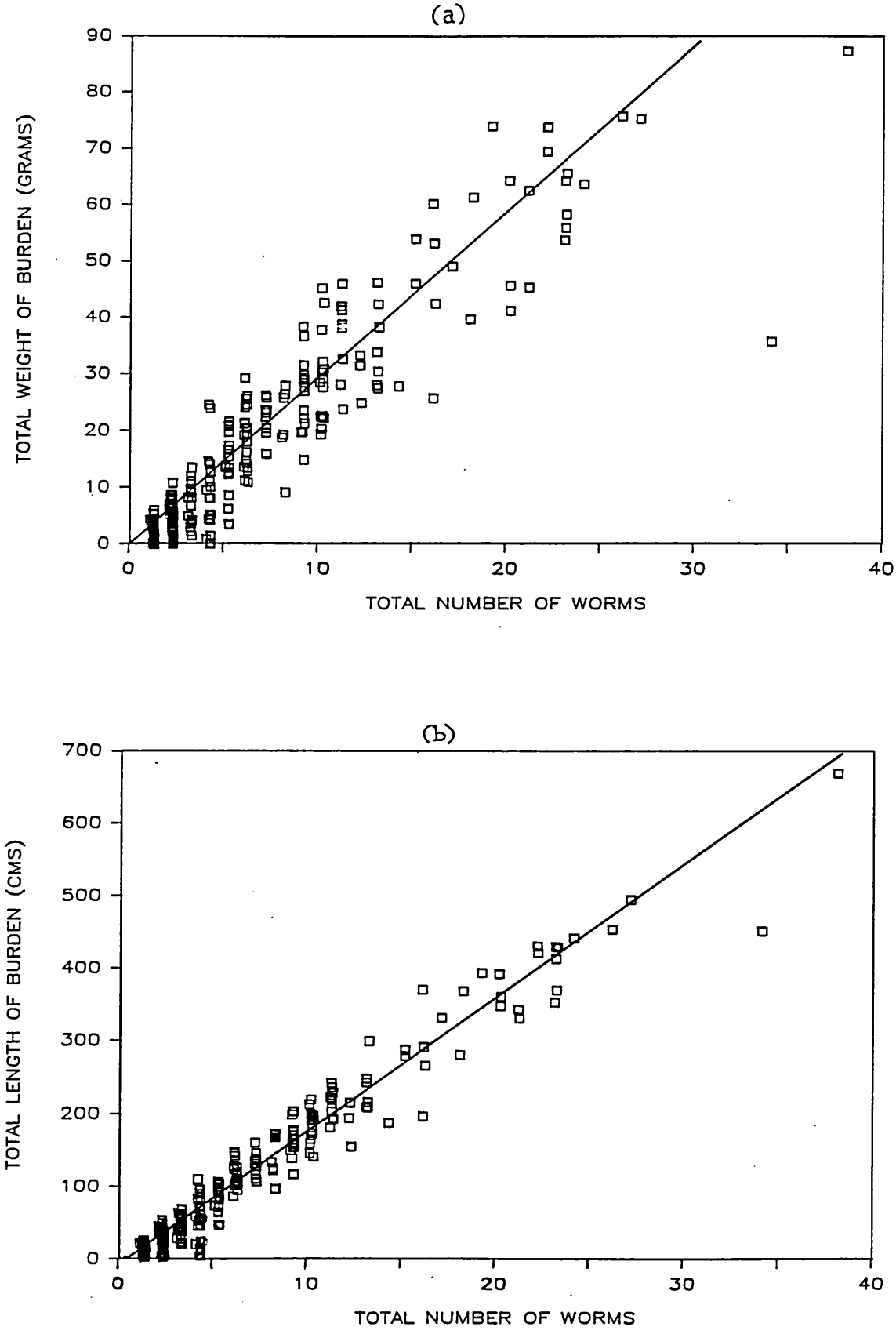


Figure 7.11

The total weight (Graph(a)) and total length (Graph(b)) of worm burdens plotted against the total number of worms harboured by 243 patients in November 1984. Linear regression of the observed data derived the equations: y= 2.64x+0.78, where y= total weight (a) ($F=1332$, $p<0.000$) and y= 17.70x-0.29, where y= total length (b)

plotted against total burden revealed no striking variation in the range of one to 25 worms (Figure 7.12(a,b)). There is no evidence from this analysis to suggest that worm weight or worm length was affected by the number of Ascaris harboured by the host.

7.2(vi) Size Distribution of Ascaris by Host Age and Sex

As shown in Chapter 4 the age and sex of the host played a strong role in determining the number of worms harboured by the host. In this section the effect of these host factors on worm growth will be examined, using the weight and length of Ascaris worms collected following treatment. As shown in Figures 7.2 (a,b) and mentioned previously, similar trends were observed for worm weight and length and between male and female worms, therefore most results presented in this section are concerned with female worm weights.

The mean weight of worms within individual burdens (the total weight of the burden divided by the number of worms harboured by the host) grouped by host age and sex following initial treatment are shown in Figure 7.13(a). Average worm weights fluctuated widely from 1.4 and 2.9 grams between age groups of both sexes. In females, the average increased with age, while in males the mean rose sharply in young groups, fell in 20 to 45 year olds and rose again in the oldest group. In contrast, the mean weight of worms recovered following reinfection varied little with the age or sex of host, ranging from 2.2 to 2.9 grams. Average weights within the worm population harboured by females were slightly higher compared to males in all age groups up to 45 years of age.

As discussed in section 7.2(iv), the distribution of Ascaris according to worm size changed dramatically following initial treatment and reinfection. The same distribution patterns were also

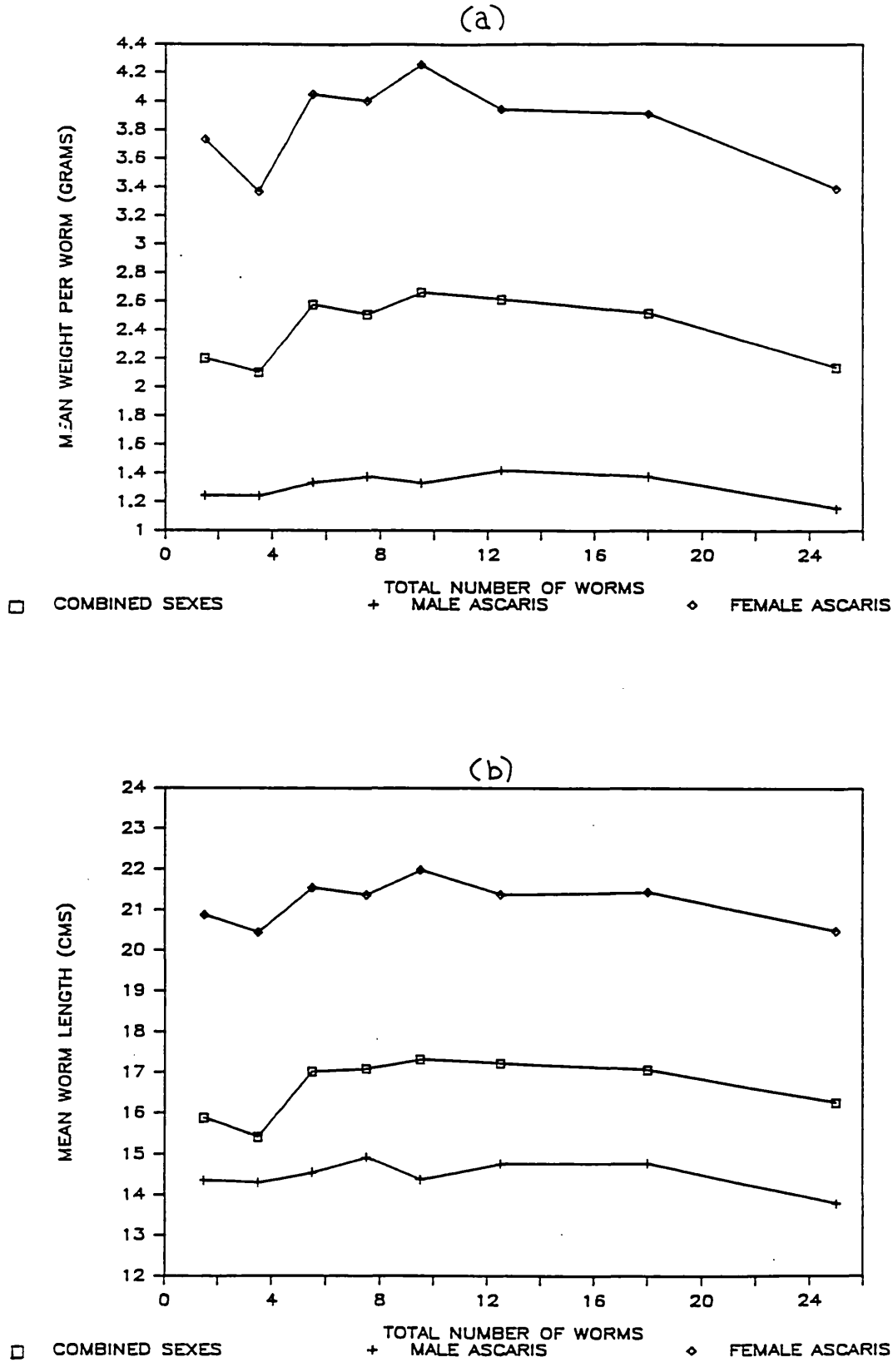


Figure 7.12

Mean worm weight (Graph(a)) and length (Graph(b)) according to the intensity of infection. The graph is based on the measurement of 1727 male and 1988 female Ascaris collected following both treatments.

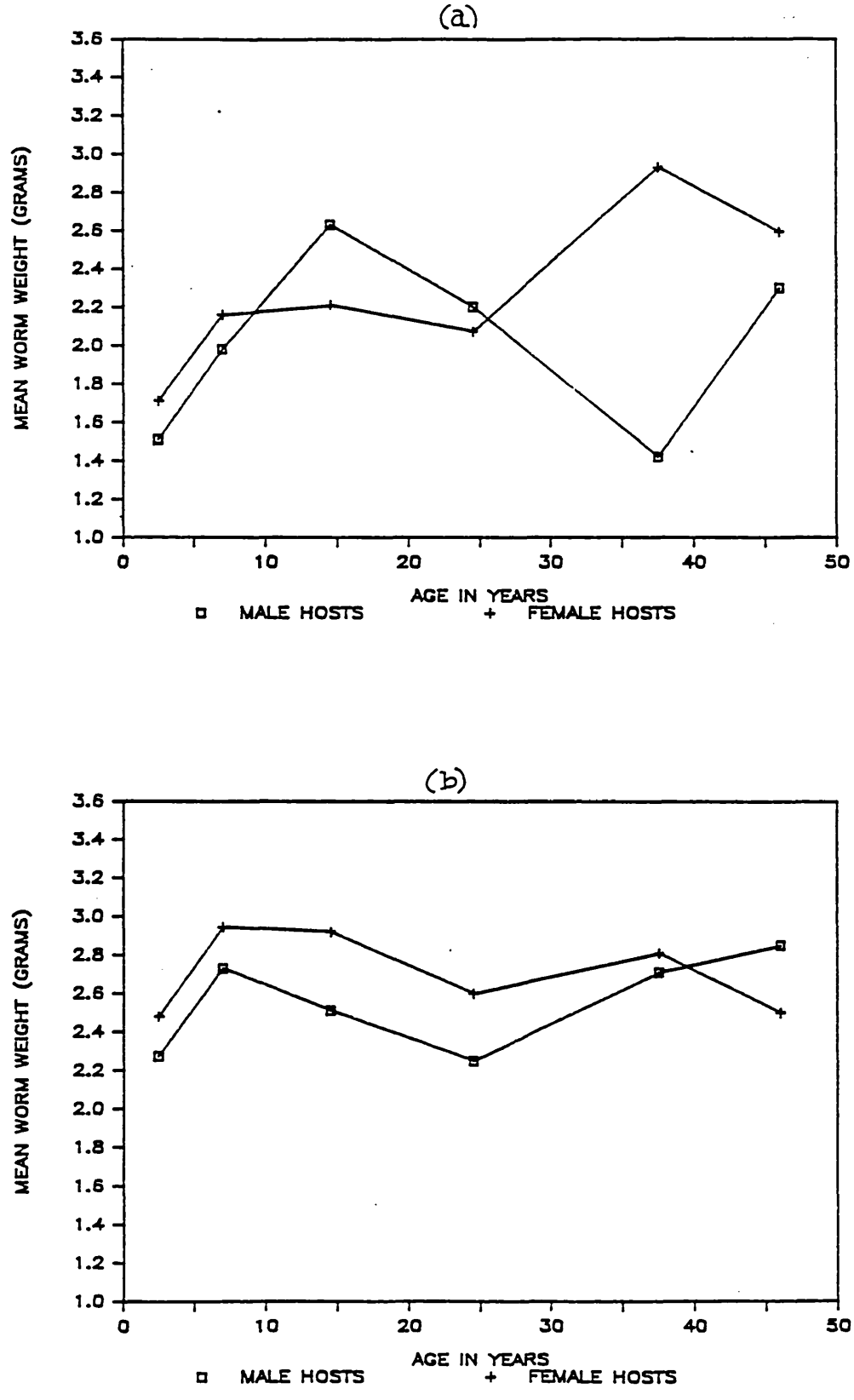


Figure 7.13

Mean weight of worms recovered during the January (Graph(a)) and the November (Graph(b)) treatment programmes according to the age and sex of host. The number of patients in each age group were 0-4: 32 and 38, 5-9: 43 and 49, 10-19: 39 and 63, 20-29: 31 and 43, 30-45: 25 and 23, 46+: 17 and 26, for January and November, respectively.

observed in worm recovered from male and female hosts examined seperately, demonstrating that the 'shift' to heavier worms was not confined to one sex (Figure 7.14(a,b)). To investigate whether the weight distribution pattern differed according to the number of Ascaris harboured, the worms recovered from 'wormy' and 'nonwormy' people were compared. In January, both groups displayed similiar worm weight patterns, regardless of whether 1 to 16 or greater than 16 worms were harboured (Figure 7.15(a)). A wide plateau between 2 to 5 grams contained the majority of the worms. Following reinfection, over 60% of the worms recovered from the 'wormy' host group (10 or more worms) weighed between 4.0 and 6.0 grams, compared to 45% of worms from 'nonwormy' hosts (Figure 7.15(b)). Surprisingly, heavily infected individuals in November harboured a larger and more homogeneous population of Ascaris than the lightly infected group.

Analysis by host age revealed a striking difference in the weight distribution of worms recovered in January (Figure 7.16(a)), but not in November (Figure 7.16(b)). The worms recovered from children (0 to 9 years of age) following initial treatment were clustered within weight classes between 1 and 4 grams. In adults (10 years plus), few worms of less than 3 grams and a large proportion of worms weighing between 4.5 and 6 grams were recovered. Following reinfection, the majority of the female worms harboured by both children and adults weighed between 3.5 and 6.5 grams, with a higher proportion of heavy worms expelled by children. Figure 7.16(b) closely resembles Figure 7.15(b), as might be expected because the majority of the 'wormy people' were under 10 years of age.

In adults (10 plus years) the patterns at both time points were similiar, with a small peak in the heavier worm weights and a relatively low percentage of lighter worms (Figure 7.17). The worms

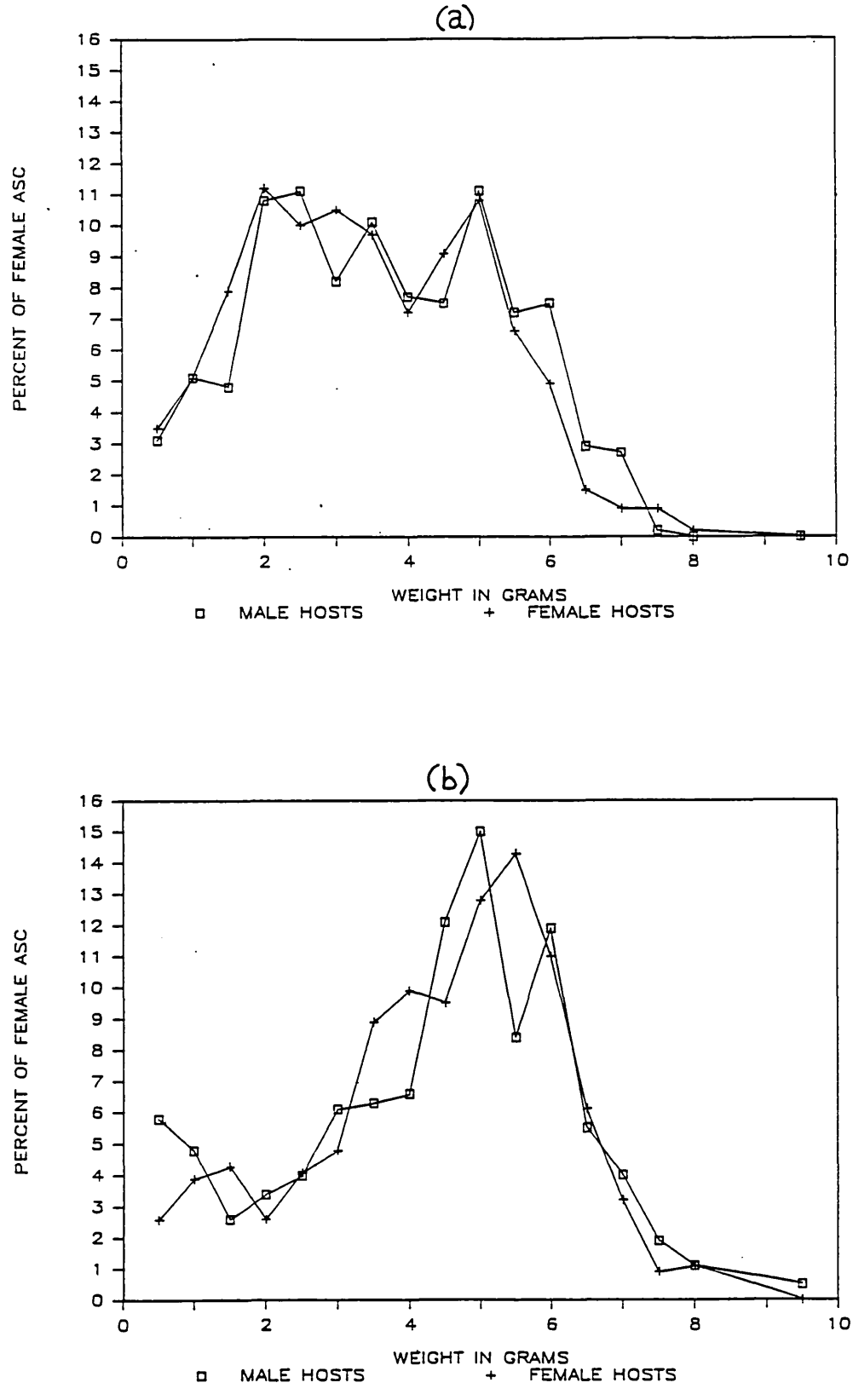


Figure 7.14

The percent weight distribution of female worms recovered from male and from female hosts during the initial (Graph(a)) and reinfection (Graph(b)) treatment programmes. Graph(a) is based on measurements of 393 and 627 worms, and Graph(b) includes analysis of 309 and 391 worms recovered from male and female hosts, respectively.

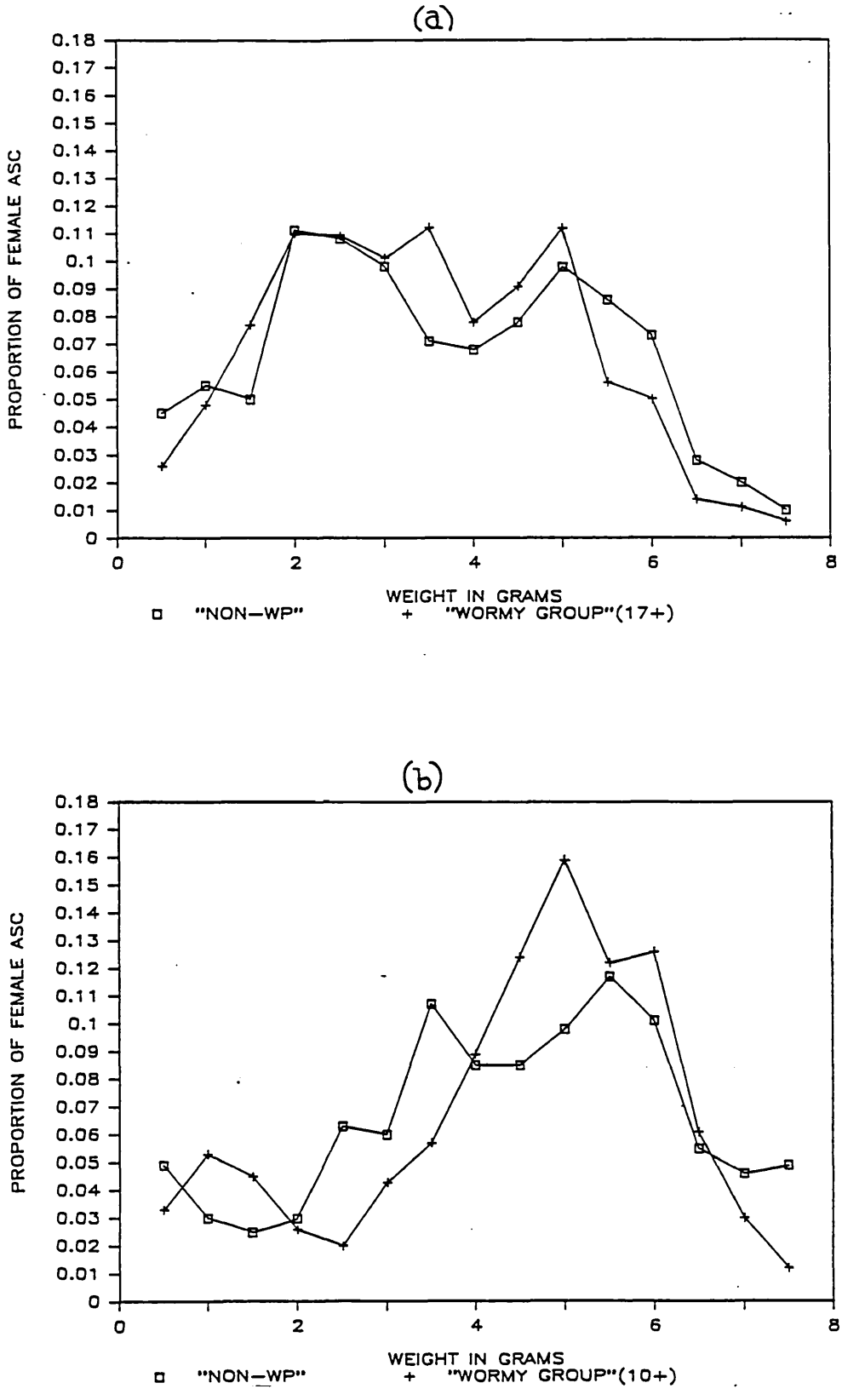


Figure 7.15

The percent weight distribution of female Ascaris recovered from lightly ("non-wp") and from heavily infected individuals ("wormy group") during the January (Graph(a)) and November (Graph(b)) programmes. The "wormy group" expelled 626 and 392 females, while the "non-wormy group" passed 394 and 308 female worms, during the initial and final treatments, respectively.

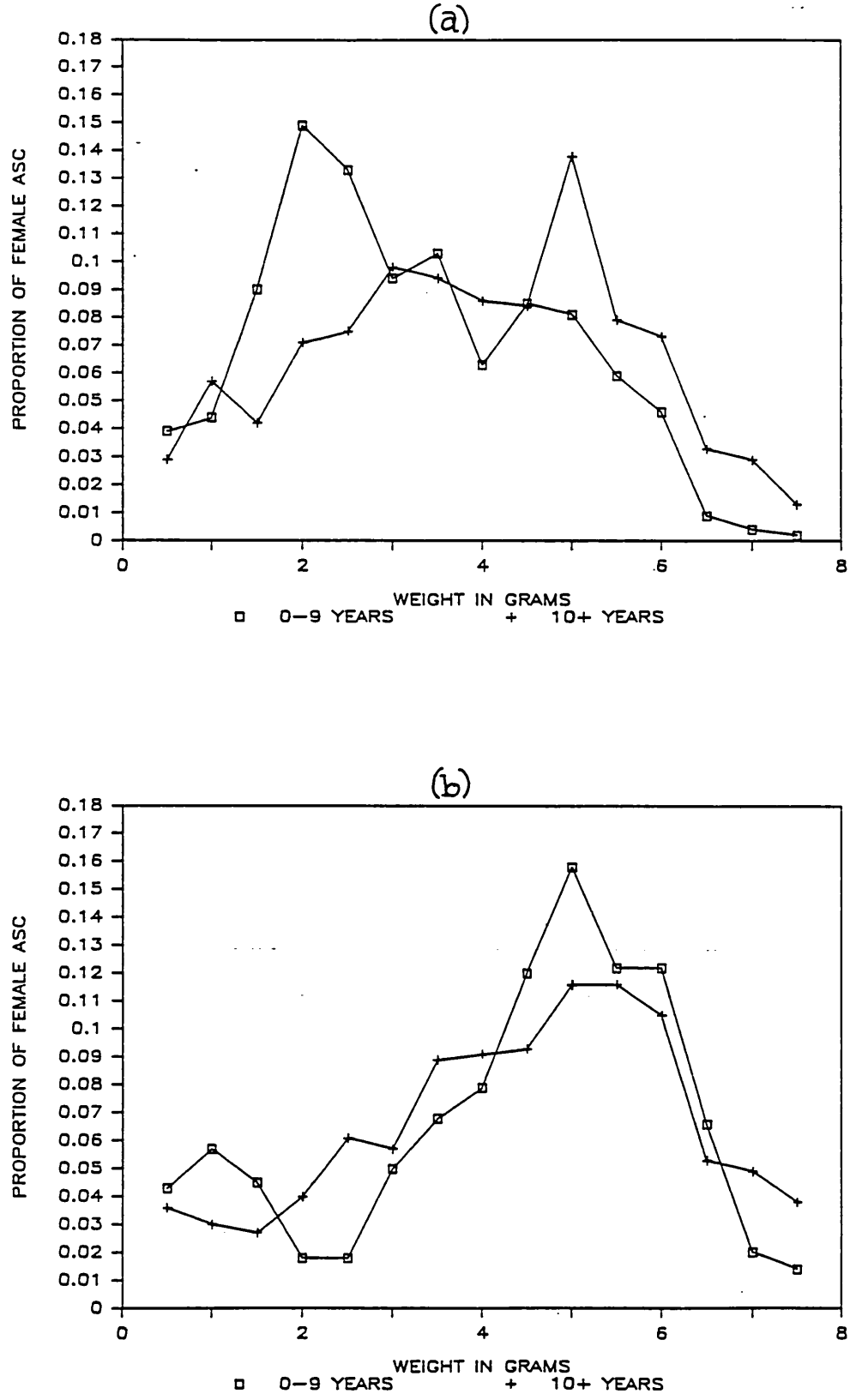


Figure 7.16

The percent weight distribution of female Ascaris recovered from children and from adults during the initial (Graph(a)) and reinfection (Graph(b)) treatment programmes. In Graph(a), the curves are based on the weight of 586 and 538 worms, and Graph(b) of 439 and 483 worms recovered from age groups, 0-9 years and 10+ years, respectively.

showed a large degree of heterogeneity in size compared to worms recovered from children. In children, the shift of both male and female worms from light classes in January to heavy classes in November (and shorter to longer worm classes) was dramatic (Figure 7.18(a,b)). When the hosts are broken into finer age groups, both the 0 to 4 and the 5 to 9 year age groups display patterns in January that were very different from 10 to 19 year olds. In the two younger groups, 75% of the worms recovered weighed less than 4.0 grams (Figure 7.19(a,b,c)), while in the 10 to 19 year group, the proportion of worms increased with weight up to 5 grams. The distributions of worms recovered following reinfection were shifted to heavier weights in the younger groups, and changed little in the older group. These observed patterns did not differ within age groups according to host sex.

7.3 Discussion

The individual and community worm burdens recovered following the initial treatment describe an undisturbed endemic *Ascaris* population. Following treatment it is generally assumed that rapid reinfection occurs until an equilibrium is re-established, barring further perturbation by climatic factors or human intervention. The size distribution of worms collected in November may reflect the sequence of time during the eleven month period of reinfection when the worms became established within the host following initial chemotherapy. An analysis of worm size may also indicate whether host factors, such as age and sex, or parasite-related factors, such as density of infection or anthelmintic treatment, play a role in controlling the growth of the worm population. The results of the weight and length analysis of *Ascaris* recovered from Vairavan Kuppam have provided a fascinating

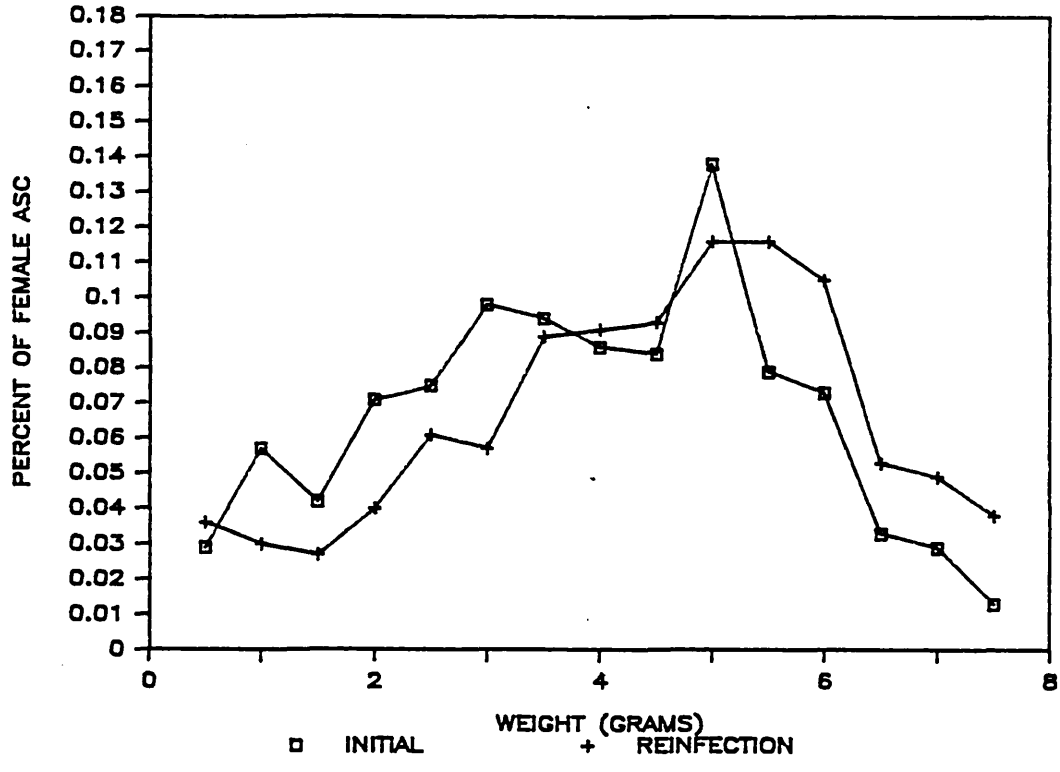
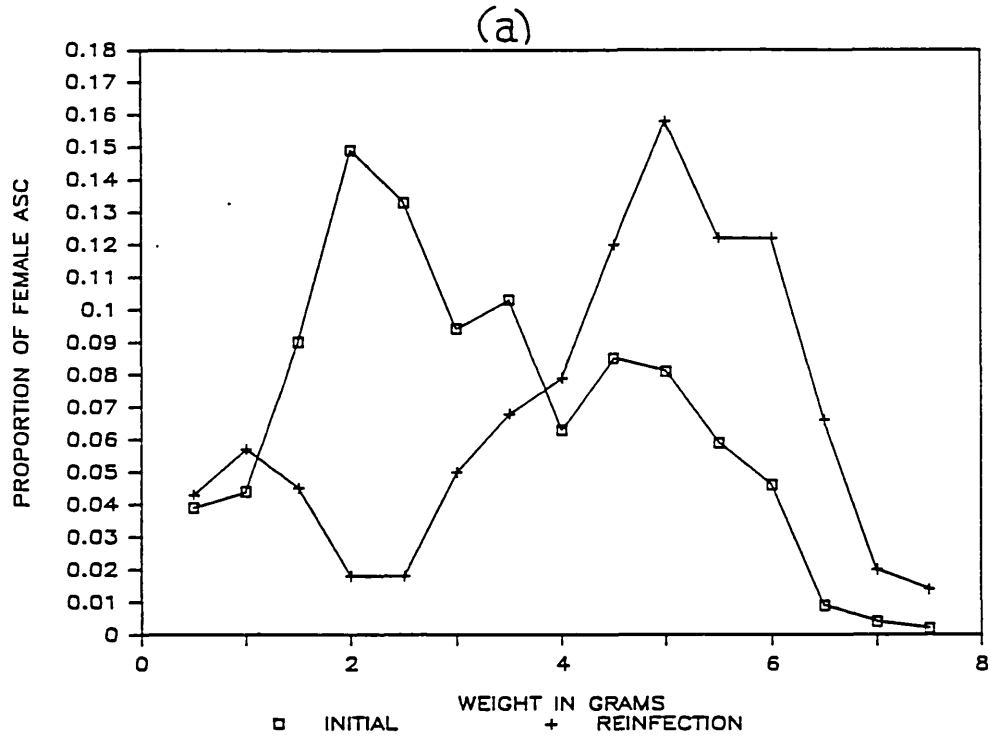


Figure 7.17

The percent weight distribution of female Ascaris recovered from adults (ages 10 years plus) in January and in November. A total of 538 and 483 female worms are represented in the initial and reinfection curves, respectively.



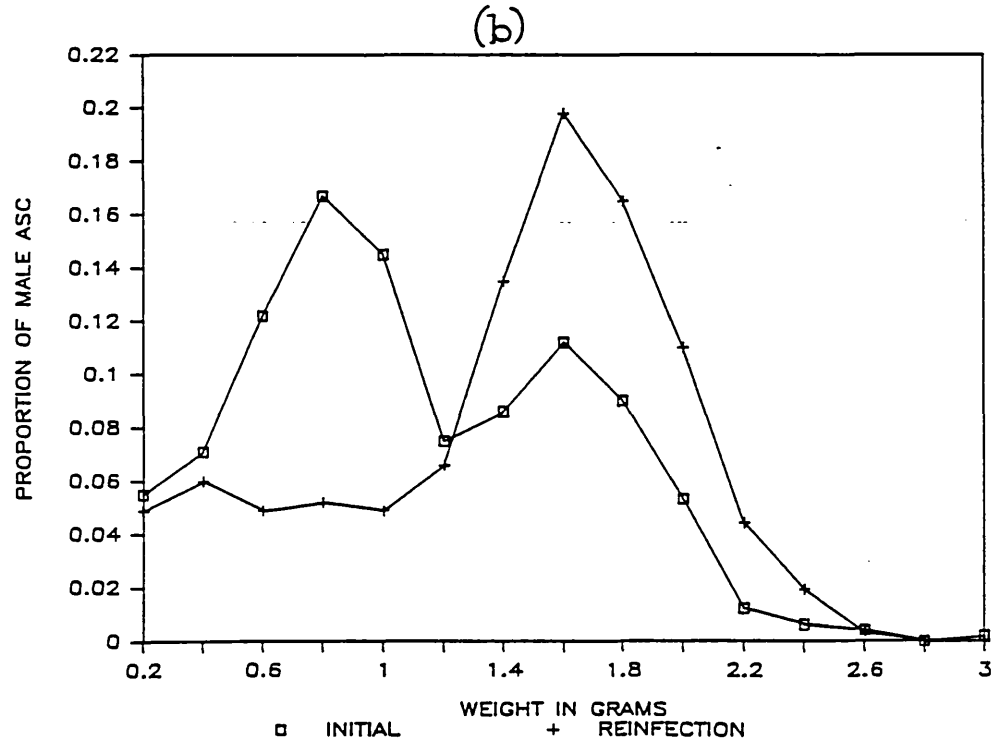


Figure 7.18

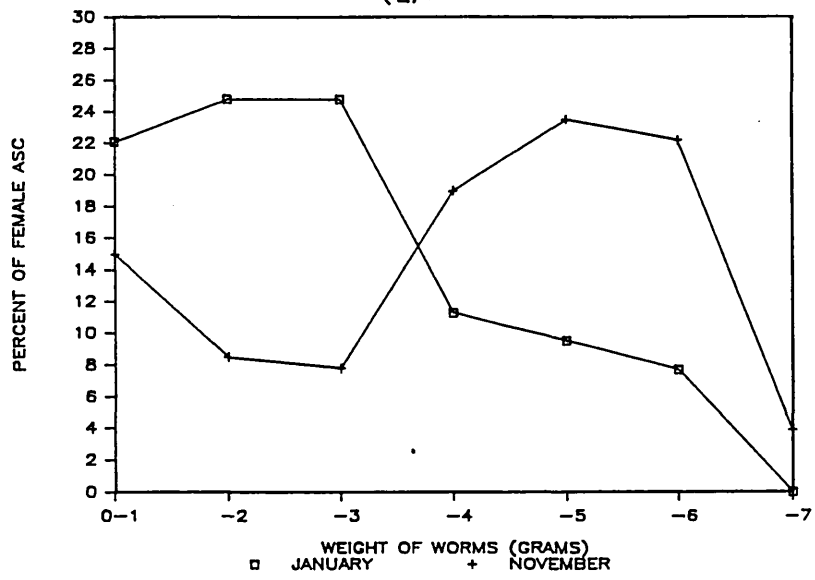
The percent weight distribution of female (Graph(a)) and of male (Graph(a)) Ascaris recovered from children (0 to 9 years of age) during the two treatment programmes. Graph(a) includes measurement of 586 and 439 female worms, and Graph(b) represents 538 and 353 male worms recovered in January and November, respectively.

Figure 7.19

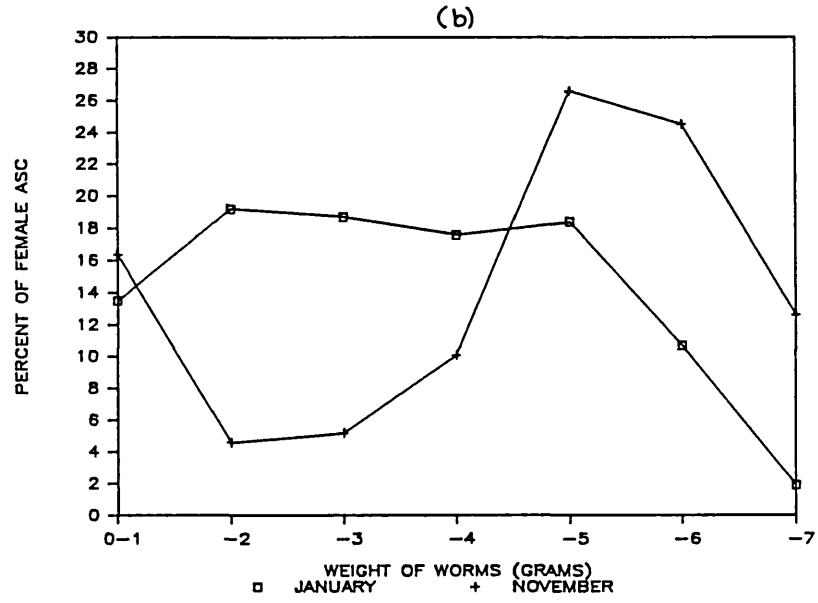
The percent weight distribution of female worms recovered in January and in November from host age groups, 0-4 (Graph(a)), 5-9 (Graph(b)) and 10-19 years (Graph(c)). Sample sizes are:

Graph	Age Group	Date	No. of Worms
(a)	0-4	January	222
		November	153
(b)	5-9	January	364
		November	286
(c)	10-19	January	252
		November	235

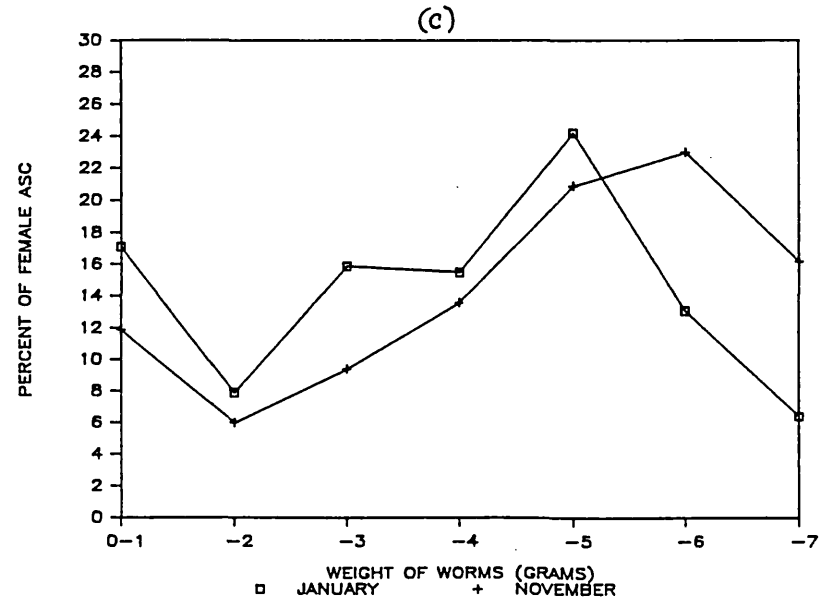
(a)


PERCENT OF FEMALE ASC
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0-1
-2
-3
-4
-5
-6
-7
WEIGHT OF WORMS (GRAMS)
JANUARY
NOVEMBER

(b)


PERCENT OF FEMALE ASC
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0-1
-2
-3
-4
-5
-6
-7
WEIGHT OF WORMS (GRAMS)
JANUARY
NOVEMBER

(c)


PERCENT OF FEMALE ASC
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0-1
-2
-3
-4
-5
-6
-7
WEIGHT OF WORMS (GRAMS)
JANUARY
NOVEMBER

look into the dynamic process of helminth infection.

A comparison of the weights and lengths of freshly passed and formalin-preserved worms provides a check of the reliability of the measurement presented in Chapter 7. In the few quantitative analyses which have been done, none have reported the effect formalin preservation may have had on their results. Fortunately, the increase in weight for length was uniform across all weight groups. It is suspected that the increase is due to the absorption of formalin across the cuticle, rather than shrinkage in length. During the initial measurements on the worms, weight was recorded first, since slight pressure on the worms (particularly mature females) caused them to burst open due to the high pressure of fluid inside. Fresh worms were not sensitive to pressure and were found to be more elastic.

Non-linear regression of worm weight versus length for all the worms collected in January and November showed a similar trend in both male and female Ascaris, with a rapid gain in length followed by an increase in weight during and after maturation. In males this has been estimated to occur between 10.5 and 11.5 cm, and in females between 12.5 and 13.5 cm (Seo & Chai, 1980). The power curves calculated from the data also reflect these close patterns of weight and length in each sex, although males did have a consistently higher mean grams per centimeter value throughout the weight groupings up to the male maximum of 2.6 to 3.0 grams. This was not readily observed in the calculated curves. Cho (1977) demonstrated that the weight of worms was highly correlated with length (combined sex) as described by the equation, $Y = 15.40\ X^{.330}$ (where X = length in cm and Y = weight in grams). The present data were described by a similar equation though the worms weighed less relative to their Korean counterparts.

Perhaps the most intriguing result which arose from the weight/length analysis of the recovered worms was the observed differences in the percent size and calculated age distribution of the worm population recovered in January and November (Figures 7.6 to 7.9). This change could not be attributed to a decrease in the ratio of males to females nor immature to mature Ascaris. The worms recovered at initial chemotherapy reflected the undisturbed weight distribution pattern, with the majority of the worms of both sexes distributed along a wide plateau of weights. Following 11 months reinfection this distribution was highly skewed to the right, with a significantly smaller percentage of worms in the lower weight classes and a large peak in the higher worm weights. Thus the worms recovered in November were both heavier and were more homogenous in size. Based on a formula derived by Seo et al (1983), the age of the majority of the worms following reinfection was estimated to be several months older than those recovered initially. This difference was not attributable to density-dependent growth restrictions, since no change was detected in the size of the worms relative to increasing numbers per burden. At both sampling points the total worm weight and length and the number of worms harboured was highly correlated and well described by a positive linear regression. However, the slope of the regression in November was significantly steeper, indicating the burdens in November were composed of longer and heavier worms independent of worm number.

Attempting to further characterize the change in worm size at reinfection, the worm weight distribution patterns were analyzed by host age and sex. The size distribution of worms recovered from male and female hosts did not differ and the observed shift occurred in both sexes (Figure 7.14). When examined by host age in January,

however, dramatic differences were observed. Children displayed a wide plateau of worms across the lower weight worm weight groupings with very few heavy worms, while the adults (10+ years of age) showed a sharper peak in the higher weight groupings. In November, however, both age groups harboured a small number of low weight worms (young worms) with the majority of Ascaris clustered in a distinct peak between 4 and 6 grams. The Ascaris recovered from hosts older than 9 years of age showed similiar worm weight distributions at both initial and reinfection treatment, and children (0 to 9 years of age) harboured worms of distinctly different weight distributions at the two time points. To summarize these observations, in the absence of treatment children harboured a large number of low weight (young or stunted) worms while adult hosts carried worms of larger size. In contrast, the worms recovered following an 11 month period of reinfection from both children and adults were of a consistently greater size. The changes described in detail concerning the weight of female worms were also clearly evident in male worms and in the length measurement of both worm sexes.

The age distribution of the worm populations were also estimated using the measurement data in an equation by Seo et al (1983). According to this equation, only a small percentage of male worms survived beyond an approximate age of six months at either sampling date. In the female population, 13.7% and 24.3% were estimated to be older than six months, based on worm recovery in January and November, respectively. These calculated ages are lower than expected according to the estimate (see Chapter 4) for the average life expectancy of Ascaris of 7 to 8 months, based on the overall proportion of immature worms. The calculated age of worms are probably underestimated, however, due to the lighter weight of the worms compared to those

found in Korea discussed above. In addition, geographical (racial variability of the parasite) or host-related (e.g. age, sex, treatment history) differences between the two study areas may also affect the growth rate.

The reasons for the observed differences in the size distributions of worms with age and date are unknown. A continual problem faced in epidemiological studies of human disease is not knowing when infection occurs. Unfortunately, it is therefore not possible to infer whether the smaller Ascaris detected were younger (more recently acquired) in age or if their growth was inhibited in children prior to initial treatment. The sharp homogeneity in large-sized worms in older hosts initially and in all hosts following reinfection may suggest that the large worms, once established, can inhibit the development of smaller worms. Some method of arrested development mediated by large adult worms may regulate maturation in Ascaris superinfections. Treatment with pyrantel pamoate may have allowed the first Ascaris entering both children and adults to grow to maximum size, thus blocking further development of incoming worms. Prior to treatment when infections in children were heavy and heterogeneous in size, a significant population of large worms may have been unable to develop due to expulsion by the host.

Though there is currently little evidence suggesting that arrested development occurs in A. lumbricoides infections, experimental evidence with A. suum infections suggests that helminths in the family Ascaridae are capable of arresting growth at the larval stage. Soulsby (1982) concluded after a series of experiments with A. suum that arrested development was observed at a stage that was specifically susceptible to host antibodies. This hypothesis has particular relevance to this work, considering the decrease in the

proportion of small worms recovered in adults and following reinfection which were approximately the size of the final moult stage. However, the present data suggest that large, established worms in the gut may also contribute to the arrestment. Another possible hypothesis is that the clearance of adults after treatment triggered the release of arrested larvae from the liver, resulting in the homogeneous distribution observed in November, with a large group of worms of similiar age becoming quickly established.

Other factors which may have contributed to these important observations include:

1) Time and level of infection. Following initial treatment, the recovered burdens were composed of worms which may have been acquired during both high and low periods of transmission. If, following initial treatment, the period between January and July is a time of high transmission, then the worms acquired after this period would be few in number, resulting in the peak found in the heavier worm weight groupings. Seasonal transmission or temporarily interrupted egg contamination in the environment due to the initial mass treatment programme (January to approximately June, see Chapter 4) may have contributed to the lack of young worms observed in November.

2) Space and resource limitation. In younger hosts, the decreased size of the small intestine combined with the lower dietary intake may limit the establishment of incoming worms and their growth. Under these restricted circumstances only the Ascaris established during the primary infection may acquire optimum space and resources for growth.

3) Host resistance. The host may also affect the worm population through inflammation of the gut. The degree of inflammation in the intestinal region may have been high in children infected with a large number of worms, causing frequent expulsion of adults. These responses may have been reduced following treatment, allowing a greater number of worms to become established and reach maximum size. In addition, host defenses are thought to be active against larvae and immature worms, limiting the survival of migrating stages. In early studies done with Ascaris suum in pigs, a spontaneous loss of immature parasites was associated with the fourth moult (Schwartz, 1959) followed by a peak in antibody production (Taffs, 1958). Antibodies directed against the surface of the cuticle can mediate leucocyte adherence, causing cuticular damage and killing of young worms. Thus, the larger average size of worms may reflect an efficient but not sterilizing immune response effective against the early, but not the later stages in development.

GENERAL DISCUSSION

This thesis describes the results of a two year field project designed to study the epidemiology of Ascaris lumbricoides in a South Indian community. The purpose of conducting a longitudinal study was to allow sufficient time to investigate an undisturbed, endemic focus, provide anthelmintic treatment and monitor the process of reinfection. A village-based study offers a number of advantages over experimental research in the laboratory. Perhaps most importantly, it allows the direct applicability of the results to the human condition - a serious short-coming with many laboratory-based studies. In addition, greater 'value for money' may be achieved through work conducted in developing countries, where staffing and overhead are relatively inexpensive. Finally, field studies can help to evaluate the relevance of findings from laboratory investigations toward the understanding and control of human disease.

Despite these advantages, however, there are also drawbacks in undertaking research in the field. Each project operates under a unique set of circumstances depending on the country, social habits of the people, environment, etc. The researcher is not just responsible for designing a project that will be suitable for local conditions, but he must also work with hundreds or perhaps thousands of people with a cultural background alien to his own and maintain the communities' enthusiasm for participation in his work. These problems, combined with technical and staffing difficulties, may complicate attempts to investigate similar questions in a different field environment. Furthermore, control strategies based on the

results of an epidemiological study in one area may not be effectively applied in other areas where the population dynamics of the parasite differ.

Epidemiological research within human populations is also hampered by the inability of the scientist to control or detect the time and frequency of exposure to the infectious stages. In addition, techniques and strategies have not been developed for the measurement of the important host responses which limit helminth establishment. Since many of the trends observed in this and other epidemiological studies could result from differences in either host resistance or frequency of exposure, it is not possible to define the relative importance of these factors in human infections.

Whether in the lab or in the field, the aim in most medical research is the control of disease. The work undertaken in Pulicat was not directly concerned with control, yet many of the methods and results would be useful if applied within a control scheme. The use of the ethyl acetate/formalin concentration technique (as modified by Hall (1981)) may be an improvement over the Kato or Stoll technique currently being used by many researchers. The ethyl acetate/formalin concentration allows preservation of the stools and requires a smaller number of immediate technical staff since egg counting need not be done promptly after collection. An additional advantage of this technique is in permitting the researcher to keep staff busy throughout the year by putting the samples aside until the inactive periods of village work, such as during the monsoons. If there is no need to monitor infection, the most accurate and rapid means of assessing intensity is via treatment and analysis of the recovered worms. Unlike egg counting, which allows two people to analyze approximately 40 samples daily, over 100 stools per day can be sieved

following chemotherapy and the worms counted, weighed and measured.

In this study, many important epidemiological trends were consistently observed throughout the observation period. The degree of worm aggregation within the population, as measured by parameter k, was remarkably similar at initial treatment and following reinfection. At both the sampling programmes, one quarter of the human population harboured approximately three quarters of the total worms of the community. Secondly, sex and age intensity trends observed following reinfection were similar to those initially recorded. The distribution pattern of the immature worms within host age classes followed closely with that of total Ascaris in both treatments. There was also a strong tendency for individual hosts to reacquire worm burdens of a similiar magnitude during reinfection relative to others in the sample population. These observations support the proposal that control programmes based on anthelmintic treatment repeatedly applied to age groups or individuals selected for their high intensity of Ascaris may be continually effective in reducing heavy infections.

The analysis of worms expelled following treatment in the present study revealed astonishing differences in the size of worms between the two sampling times. These results have important implications for the use of anthelmintics in control programmes aimed at reducing the numbers of Ascaris eggs in the environment and the average burden in the community. Larger worms may release more eggs into the environment, increasing the level of transmission. This observation further supports the idea that treatment must be applied at frequent intervals. Treatment at longer intervals may actually increase egg production by altering the age distribution of the parasites and reducing the density-dependent regulation of egg fecundity.

The results suggest a great need of further investigation into the dynamics of reinfection and the relationship between Ascaris lumbricoides and the human host. Using the effective anthelmintics now available (which cause a rapid expulsion of helminths), this work could be done on humans at the individual and community level. Perhaps what is needed most at this time is a large scale application of the proposed control strategies in endemic areas, with careful monitoring of the results. The latter would provide an immensely valuable body of data on Ascaris infection at the population level and would be a great contribution towards understanding the mechanics of infection. This has been the strategy of Korean and Japanese scientists who have played a major role in the near eradication of Ascaris lumbricoides within their countries.

SUMMARY

1) Ascaris infections are acquired early in life in Vairavan Kuppam where 46% of the 0 to 2 year age class excreted eggs in the faeces. By age 9 years, the prevalence attained a peak value of 96%, thereafter a slight decline was recorded with a value of around 80% in the oldest age class. The age prevalence pattern was similiar between male and female hosts.

2) On a community basis, egg and worm counts of individuals were found to be highly correlated in both treatment programmes. In general, the measures of intensity based on egg counts provided a good description of the age-related changes in average worm burden. Intensity peaked in young age groups and declined with age. Females were more heavily infected than males in most age classes and the overall difference was highly significant.

3) The proportion of immature worms in the parasite populations recovered in each age class of the community did not vary significantly with host age. Parasite recruitment clearly took place in all age classes, and there is no evidence that acquired resistance mechanisms influenced the proportion of young worms (recent recruits) in older people with long periods of past experience of Ascaris infection. The distribution pattern of immature worms within host age classes followed closely with that of total Ascaris.

4) At reinfection, neither the prevalence nor the egg intensity curves accurately reflected the overall intensity of worm burdens. The overall mean egg output (EPG and EPD) reattained pretreatment levels by 6 months reinfection and continued to rise in the final 5 months. In contrast, intensity according to worm burden was reduced by nearly half of the initial levels following 11 months of reinfection. Both male and female hosts harboured significantly lower mean worm burdens at reinfection, with a lower proportion of males becoming reinfected compared to those initially infected. Initial and reinfection prevalences in females did not differ.

5) The negative binomial probability model provided a good description of the distribution of Ascaris at initial treatment and following reinfection. The within age group frequency distributions were aggregated in form, and the negative binomial model was an acceptable model of observed trends within all age groups. Ascaris was more highly aggregated in male hosts, as exhibited by lower k values at both inital and reinfection treatments.

6) Anthelmintic treatment repeatedly applied to age groups selected for their high intensity of Ascaris may achieve continual suppression of Ascaris populations. If treatment had been targetted toward infections in children aged 2 to 9 years, over 50% of the total Ascaris and the maximum source of egg production would have been eliminated. Treatment should be applied at intervals of 6 months or less due to the rapid recovery of egg output, particularly in females of this age group.

7) There was a strong tendency for individual hosts to reacquire worm burdens of a similiar magnitude following reinfection relative to others in the sample population. Following treatment in January, the initial 'wormy people' reaccumulated Ascaris at a faster rate than lightly infected individuals according to faecal egg count scores during the 11 months reinfection and the worms collected following treatment in November.

8) The 5 to 9 age class contained half of the 'wormy people' in both treatments. Age-targetted treatment of the 5 to 9 year olds is probably a more appropriate control strategy than selective treatment since this age group was highly susceptible to heavy burdens.

9) The technique employed for this study, formalin/ ethyl acetate sedimentation of samples from mixed, whole stools, yielded a 65% success rate in identifying 'wormy people'.

10) No significant variation was detected in per capita EPG and EPD according to age class, sex of the host, or date of treatment.

11) The mean stool weights of the 'wormy' and 'non-wormy' groups did not differ significantly at either time point.

12) A highly significant correlation was detected between 24 hour faecal output and daily egg fecundity (EPD per mature female).

13) Fecundity was reduced at low and high worm burdens as a consequence of mating probabilities and density-dependent restrictions, respectively. Negative correlations between per capita EPG and EPD were detected in the total population, in children (0 to 14 years) and in male hosts in January. Positive correlations were more prominent following reinfection where worm burdens were lighter.

14) The regression of worm weight on worm length generated a power curve that varied little by sex of Ascaris or by treatment programme. An initial rapid gain in length was followed by an increase in weight during and after maturation. Worm weight and length was unaffected by the number harboured by the host. There was a consistently higher weight for length ratio in worms preserved in formalin, however, compared to fresh worms at all weight classes.

15) The size distribution of worms recovered at initial treatment from children (0 to 9 years) and adults (10 years plus) differed dramatically. The worms recovered from children following initial treatmeny were clustered within weight classes between 1 and 4 grams. In adults few worms of less than 3 grams and a large proportion of worms weighing between 4.5 and 6 grams were recovered.

16) The male to female and immature to mature worm ratios did not differ with time of treatment, although children following anthelmintic treatment reacquired burdens that were composed of heavier and longer (perhaps older) worms, averaging a greater grams/centimeter compared to initial treatment. The overall weight for length ratio increased 12% at reinfection.

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APPENDIX 1

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APPENDIX 2(a)

LIST OF MEDICATIONS PROVIDED AT THE CLINICS

A. VITAMINS

Vitamin A & D Capsules
F.S.T. (Iron) Tablets
Multivitamins
Vitamin C Tablets
B Complex Tablets
Vitamin B_{12} Injections

B. OINTMENTS, PAINTS AND DROPS

Sulphur Ointment
Benzoic Acid
Ointment(Whitfield's)
Gentian Violet(1%)Aqueous
Ichthammol Paint
Tetracycline (1%) Eye Drops
Boric Acid Ear Drops
Cough Syrup

C. TABLETS AND CAPSULES

Acriphylline
Aminophylline
Ampicillin
Antacids
Antihistamine
Antispasmodics
Aspirin
Chloromycetin
Chlorostreptomycin
Chloroquine
Doxycycline
Ephedrin
Erythromycin
Ethambutol
Inseptin
Isokin
Metronidazole
Paracetamol
Salbutamol
Septrin
Stomatil
Sulphaguanidine
Tetracycline
Tinidizole
Zylocaine

D. MISCELLANEOUS

Cotton
Scissors
Gauze
Tape

APPENDIX 2(b)

EQUIPMENT FOR MEDICAL CLINICS

1. 3 Tables
   i) I.D. Desk
   ii) Doctor's Table
   iii) Measurement Area

2. 2 Long Benches
   i) For Patients
   ii) To Place the Baby Crown-Heel Measuring Board on

3. 8 Chairs
   i) I.D. Desk
   ii) Doctors
   iii) Patients under Examination
   iv) Weight and Height Recording

4. Weighing Machine (Balance Scale) and Reference Weights

5. Baby Crown-Heel Length Measurement Board

6. Height Scale

7. White Sheets and Ropes (to construct screen for private examination)

8. Pens, Pencils, Clipboards

9. Health Forms, Family Cards, Extra Pink Admission Cards

10. Medications

11. Prescription Pads for each Doctor

12. Extra Stool Containers, Labels and Plastic Bags for Collection

13. Flashlight for Examining Eyes and Throat

APPENDIX 3

BIRTHS AND DEATHS IN VAIRAVAN KUPPAM DURING OBSERVATION PERIOD

BIRTHS:

DATE	SEX	
1983	Males	Females
Jan.-March	2	2
April-June	2	3
July-Sept.	0	2
Oct.-Dec.	0	4
1984		
Jan.-March	2	3
April-June	1	1
July-Sept.	1	1
Oct.-Dec.	3	1

DEATHS:

1984	AGE/SEX
January	1 year, Female
March	<1 year, Female
June	31 years, Male
October	2 years, Female
October	2 years, Male
November	60 years, Female
December	6 years, Male

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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

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Abstract

The study design of a project to investigate the epidemiology, population dynamics and control of intestinal nematode infections in fishing village communities in Southern India is described. The paper focuses on *Ascaris lumbricoides* infection and describes changes in prevalence and intensity (worm burdens) with host age, the aggregated frequency distributions of parasite numbers per person, a density-dependent relationship between parasite fecundity and worm burden and rates of reinfection following chemotherapeutic treatment. The age-intensity of infection profile is convex in form, where maximum worm burdens are attained in children in the age range five to nine years. On the basis of juvenile to adult worm ratioos, the life expectancy of *Ascaris* in man is estimated to be of the order of one year. Rates of reacquisition of worms after chemotherapy are shown to be dependent on host age. Wormy individuals with heavy infections are shown to be predisposed to this state such that they reacquire heavier than average worm burdens following treatment.

Introduction

Direct life-cycle intestinal nematodes are amongst the most prevalent of all infectious disease agents of man. Between 500 and 1000 million people are estimated to be infected with roundworm (*Ascaris lumbricoides*), hookworm (*Ancylostoma duodenale* and *Necator americanus*), whipworm (*Trichuris trichiura*) and pinworm (*Enterobius vermicularis*) (PETERS, 1978; WALSH & WARREN, 1979). The distribution of the diseases caused by these infections extends throughout communities of poor and socio-economically deprived people, in countries of the tropics and subtropics. Intestinal nematodes are primarily a cause of morbidity as opposed to mortality and, as such, the study of their epidemiology and control is somewhat neglected by comparison with the degree of attention devoted to diseases such as malaria, schistosomiasis and onchocerciasis. The morbidity statistics for the directly transmitted nematode infections are ill-defined at present and yet their high global prevalences imply that many people are ill and many more are at serious risk from infection. Morbidity is particularly acute in children who are, in general, most at risk with respect to heavy infection. Nutritional deficiencies caused by poor diet are aggravated by heavy worm loads and these effects are particularly serious during the years of child development and growth (STEPHENSON, 1980; STEPHENSON *et al.*, 1980; TRIPATHY *et al.*, 1971; BROWN *et al.*, 1980; APCO, 1980a, 1980b).

The remarkable population stability of helminth parasites to perturbations induced by man's activities or climatic factors has important implications for control (ANDERSON, 1982; ANDERSON & MAY, 1982; ANDERSON & MEDLEY, 1985). Traditional methods of mass chemotherapy, in the absence of concomitant improvements in sanitation, hygiene and education, have done little to alleviate parasite abundance. Man is able to mount immune responses to infection, which may help to limit parasite survival, establishment and fecundity. Acquired resistance, however, does not create sterile immunity and thus reinfection tends to be rapid following treatment. In the case of *Ascaris* and *Trichuris*, for example, worm burdens tend to return to their pre-treatment levels 9 to 12 months after chemotherapy (CHO, 1977; SEO *et al.*, 1980). Annual blanket chemotherapy in endemic areas is therefore of little value in the long term suppression of parasite abundance. Extensive studies in Japan, Thailand, Korea and Burma, for example, suggest that bi-monthly treatment intervals are optimal for the long term control of *Ascaris* (APCO, 1980a, 1980b). Recent theoretical studies, employing mathematical models of parasite transmission, confirm the need for frequent mass treatment maintained over long periods of time (ANDERSON, 1982; ANDERSON & MAY, 1982, 1985; ANDERSON, 1985; ANDERSON & MEDLEY, 1985). Thus, despite the availability of cheap and effective drugs, the frequent use of large quantities of anthelmintics (required for effective long term community control) is prohibitively expensive for most developing countries.

Such economic factors, linked with logistic and organizational problems, have stimulated the search for more effective ways of employing anthelmintics within communities. Selective or targetted chemotherapy is one alternative to mass application (SMILLIE, 1924; WARREN, 1982; ANDERSON & MAY, 1982; ANDERSON, 1985; ANDERSON & MEDLEY, 1985). Selection for treatment can be based on the individual (those with high worm loads) or a group of people (e.g., age, sex, religious or occupational classes with higher than average parasite burdens). One particular feature of the population biology of helminths makes selective treatment an attractive option. It has been repeatedly shown that the distributions of worm numbers per person are highly aggregated in form (well described empirically by the negative binomial probability model) such that most people harbour a few or no worms, and a few people harbour most of the total parasite population (SEO *et al.*, 1979; APCO, 1980a, b; CROLL *et al.*, 1982; ANDERSON &

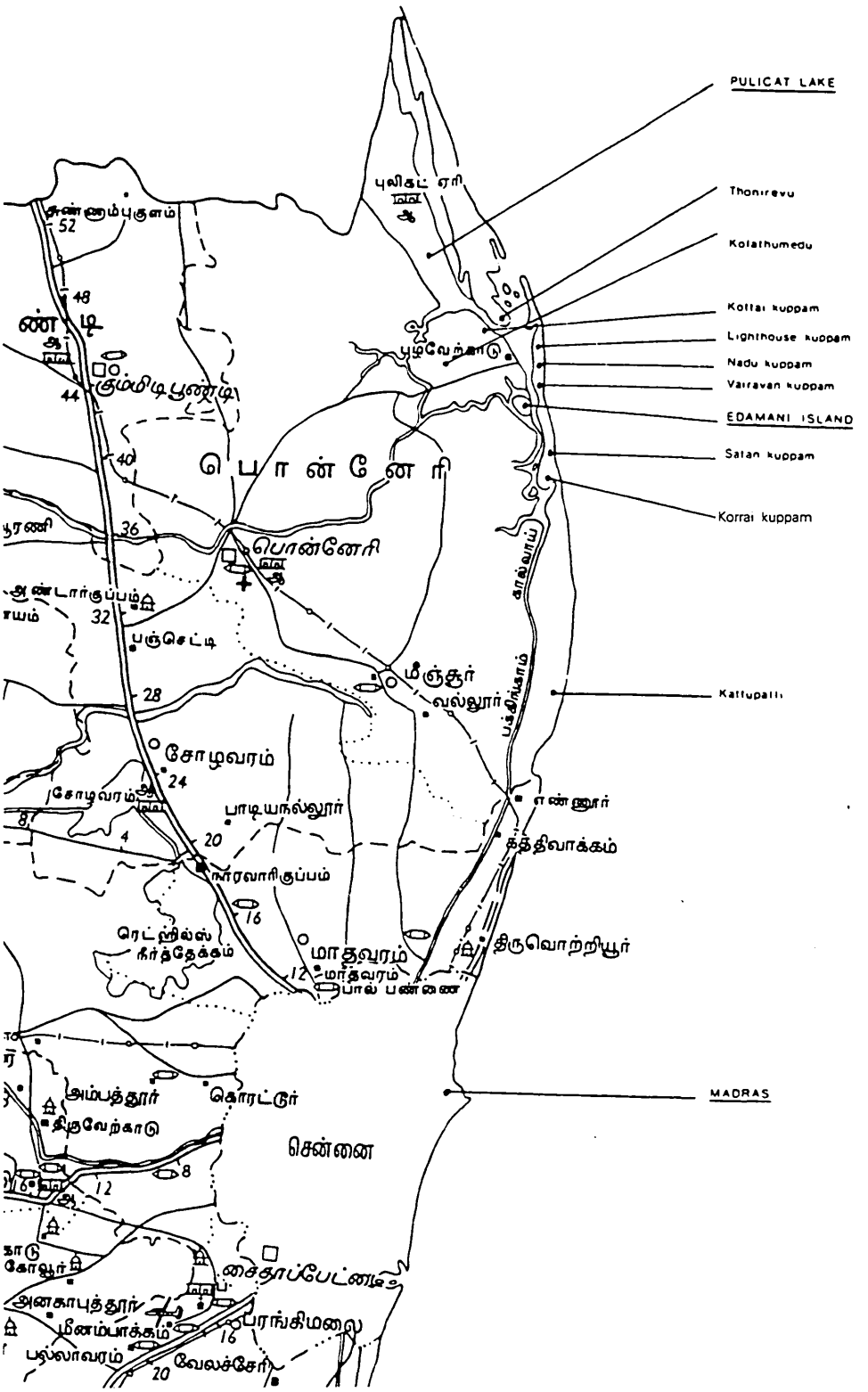


Fig. 1. Map of the study in the Pulicat Lake region of Southern India recording the locations of village communities.

MAY, 1982; MARTIN *et al.*, 1984; BUNDY *et al.*, 1985; HLIANG *et al.*, 1984). Selective treatment of the 'wormy' fraction of a community would therefore act to reduce substantially both morbidity (given that worm load and morbidity are positively correlated) and the release of transmission stages into the environment. In economic terms, selectivity clearly acts to reduce the amount of drug usage when compared with blanket application. In practical terms, however, many problems surround a selective approach, not least of which is the extra cost entailed in identifying those to be treated within a population. The significance of this problem diminishes if heavily infected individuals are predisposed to this state, not by chance, but by demographic, genetic (perhaps immunological), social, behavioural or spatial factors (ANDERSON & MAY, 1982; ANDERSON & MEDLEY, 1985; ANDERSON, 1985). If predisposition exists, then targetted treatment can be repeatedly aimed at those identified to be heavily infected during the pre-control survey. Recent research provides encouraging support for the hypothesis of predisposition, for both hookworm and *Ascaris* infection (SCHAD & ANDERSON, 1985; HLIANG, 1985).

This paper is the first in a series reporting the results of an epidemiological study of intestinal helminth infections in villages in the Pulicat Lake region of Southern India. The communities studied are predominantly involved in fishing activities (both sea and lake based fishing) and harbour a wide range of intestinal parasites. The principle aims of the study are twofold. The first is to examine the question of predisposition to heavy or light infection, by treatment and reinfection studies. The second is to assess the relative practical merits of selective and mass chemotherapy in the control of intestinal nematodes.

The first paper describes the study communities and sampling programme, and considers the epidemiology and population biology of one of the most abundant helminths, namely, *Ascaris lumbricoides*. Emphasis is placed on the results of horizontal cross-sectional surveys for parasite prevalence and intensity, on longitudinal studies of reinfection and on the issues of predisposition and density-dependent parasite fecundity.

Study area and design

A preliminary survey of intestinal helminths in children among five different social communities near Madras, India in 1980 revealed that the fishing communities at Pulicat Lake (see Fig. 1) were heavily infected with *Ascaris* and other helminths (ELKINS, 1984). The Pulicat location was adopted for the present project for the following reasons: (i) a high prevalence and intensity (mean EPG) of *Ascaris*; (ii) the villages are small and isolated with limited between-village contact; (iii) no previous control programmes or research projects, and no effective local health care delivery system have been implemented; and (iv) the availability of laboratory facilities at Pulicat (made available by Madras Christian College). Informed consent was obtained at all stages of the study.

Area

The study villages lie 55 km north of Madras, Tamil Nadu in South India, along the coast of the Bay of Bengal. They are located on a narrow, sandy peninsula, bounded by the sea to the east, and shallow backwaters of a large brackish water to the west. The villages are isolated from each other by expanses of sand and cassuarina groves. The project was initiated at the end of a severe three-year drought in South India. The average annual rainfall was 1215.3 mm, falling during the monsoon season of October-November and in August. The maximum and minimum mean daily temperatures were 32·9°C and 24·3°C respectively, and the average annual relative humidity is 74%. A mild to strong breeze blows in from the sea throughout the year.

Houses are arranged in fenced compounds comprising living quarters, a cooking area and a bathing area. Most houses are mud/dung with thatched roofs, while some are constructed of cement. The ground surrounding the houses and streets is loose sand in all of the seaside villages. All villages have at least one Hindu temple for worship and most have a government building which serves as a school. Many children attend primary school, but adults are generally uneducated. Men in seaside villages fish in the ocean on log rafts with nets, while in lakeside villages men catch lake fish and prawns. The daily catch is sold at the nearby market town by the women who in turn buy rice, dahls and vegetables. Both fresh, salted and dried fish plus prawn are eaten. Water is taken from open public wells or private hand pumps. Women and men use separate preferred defaecation sites within cassuarina groves, while children defaecate indiscriminately at the seaside or around the perimeter of the village.

A general description of each of the seven villages under study is given in Table I.

Table I—General description of the seven villages studied

Village	Reference number	Population	No. of houses	Method of parasite control	Location-Livelihood
Korrai kuppam	I	217	40	No control	Seaside/Sea fishing
Vairavan kuppam	II	457	70	Mass	Seaside/Sea fishing
Nadu kuppam	III	198	37	Selective	Seaside/Sea fishing
Lighthouse kuppam	IV	445	92	Selective	Lakeside/Lake fishing
Thonirevu	V	448	95	Mass	Lakeside/Lake fishing
Kattupalli	VI	153	31	Random	Seaside/Sea fishing
Kolathumedu	VII	245	55	No control	Landlocked/Prawn picking

Design

The design and plan of the investigation may be summarized as follows:

(1) Choice and characterization of a suitable study area, including mapping and demography, plus assignment of mass chemotherapy, random chemotherapy, selective chemotherapy and control villages.
(2) Introductory meeting and slide show in each community to explain the programme and request co-operation by majority consent.
(3) Collection of full 24-hour stool output and clinical information, including height and weight, from each resident.
(4) Anthelmintic treatment of each village according to the following schedule:
    (a) *Mass Village*: All residents (both infected and uninfected) given anthelmintic initially and the treatment to be repeated when egg output attains pre-chemotherapy levels.
    (b) *Selective Village*: Treatment given only to the most heavily infected 20% of the population (as determined by EPG and EPD) at two-month intervals.
    (c) *Random Village*: Treatment given to a 20% sample of the population (chosen at random) at two-month intervals.
    (d) *Control Village*: Treatment withheld until the completion of the study.
(5) 48-hour stool collection in mass village immediately after initial and final mass chemotherapy to determine worm abundance and distribution within the community, to identify, by worm and pre-chemotherapy egg counts, individuals harbouring the heavy infections, and to evaluate the accuracy of EPG and EPD as measures of worm burden.
(6) Further observations over a one year period comprising:
    (a) Quarter-yearly 24-hour stool collections from all residents in each village to measure reinfection rates (mass village), seasonal variation in egg output (control village) and effects of repeated random and selective treatment on the village population (random and selective villages);
    (b) Quarter-yearly medical clinics to monitor clinical, height and weight changes among residents of each village.

This paper records the initial results from the mass chemotherapy village, but additional detail is provided for reference in subsequent publications.

Materials and Methods

(a) *Sampling schedule*

Twenty-four-hour stool collections were initiated in a control (untreated) village (No. 1, see Table I) in September 1983 and repeated in February, July, and October 1984 and February and May 1985, followed by mass treatment. A mass chemotherapy village (No. II) was initiated in January 1984, and follow-up collections were done in March (to check drug efficacy), July and November 1984. Mass chemotherapy and 48-hour collections were repeated in November 1984.

Initial collection in selective villages, numbers III and IV, were completed in March 1984. Selective treatment began in July 1984 together with a general 24-hour stool collection to establish initial egg output intensities. Treatment was repeated bimonthly thereafter, with general collections in January 1985 and April 1985. Mass chemotherapy and 48-hour stool collections were performed in April 1985.

Sampling techniques

(i) *Stool Collections*. Village programmes were initiated with an evening lecture to explain the purpose of the research project and a slide show illustrating the health problems associated with intestinal helminths. At the close, the village head conducted a vote to record those willing to co-operate and to donate stool samples. The following day, the field team visited each house to record names, ages, family relationships and house location. On future visits births, deaths and movements in and out of the village were recorded. Also, during the first visit, one-litre airtight plastic containers (Snackie-Box, Bombay) labelled by an identification (ID) number (village initial, house number and position in family by age) and name in English and Tamil, were distributed to each family members. Pictures and numbers were drawn on the person's forearm using indelible ink and on his/her container to facilitate identification. Field workers fluent in Tamil explained to each family that all faeces should be passed in the container for a specified time period. 24 hours later, the team returned to collect used containers and distribute cards to those donating stools, to enable them to attend forthcoming medical clinics. Individuals who did not donate stools the first day were allowed an extra 24 hours to return their faecal output.

(b) *Clinics*. On the fourth day of the programme, a medical clinic was held in the village, and three medical doctors examined all attending. Drugs and vitamin tablets were dispensed at no charge. Cases of serious disease, requiring further attention, were referred to government hospitals in Madras. Heights and weights (Avery beam scale, sensitivity-50 g) and any clinical and nutritional disorders detected by the doctors were recorded.

(c) *Treatment for helminth infection*. Pyrantel pamoate (Nemocid, Mexin Pharmaceuticals, Bombay) was given in a single dose of 10 mg/kg following meals. Children under one year of age, pregnant women and adults over 70 years were excluded from treatment. Whenever possible the tablets were taken in the presence of the attending doctor. No serious side effects were reported. Two-litre plastic containers, labelled as previously described, were immediately given to treated individuals with instructions to pass all stools in the box for the following 48 hours, the approximate time within which most of the worms are expelled (CHO, 1977). Used containers were collected and clean ones distributed over the two days. Each individual was questioned whether any stools were passed elsewhere, and data from incomplete 48 hours ouput were excluded from the results.

(d) *Drug efficacy*. Within eight weeks after mass chemotherapy in village No. II (see Table I), a second 24-hour stool collection was carried out to establish: (i) the conversion rate of egg counts from positive to negative following treatment with pyrantel pamoate; (ii) the post-chemotherapy prevalence of *Ascaris*; (iii) the accuracy of worm expulsion counts as measures of total worm burden. Of the 223 stools collected, 199 were from treated patients and, of these, 95% were negative for *Ascaris* eggs. Of the 10 patients still egg-positive for *Ascaris* after treatment, five passed only unfertilized eggs. The true efficacy of the drug may be lower than the 95% level recorded due to the inability to detect male worms by faecal egg count methods. Of the worms passed in the stools following treatment, more than 73% were found in the first 24-hour stool collection (see Fig. 2).

Laboratory techniques

(a) *Egg count determinations*. Pre-chemotherapy stools were immediately transported to the laboratory after collection and weighed (Port-O-Gram, O'haus, Germany) to determine 24-hour faecal output. After thorough homogenization, a 1·8 to 2·6 gram sample of faeces was placed in 8 ml of 10%

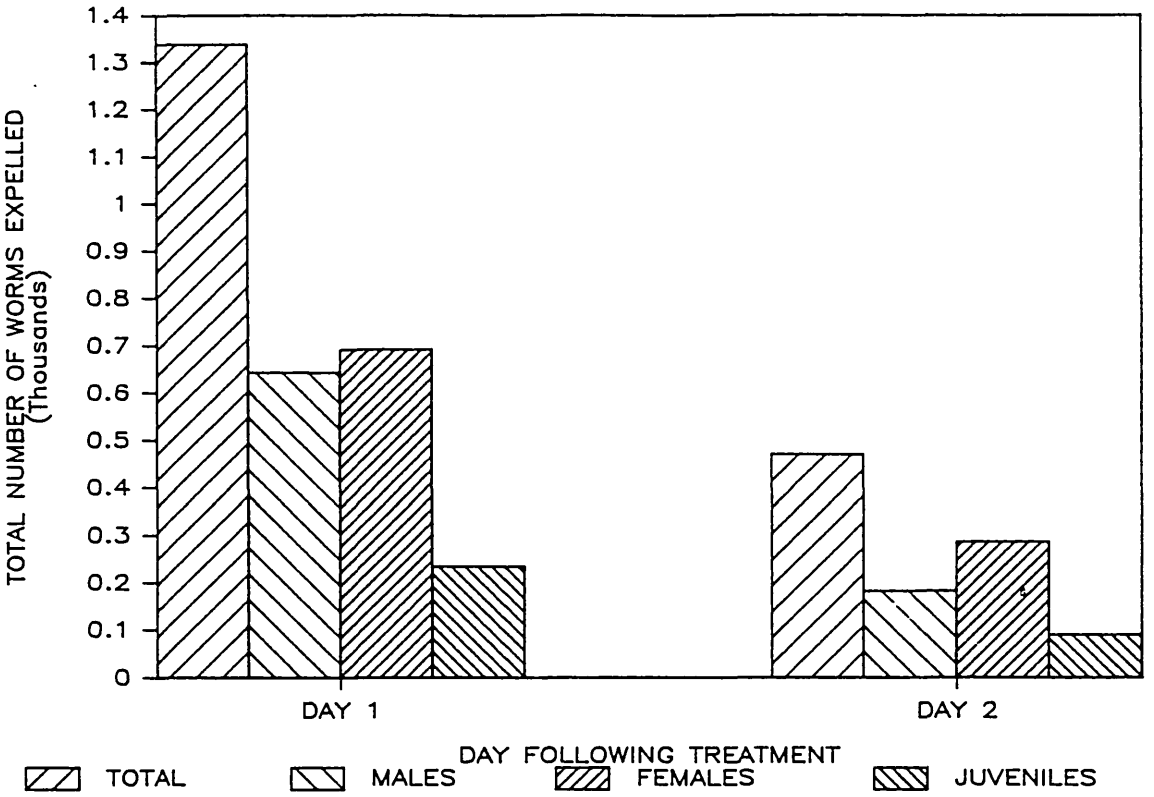


Fig. 2. Total numbers of adult male, adult female and juvenile *Ascaris* expelled in 24-hour stool collections on the first and second day post-chemotherapy (see text for further details).

formalin, shaken vigorously and stored. Samples were processed according to a modified formalin-ether sedimentation technique described by HALL (1981a, b) to concentrate helminth eggs and larvae. This technique was chosen because it allows preservation of eggs as well as quantification. Briefly, the samples were poured into 15 ml conical tubes and washed once. The pellets were re-suspended in 3 ml of water, then passed through copper mesh (34 gauge) and flushed with 12 ml water. After centrifugation, samples were shaken vigorously (60 sec) in 7 ml water plus 3 ml of ethyl acetate (equivalent to diethyl ether, TRUANT *et al.*, 1981). Following centrifugation, the detritus rings and the supernatants were decanted. The egg bearing pellets were suspended in a small amount of formalin and transferred to screw top tubes.

To determine egg counts, the total number of drops of sediment were counted and, after vigorous resuspension, one drop was placed on a microslide for examination. The total number of fertilized *Ascaris*, unfertilized *Ascaris*, *Trichuris* and hookworm eggs were recorded and the presence of *Hymenolepis nana* and *Strongyloides* noted. Eggs per gram was calculated by: EPG = (hV/vw), and eggs per day: EPD = (hVW)/vw, where h = the number of eggs counted, V = total volume of sediment in drops, v = the number of drops examined, w = the weight of the preserved sample and W = the 24-hour stool weight.

(b) *Worm extraction*. Post-treatment stools were preserved in 10% formalin and stored. To remove and count mature and immature *Ascaris*, stools were flushed through two grades of sieves (1·0 mm and 300 μm, Endecott, London) with water. Worms and sediment were washed out of the sieves and preserved in 10% formalin. Large worms (10·0 cm and over) were weighed (Port-O-Gram), measured for length and sexed by dissection. Small worms (0·2 to 9·9 cm) were located in the sediment by magnifying glass and placed in tubes in 10% formalin. The number of each species, *Trichuris*, hookworm, pinworm or immature *Ascaris*, was determined by means of a dissecting microscope.

Results

Demography

The age and sex structure of the population of Vairavan kuppam is displayed in Fig. 3. Of the total population of 457, 50% are below the age of 15 years. Life expectancy (from birth) is approximately 35 years and the net annual birth rate is roughly 34 per 1000 head of population (approximately three times the current birth rate in England and Wales, and the USA). The demographic pattern in Vairavan kuppam is typical of many developing countries.

Co-operation and faecal production

Co-operation in the first round of stool collection was 76% of the total population and 68% received anthelmintic treatment (5% were ineligible due to age or pregnancy status). Full 48-hour post-treatment stools were provided by 49% of the community with the highest level of co-operation provided by young children and elderly people.

The average 24-hour stool weights by age and sex are recorded in Fig. 4. Faecal output rises steadily with age for both males and females. After the age of

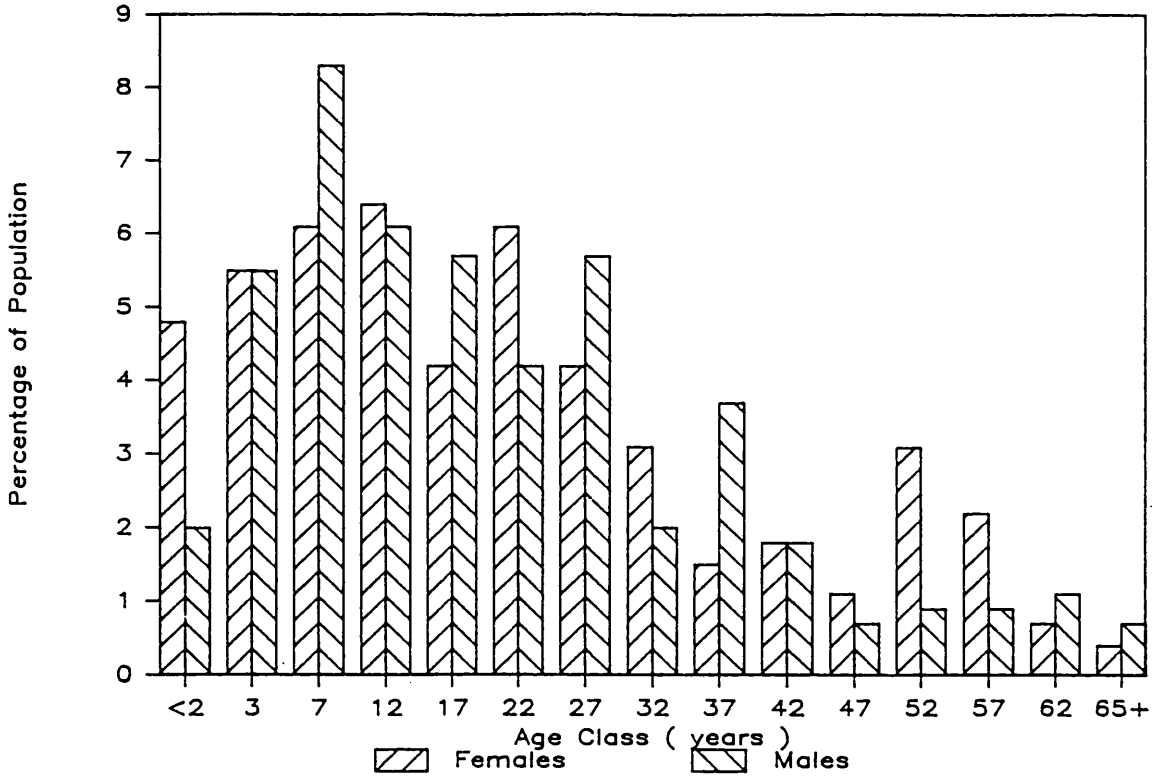


Fig. 3. Age and sex distribution of the population of Vairavan kuppam.

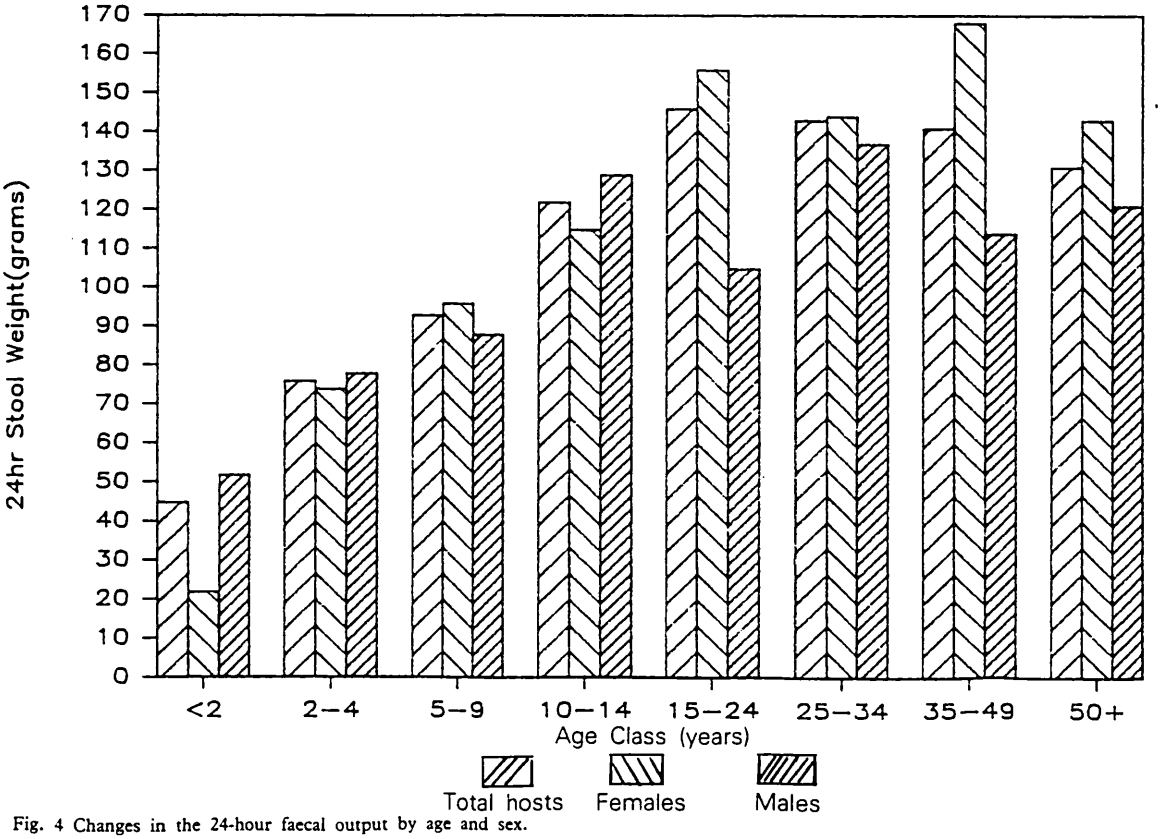


Fig. 4 Changes in the 24-hour faecal output by age and sex.

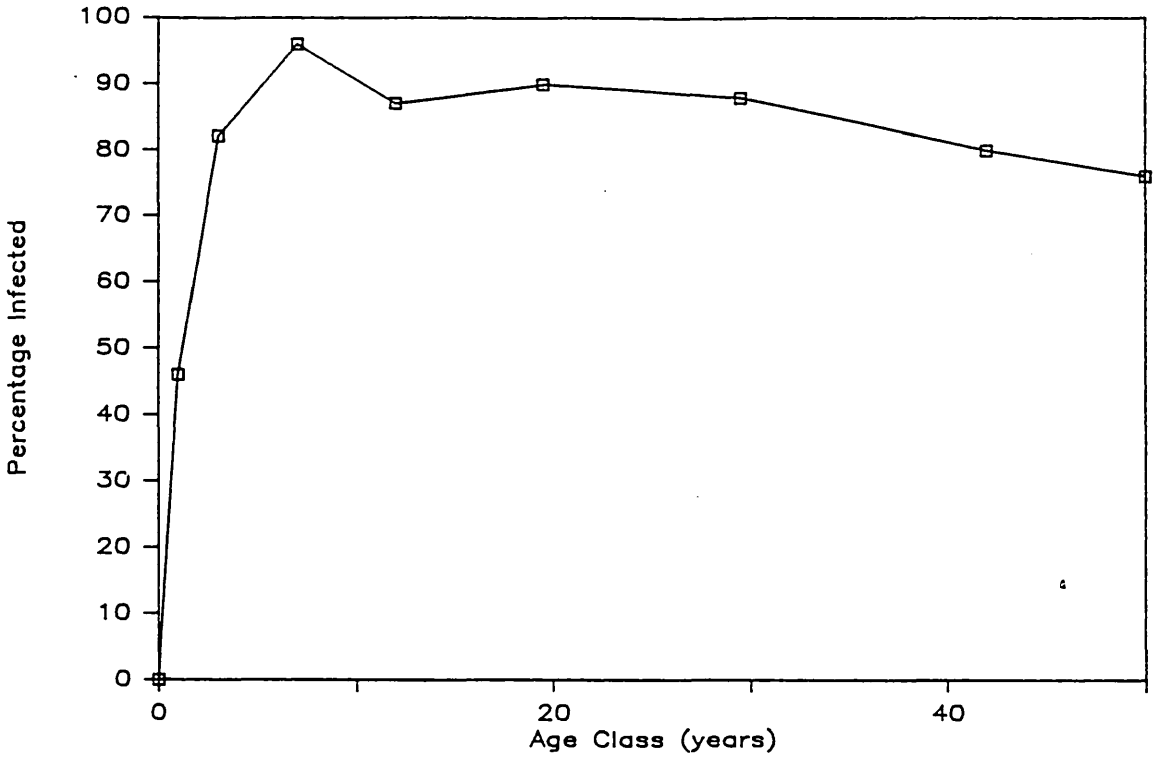


Fig. 5. Horizontal age-stratified profile of the prevalence of *Ascaris* infection in Vairavan kuppam.

14 years male output consistently exceeds that produced by females. Variation in stool weight is high both within age groups and in individuals on a day-to-day basis.

Helminth prevalence and intensity (horizontal survey)

At the pretreatment survey in Vairavan kuppam the prevalences of *Ascaris*, *Trichuris* and hookworm infection were 85%, 95% and 32% respectively. *Strongyloides* and *Hymenolepis nana* was also present with respective prevalences of 5·5% and 3·0%.

Ascaris infections are acquired early in life with 46% of the nought to two-year age class excreting eggs in the faeces (Fig. 5). By age nine years, the prevalence attains a peak value of 96%; thereafter a slight decline was recorded with a value of around 80% in the elderly age classes. Changes in prevalence with age did not differ significantly with sex. Horizontal age-intensity of infection profiles are recorded in Fig. 6; graphs (a) and (b) denote pre-chemotherapy values of EPG and EPD respectively while graph (c) records worm counts (juvenile and adult worms) derived by expulsion chemotherapy. All three graphs (Figs 6a, b and c) show similar trends, with a peak intensity of infection occurring in the five to nine-year-old children. The intensities show a marked decline in older age classes (more marked than that observed for the prevalence of infection, Fig. 5). Females display higher intensities of infection in most age classes. This trend is most striking in the two- to four-year-olds where girls have an average burden of 21·3 worms while males have an average burden of 5·8 worms. In general the intensity measures based on egg counts provide a good description of the age-related changes in average worm burden (compare graphs (a), (b) and (c) in Fig. 6).

Over half of the total number of worms recovered by expulsion chemotherapy were found in children between the ages of two and nine years (Fig. 7). This age group constitutes 34% of the total village population (Fig. 7). A similar pattern arises for the distribution of immature worms (male worms under 10·5 cm in length and female worms under 12·5 cm in length) where 53% of all immature parasites were harboured by the two- to nine-year age group (SEO & CHAI, 1980). Immature worms were recovered from all age classes and of the total of 1811 worms recovered 18% were immature (Fig. 8). This percentage can be used to obtain a crude estimate of worm life expectancy ($1/\mu$, where μ is the per capita death rate). If the death rate, μ, is constant and independent of age, then the proportion of immature worms in the population, p, is given by

$$p = [1\text{-}e^{-\mu T}] \quad (1)$$

where T is the time to maturation of *Ascaris*. Taking T to be approximately 50-60 days, eqn (1) yields an estimate of $1/\mu$ of just in excess of one year (where p = 0·18). The over-all female to male sex ratio of worms was approximately 1:1.

It is interesting to note from Fig. 8 that the proportion of immature worms in the parasite popula-

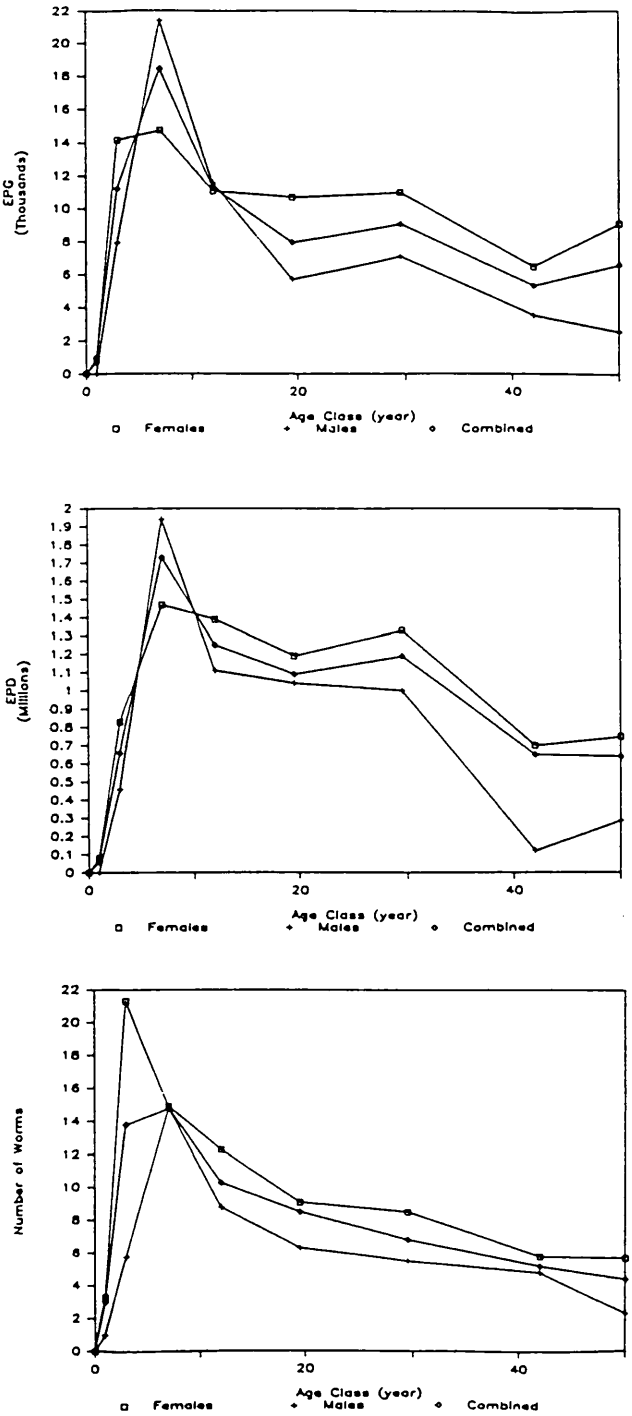


Fig. 6. Horizontal age- and sex-stratified profiles of the average intensity of infection with *Ascaris* in Vairavan kuppam. *Graph (a)*(upper). Changes in eggs/gram of faaeces (EPG) with age. *Graph (b)*(centre). Changes in eggs/day (EPD) with age. *Graph (c)*(lower). Changes in worm burden (juvenile plus adult worms), assessed by expulsion chemotherapy, with age. The sample sizes for each age group were as follows: <2 yrs, 13; 2-4 yrs, 38; 5-9 yrs, 54; 10-14 yrs, 46; 15-24 yrs, 60; 25-34 yrs, 49; 35-49 yrs, 35; >50 yrs, 33.

tions recovered in each age class of the community did not appear to vary greatly with host age. Parasite recruitment clearly takes place in all age classes and acquired resistance (if present) does not seem to decrease the proportion of young worms (recent recruits) in older people with long periods of past experience of *Ascaris* infection. This pattern, however, must be interpreted with caution, since the proportion of immature worms in different age classes of people could remain approximately constant, and independent of age, if a decline in parasite establishment (due to acquired resistance to infection) in older people acts concomitantly with a decrease in worm life expectancy and/or an increase in parasite maturation time.

Worm burden and parasite fecundity

The good agreement between age-related changes in egg counts and worm burdens (Fig. 6) suggests that EPG and/or EPD are satisfactory measures of parasite numbers per person. The mean egg output (EPG) per female worm shows no significant association with the age or the sex of the host (Fig. 9).

However, an examination of the relationship between *per capita* female worm egg production and total worm burden reveals a pattern of density dependent fecundity. Fig. 10 records *per capita* daily output of eggs/female worm (f) in various worm burden classes (the observed values denoted by square symbols are averages for a range of worm burden class, e.g., 1-4, 5-8, 9-12, 13-16, 17-20, 21-24, and 25+ worms with sample sizes, 46, 29, 19, 14, 17, 8 and 12 respectively). The solid lines denote the best fit exponential model of the form.

$$f = a \exp(-bp) \qquad (2)$$

where a and b are constants and p is total worm burden. In the lowest worm burden class (one to four worms) a proportion of the samples (16 of 46) contained only unfertilized eggs (probably as a consequence of single female worm infections) (SEO *et al.*, 1979). The squares in Fig. 10 denote the *per capita* fecundities for the samples which contained fertilized eggs (best fit exponential model has parameters $a = 4{\cdot}563 \times 10^5$, $b = 0{\cdot}0374$, $r^2 = 0{\cdot}89$) while the diamond in the one to four worm burden class denotes the *per capita* fecundity of eggs based on all samples (whether containing fertilized or unfertilized eggs) (best fit exponential model has parameters $a = 3{\cdot}6557 \times 10^5$, $b = 0{\cdot}0264$, $r^2 = 0{\cdot}79$). In both cases the exponential model provides a good empirical description of the density-dependent trend, where both slopes are significantly different from zero (at the 5% level). The severity of decline in fecundity as worm burden rises is insufficient to influence the value of egg counts as measures of parasite density.

Similar patterns of density-dependent *Ascaris* fecundity have been reported in a number of previous studies (ANDERSON, 1985; HLIANG *et al.*, 1984; MARTIN *et al.*, 1984; SINNIAH, 1982; CROLL *et al.*, 1982). Over 50% of the community harbour fewer than six parasites per person. The negative binomial probability model provides a good description of the observed pattern ($\chi^2 = 9{\cdot}55$, df = 22, $p > 0{\cdot}05$) and the aggregation parameter of the distribution, k (which varies inversely with the degree of parasite

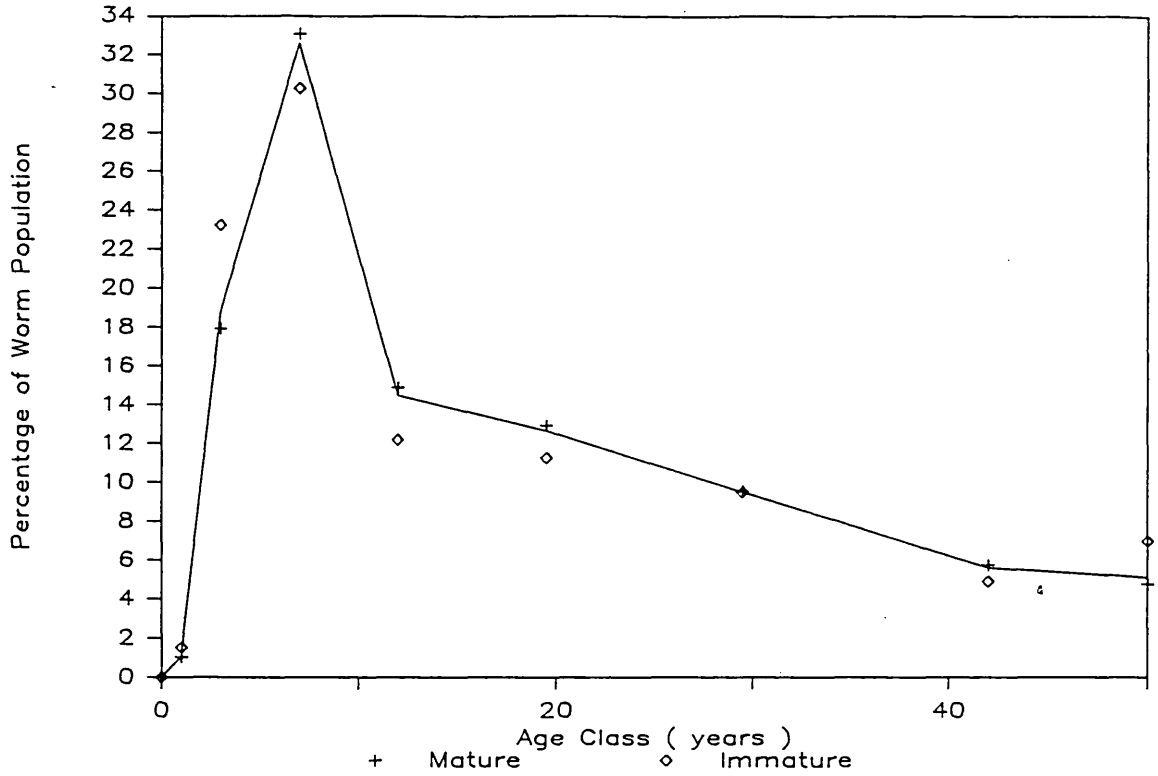


Fig. 7. The percentages of the total worm population recovered in each age class of the Vairavan kuppam community.

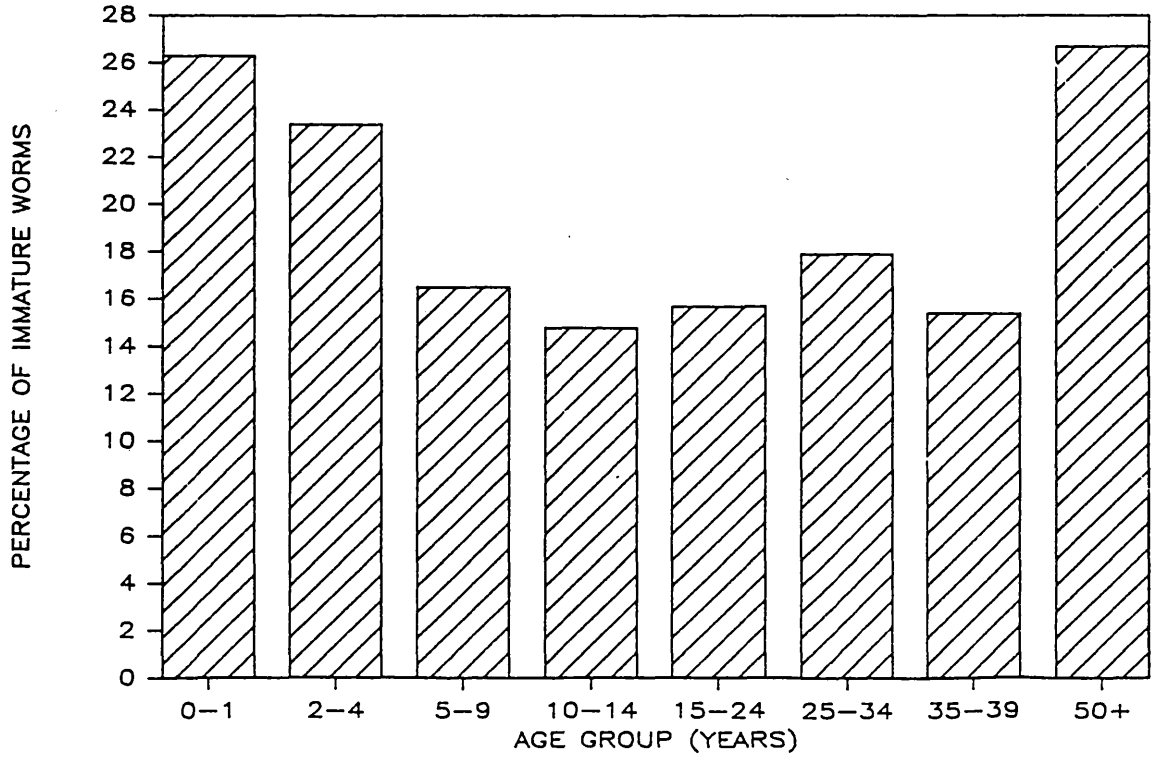


Fig. 8. The percentage of juvenile (= immature) *Ascaris* (see text for further details) in the populations of worms recovered from different age classes of the Vairavan kuppam community.

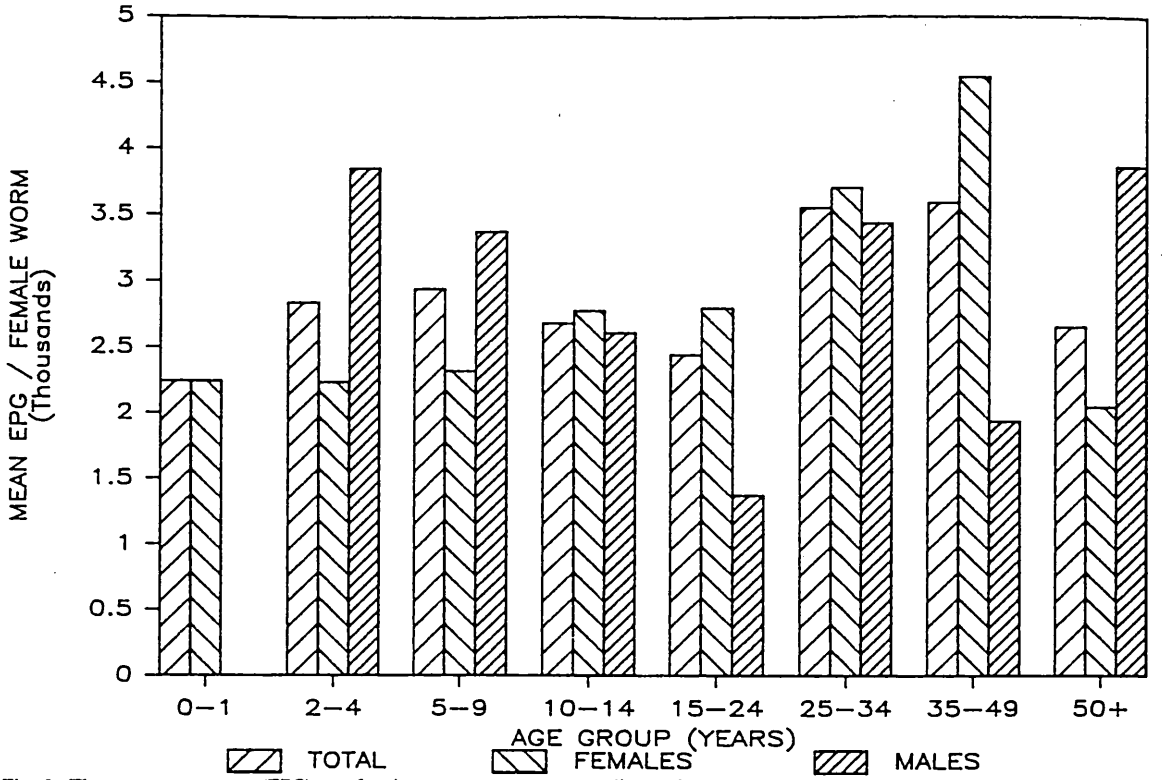


Fig. 9. The mean egg output (EPG) per female worm per person according to host age and sex.

clumping), has a value of 0·8 (derived by the maximum likelihood method described by BLISS & FISHER, 1953) (Fig. 11). Over 60% of the total population of parasites are harboured by 22% of the community. The subpopulation of people who expelled 17 or more *Ascaris* (hereafter referred to as 'wormy persons') is dominated by children between the ages of two and nine years (Fig. 12).

The within age group frequency distributions are again aggregated in form (see Table II) and the negative binomial model is, on average, a good model of observed trends (except for the distributions within the 10 to 24-year-old age classes) (Fig. 12). The degree of parasite aggregation (measured inversely by the parameter k) tends to be high in young children, low in older children, teenagers and young adults, and high in the older adult age groups (see Table II). In addition, aggregation is consistently higher among males in most age classes when compared with females.

The generative mechanisms of helminth parasite aggregation within human communities are probably many and varied (ANDERSON, 1983; ANDERSON & MAY, 1985b). For *Ascaris*, they are likely to include heterogeneities induced by social, behavioural, spatial and genetic (i.e., ability to resist invasion and acquire resistance to reinfection) factors. The processes that induced age related changes in the degree of contagion are unknown at present but they may include variation (perhaps genetically based) in the ability to acquire resistance to parasite invasion and establishment (ANDERSON, 1985).

Reinfection following chemotherapy

Following treatment in the village of Vairavan kuppam in January 1984, rates of reinfection were assessed by faecal egg counts after a period of 6 months in July 1984. A post chemotherapy survey was performed in March 1984 to assess the efficacy of treatment. The intensities (EPG) and prevalences of infection, stratified according to age, are recorded in Fig. 13 for the sampling times of January, March and July 1984. It can be seen that reinfection occurred rapidly such that in the younger age groups (0-9 years) the average intensities of infection (as estimated by egg output) had reached (or exceeded) their pre-treatment levels by the end of the six month period of reinfection (January to July) (Fig. 13a). The prevalence of infection is a less sensitive measure of reinfection, as a direct consequence of the aggregated distribution of worm numbers per person (see ANDERSON & MAY (1985b)). For contagious distributions, relatively large changes in average worm load may induce only small changes in overall prevalence.

Of greatest interest is the relative rate of reinfection in the different age classes of the population. As displayed in Fig. 14, the rate of reinfection over the six-month period (defined as the average egg output (EPG) after the six months of reinfection divided by the pre-treatment average intensity) is high in the young age classes, but declines rapidly with age. This could either be due to age-related changes in the degree of contact with infective stages, acquired resistance to reinfection (possibly of greatest magnitude in the older age classes with long past experi-

ences of infection) or a combination of both mechanisms. In an untreated control village (Korrai kuppam, village No. I in Table I) average intensities and prevalence of infection (stratified according to age) changed little over the six-month experimental period (Fig. 15).

Predisposition to heavy or light infection

The reinfection studies in Vairavan kuppam provide information on predisposition to heavy or light infection with *Ascaris*. These data can be analysed by reference to the division (discussed earlier) of the population into 'wormy' (those with >16 parasites) and 'non-wormy' (those with <17 parasites) segments, on the basis of the pre-treatment faecal egg counts and post-treatment worm counts. The rates of reinfection over the six-month period January to July in these two segments of the population, stratified according to age group, differ markedly (Fig. 16). Those who had heavy worm burdens before treatment tended to reacquire heavier than average burdens over the six-month reinfection period (assessed at the end of the period by faecal egg counts). Similarly those with light infections before treatment tended on average to reacquire light burdens (Fig. 16).

This analysis can be refined by considering reinfection within each individual in the sample population (see SCHAD & ANDERSON, 1985). Non-parametric statistical tests (Spearman's rank and Kendall's r test) reveal a significant correlation (at the 1% level) between pre-treatment and the six-month reinfection faecal egg counts for the sample population (n = 171) (Table III). This interesting positive association could arise as a consequence of differences in the intensity of infection in various strata of the population, such as age groups or sexes (see Fig. 6). Rank correlation tests based on age and sex are presented in Table III. The positive associations between pre-treatment EPG and similar counts after six months of reinfection following treatment are significant in the three broad age classes of 0 to 9 years, 10 to 24 years and 25 to 50 years. These broad groupings are necessary to obtain reasonable sample sizes for each class. Analyses based on sex within the age groups revealed an interesting trend. A significant positive association was found for females in the child and teenage plus young adult groups, while for males the correlation coefficient was significant only in the child age class. In the adult age class (25 to 50 years) the coefficients (r) for both sexes were not significant (Table III). The failure to detect significant positive associations in the adults may be a consequence of the low intensities of infection in this group when compared with the children (see Fig. 6). Of most interest, however, is the greater tendency of predisposition to heavy infection in females as opposed to males.

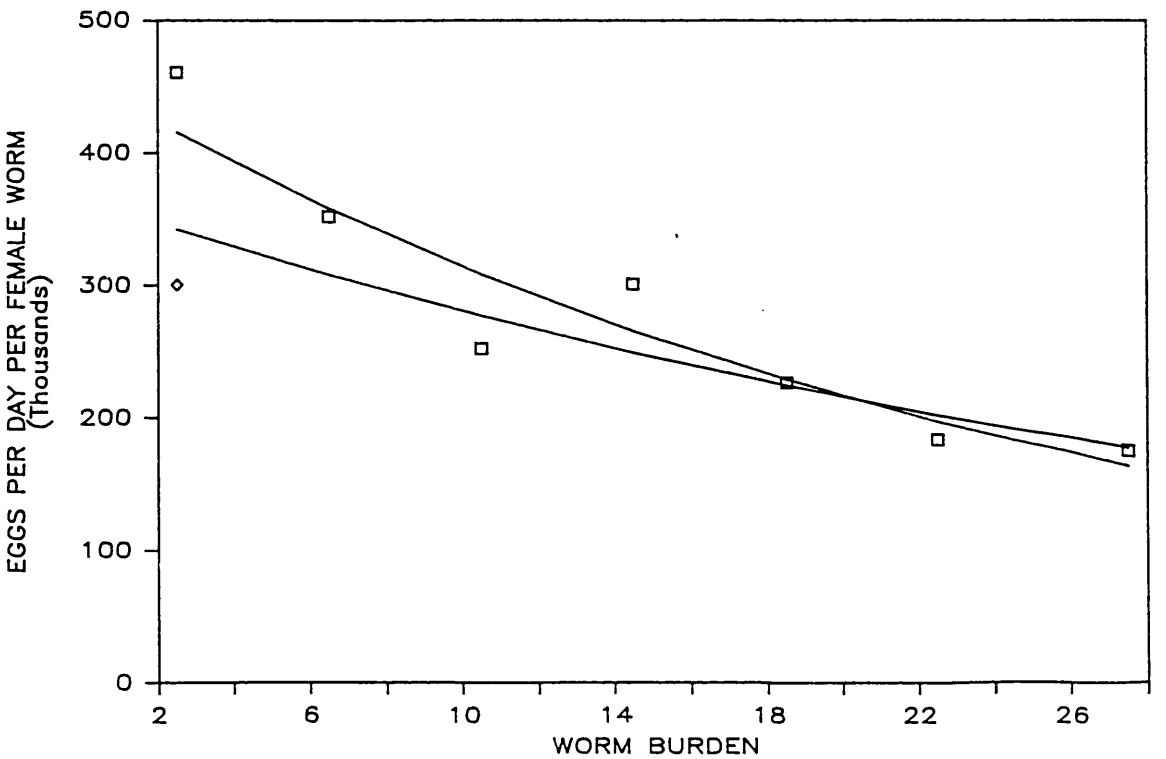


Fig. 10. Density-dependent fecundity of *Ascaris*. The graph records the *per capita* (female worms) egg output per 24-hour period as a function of total worm burden (juveniles plus adults). The squares record observed egg output (both fertilized and unfertilized eggs) where fertilized eggs were present. The diamond records egg fecundity (in the low burden class) of all samples including stools which contained only unfertilized eggs. The solid lines denote the best fit exponential decay models (see text for details). The top line (at the low worm burdens) denotes the best fit model for the observations recording fertilized and unfertilized egg output while the bottom line (at the low worm burdens) records the best fit model for the observations recording egg output in all individuals.

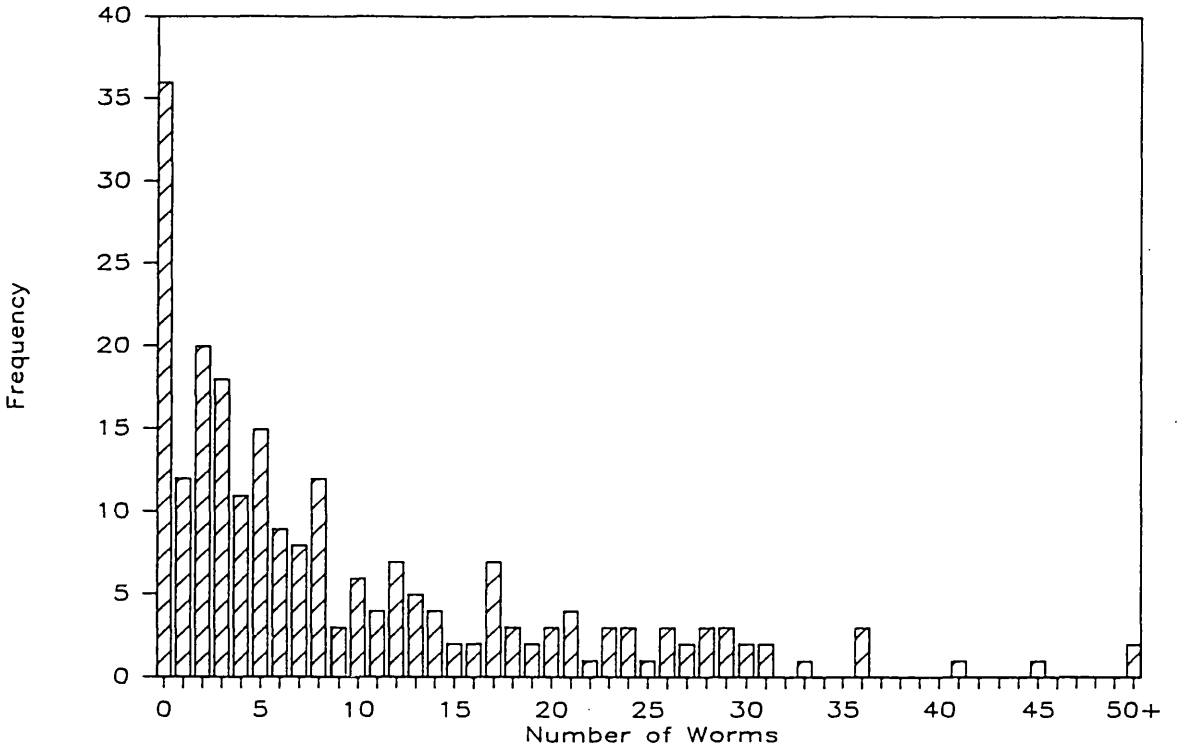


Fig. 11. Observed frequency distribution of the number of *Ascaris* (juveniles and adults) per person in the Vairavan kuppam community (sample size = 224).

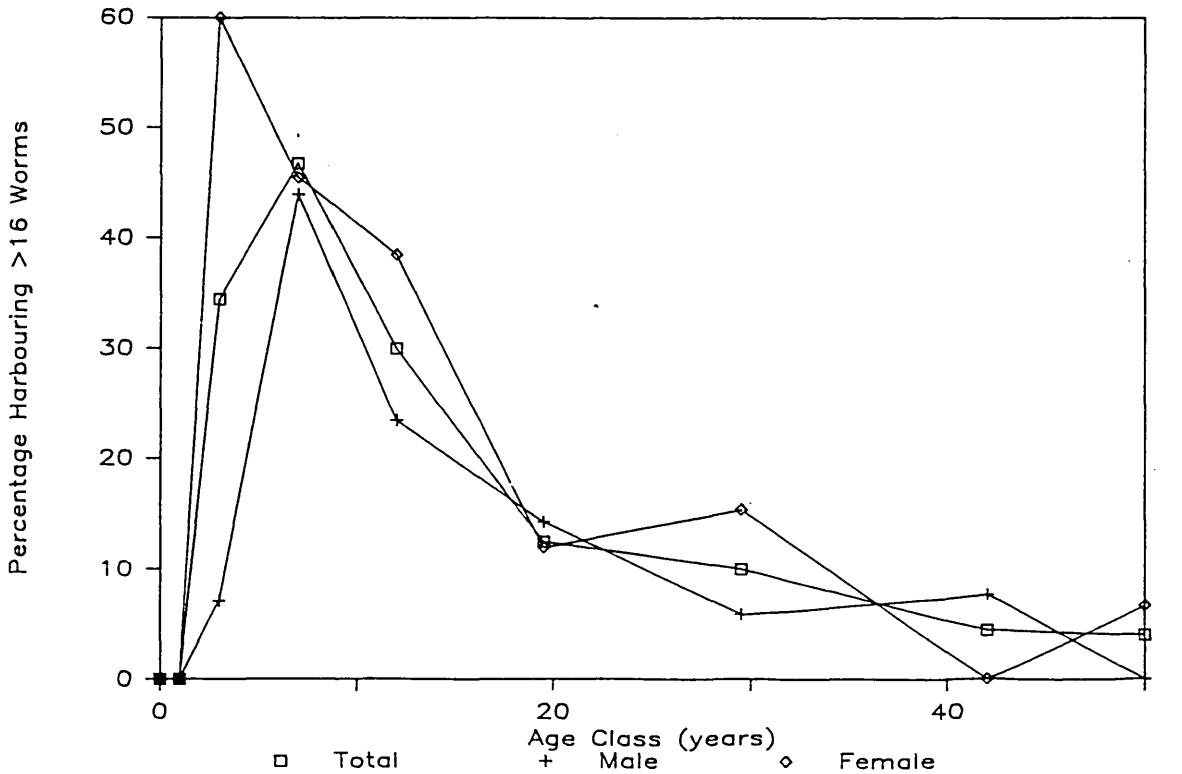


Fig. 12. The percentage of individuals harbouring more than 16 worms ("wormy" people) in each age and sex class of the community. In the total population, 81% of wormy females and 84% of wormy males were in the age class 0-14 years.

Table II—The mean ($\bar{x}$), Variance (s^2), aggregation parameter (k), and the goodness of fit of the negative binomial model for *Ascaris* frequency distributions in Vairavan kuppam (sample population 224) by age group and sex

Age	Group	Population	$\bar{x}$	s^2	k
<2	total	8	3·0	14·8	0·77
2-4	total	29	13·8	217·6	0·85 NS
	males	14	5·7	60·6	0·64
	females	15	21·3	253·9	1·94
5-9	total	47	14·8	169·6	1·40 NS
	males	25	14·8	197·5	1·25
	females	22	14·8	145·7	1·68
10-14	total	30	10·3	71·8	1·73*
	males	17	8·8	75·2	1·15
	females	13	12·3	65·7	2·83
15-24	total	32	8·5	86·5	0·90*
	males	7	6·2	59·9	0·73
	females	25	9·1	95·0	0·92
25-34	total	30	6·8	41·0	1·34 NS
	males	17	5·4	36·1	0·93
	females	13	8·5	45·2	2·00
35-49	total	23	5·2	44·2	0·64 NS
	males	13	4·7	57·6	0·40
	females	10	5·8	30·6	1·42
50+	total	25	4·3	31·8	0·78 NS
	males	10	2·3	6·4	1·28
	females	15	5·7	45·3	0·89
Over-all		224	9·5	113·3	0·80 NS
0-14	males	57	9·7	130·4	0·85 NS
	females	57	16·0	161·4	1·75 NS
15+	males	47	4·7	38·5	0·57 NS
	females	63	7·6	62·7	1·05 NS

Fit of the negative binomial indicated by * and NS:
* = P<0·05, significant at 5% level
NS = P>0·05, not significant at 5% level

Table III—Comparisons of pre-treatment egg counts with egg counts obtained after a 6-month period of reinfection on an individual patient basis (Vairavan kuppam)

Age group		Sample size n	Kendall's r test
All ages	Combined	171	0·24 **
	Male	73	0·24 **
	Female	98	0·29 **
0-9	Combined	65	0·20 **
	Male	29	0·09 NS
	Female	36	0·35 **
10-24	Combined	46	0·18 *
	Male	19	0·19 NS
	Female	27	0·33 *
25-50	Combined	60	0·16 *
	Male	25	0·16 NS
	Female	35	0·17 NS

** significant at 1% level
* significant at 5% level
NS not significant

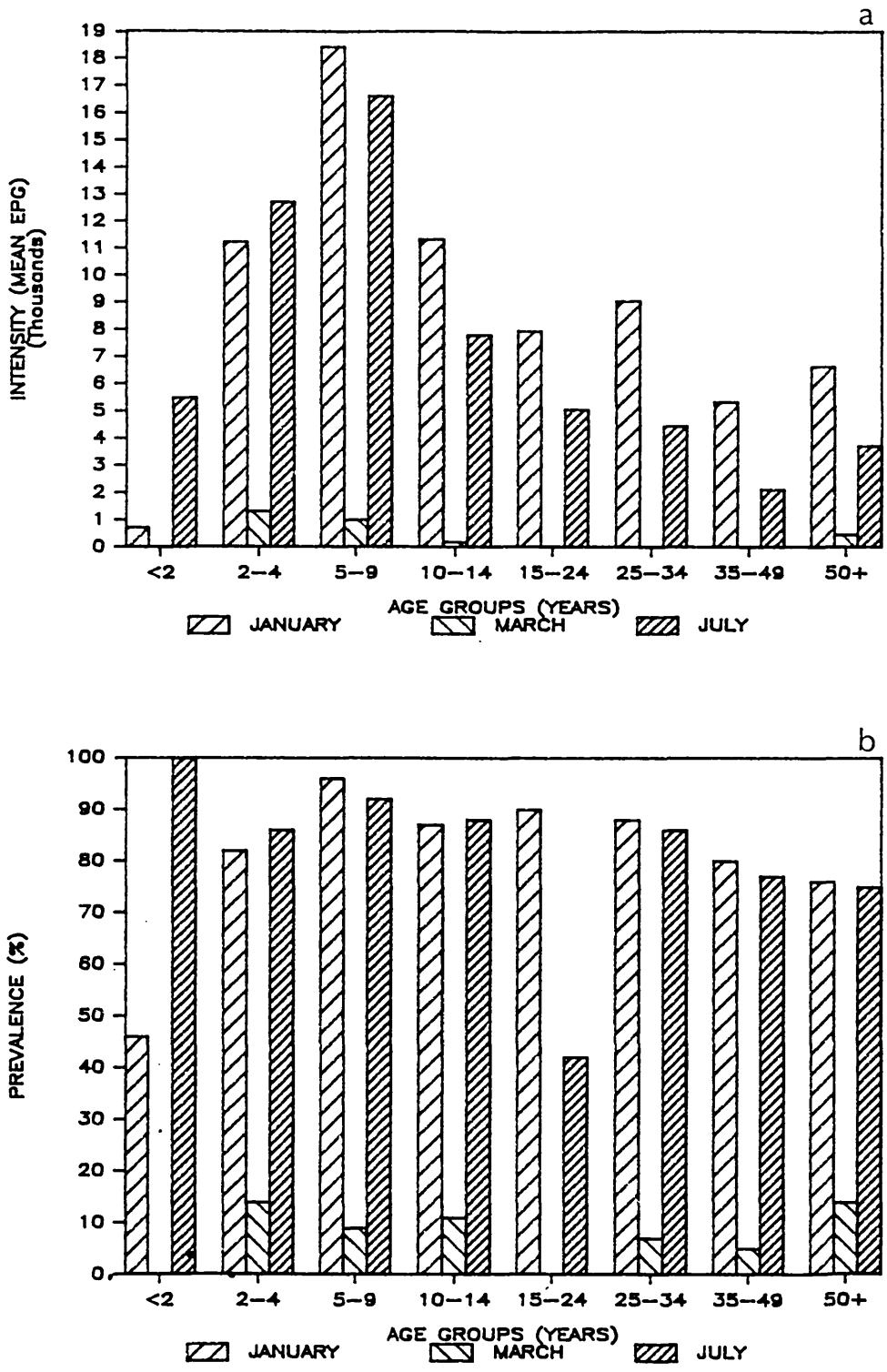


Fig. 13. Changes in *Ascaris* average egg output (EPG) (*graph a*) and prevalence (*graph b*) in different age classes following chemotherapy and a 6-month period of reinfection. The January data are pre-treatment observations, the March data were collected 8 weeks post-treatment (to check on drug efficacy) and the July data were collected 6 months post-treatment (see text for details).

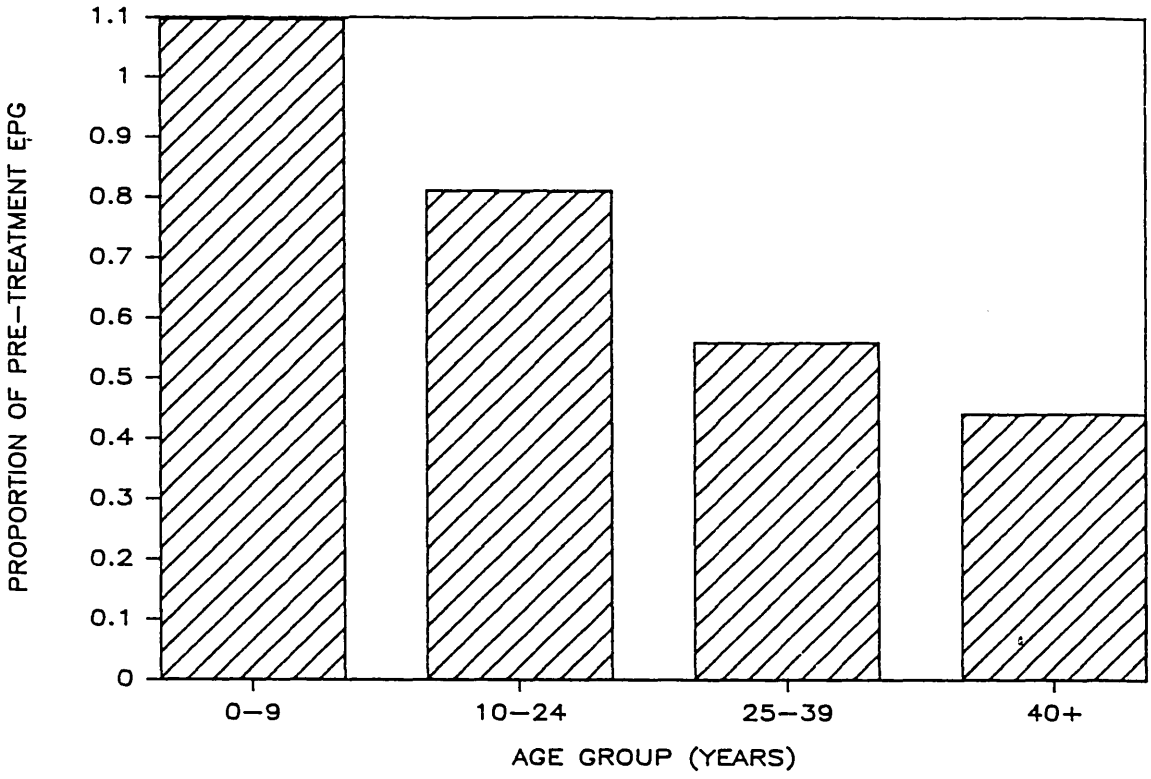


Fig. 14. Average faecal egg counts (average per person per age class) following a 6-month period of reinfection post-chemotherapy, recorded as a proportion of pre-treatment counts, in various age classes of the Vairavan kuppam community.

Discussion

The patterns of change of *Ascaris* prevalence and intensity of infection with age (horizontal trends) recorded in this study are typical for intestinal helminth parasites in communities with stable endemic infection (Fig. 6). The prevalence increased rapidly with age and remained at a plateau in the teenage and adult classes. By contrast, following a rapid rise, the intensity of infection decayed to a low level in the older adult age classes. A comparison of the age-related trends in these two epidemiological statistics (prevalence and intensity) reveals the limitations inherent in a prevalence measure for the epidemiological study of helminth parasitic infections. The relationship between the two statistics is determined by the frequency (or probability) distribution of parasite numbers per host; where aggregation or clumping of parasites is severe (as it is for most helminths) large changes in intensity may have little impact on prevalence (this point is clearly illustrated in Fig. 6). In practical terms, this observation implies that any assessment of the efficacy of control measures within a community must be based on intensity as opposed to prevalence statistics (ANDERSON & MAY, 1985b; ANDERSON, 1982).

The reason for the decay in intensity in older age classes is unclear at present. It could be due to age-related changes in contact with infective stages (due to diet, hygiene, activity etc.), resistance to helminth invasion and establishment acquired by exposure in the child and teenage years, or a combination of both mechanisms. The reinfection studies revealed a clear difference in the rate of reacquisition of worms in the different age classes (see Fig. 15), with high rates in children, and low rates in adults. Here again such differences could be due to age-dependent transmission, acquired resistance or a combination of the two processes. In the absence of experimental infections, the elucidation of the generative mechanisms of the decline in average intensity of infection in adult groups remains one of the most intriguing challenges facing epidemiologists interested in helminth parasitic infections. In future work on intestinal parasites, it will be desirable to attempt to record quantitative measures of behaviour relevant to transmission, and to monitor antibody and cellular activity within blood samples taken from different age groups in communities with endemic infection.

A special feature of the study reported in this paper was the recording of worm counts, for individuals in different age classes, by means of expulsion chemotherapy techniques. This approach produces information on the accuracy of faecal egg counts (both EPG and EPD) as measures of worm burden, on the relationship between parasite fecundity and parasite load and on the frequency distribution of parasite numbers per host (see Figs. 6c, 10, and 11). As

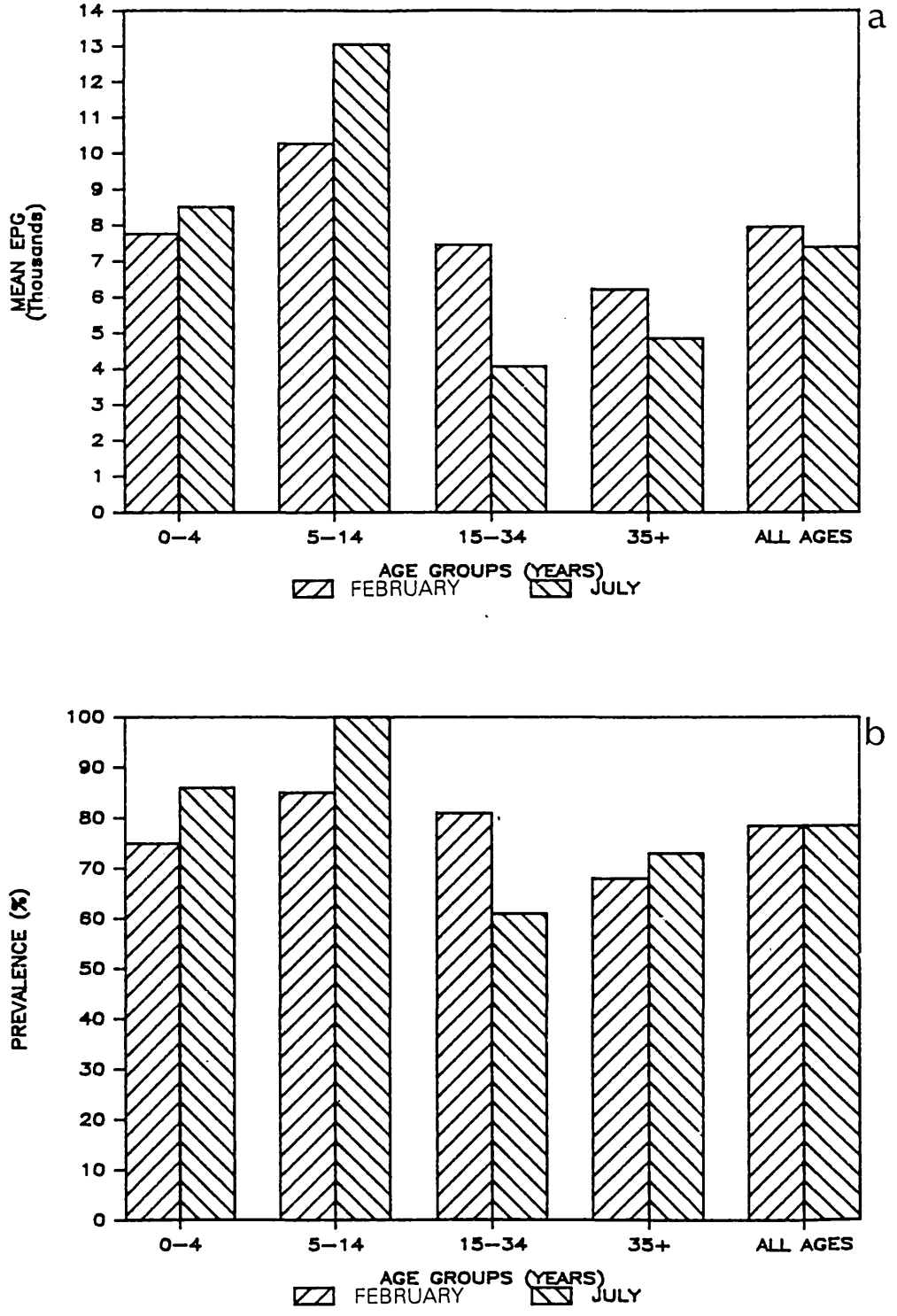


Fig. 15. Changes in the prevalence (graph (b)) and average egg output (EPG) of *Ascaris* by age class (graph (a)) in the control village, Korrai kuppam, (over the period February to July 1984) where no treatment took place.



Fig. 16. Rates of reinfection (measured by EPG) over a 6-month interval following treatment in "wormy' (>17 worms before treatment) and "non-wormy" (<17 worms before treatment) individuals in different age classes of the Vairavan kuppam community. The January counts are pre-treatment, the March counts 8 weeks post-treatment, and the July counts 6 months post-treatment.

Table III—Comparisons of pre-treatment egg counts with egg counts obtained after a 6-month period of reinfection on an individual patient basis (Vairavan kuppam)

Age group		Sample size n	Kendall's r test
All ages	Combined	171	0·24 **
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	Male	25	0·16 NS
	Female	35	0·17 NS

** significant at 1% level
* significant at 5% level
NS not significant

revealed in Fig. 6, egg counts provide a reasonable measure of the change of average worm burden with age. However, a careful examination of *per capita* female egg production (EPD/female worm) and worm burden revealed clear evidence of a density-dependent relationship (Fig. 10). The severity of this association, however, is insufficient to invalidate faecal egg counts as measures of average parasite intensity (Fig. 6). This result is clearly of practical value with respect to future epidemiological studies of *Ascaris* infection. With respect to the regulation of *Ascaris* transmission and abundance, it is of particular interest to note that the density of *fertilized* eggs is influenced adversely at very low parasite burdens as a consequence of the dioecious habits of the parasite (the probability of successful mating is adversely influenced in hosts with few worms) (Fig. 10). As such, the density-dependent association between parasite fecundity (the production of fertilized eggs) and parasite burden per host is markedly non-linear. Fecundity is reduced at low and high worm burdens as a consequence of mating probabilities and density-dependent restrictions, respectively. The mechanisms that reduced fecundity at high worm loads are unknown at present, but may involve limitations in resources or host response to the parasites' presence in the gut (ANDERSON, 1982; KEYMER, 1982).

The distribution of worm numbers per person was highly aggregated in form, such that most people harboured few worms and a few harboured heavy infections. Aggregation (as described by the negative binomial parameter k) was present in all age classes within the population, and within both sexes. In combination with density-dependent fecundity, parasite aggregation acts to create a strong regulatory constraint on parasite transmission (via the production of transmission stages) within the community. The effects of density-dependence are most marked within the few individuals who harbour most of the total parasite population. It is probable that parasite establishment and survival are also influenced by density-dependent mechanisms. However, at present it is not possible to quantify such effects.

The ratios of immature to mature worms within the different age classes of the populations provide information on the life expectancy of *Ascaris* in man. The estimation procedure adopted is crude, since it is based on the assumption that survival is independent of parasite density and parasite age. These assumptions are clearly questionable, but it is interesting to note that the estimate produced suggests a worm life expectancy of around one year. The calculation is in good agreement with figures widely quoted in past studies (see ANDERSON, 1982; CROLL *et al.*, 1982), but it should be noted that previous estimates have tended to be based more on guesswork, as opposed to quantitative information.

The most intriguing result obtained in this study concerns the question of predisposition to heavy or light *Ascaris* infection. Within the population as a whole, there is clear evidence of a positive association between pre-treatment intensities of infection and intensities after a six-month reinfection period following chemotherapy (Fig. 17). Wormy individuals (defined as those harbouring >16 parasites at the time of treatment), particularly children (0 to 14 years), tended to reacquire heavier burdens of *Ascaris* than their lightly infected counterparts (Fig. 16; Table III). Predisposition was most apparent in young age classes and in females. The generative mechanisms underpinning these observations are unknown at present. They are likely to include some combinations of social, behavioural, habitat (= spatial) and genetic factors. Work in progress on behavioural and genetic factors will, we hope, provide further information on this topic. However, irrespective of the mechanisms that generate these patterns, the results are of practical significance with respect to the community wide control of *Ascaris* by chemotherapy. They suggest that selective or targetted treatment of those predisposed to heavy infection may be a practical alternative to mass or blanket treatment. A more detailed discussion of this topic, plus further observations on the relative merits of different programmes of chemotherapy, will be described in future publications.

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A survey of intestinal helminths among children of different social communities in Madras, India†

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Summary

Approximately 500 children from five social communites in Madras, Tamilnadu, India were surveyed for intestinal helminth infection. The results of the survey indicated that the extent and degree of helminth infections vary significantly ($p<0.001$) between communities in Madras. The frequency and intensity distributions in two of the populations, the elite and the fishing communities are discussed. It is suggested that in view of the wide variability, health education should be geared toward each type of community and its specific routes of helminth dissemination rather than mass education, much of which may be useless to large segments of the general population.

Introduction

The helminth problems of India have changed little in the past century despite organized and active health programmes. This is due, in part, to a tremendous population increase combined with a rapid and overwelming influx of agricultural people into urban areas (JOSHI *et al.*, 1980).

Many surveys of the problem have been made but unfortunately these are difficult to correlate and compare because different techniques were used. Furthermore, many surveys, with the notable exceptions of CHANDLER (1927), MITRA (1970), CHOWDHURY & SCHILLER (1968), CHOWDHURY *et al.* (1968) and RAO & RAO (1975) were carried out in institutions where local epidemiological conditions could not be correlated with infections. The present survey was designed to survey the children of the Madras, Tamilnadu area, and to determine the qualitative and quantitative differences in intestinal helminths existing between the major social communities found therein.

Materials and Methods

Five basic types of social communites are found within the Madras area: (i) farming; (ii) fishing; (iii) slum; (iv) tribal; (v) elite. Three villages or neighbourhoods from each type of community were chosen as the source of data for the survey. Within each village, 25 to 35 stool samples were obtained from children between the ages of one and 12 years, thus totalling about 100 samples from each of the five communities.

Stools were analysed for the extent and degree of infection with the qualitative Merthiolate-Iodine-Formaldehyde Concentration technique (MIFC) and the quantitative Stoll egg-count technique (MELVIN & BROOKE, 1980).

Results and Discussion

The results of the survey indicated a significantly ($p<0.001$) diverse range in helminth prevalence and intensity between the children of these communities in the Madras area. Two groups which exhibited the greatest differences in number and density of intestinal helminths were the elite and fishing community children, the former defined as coming from homes with flush toilets and city water (Table I). Only 40·2% of the elite children were positive for one or more

†The work was done at Madras Christian College, Tambaram, Tamilnadu, India.

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Table I—The frequency and intensity of helminth infections in children of the elite and fishing communities in Madras, India

Parasite	Fishing Community (N=113)				Elite Community (N=97)				Total Survey (N=491)[2]			
	% infec.	(No.)	e.p.g.[1]	range	% infec.	(No.)	e.p.g.	range	% infec.	(No.)	e.p.g.	range
Ascaris lumbricoides	92.2	(107)	559	28-3704	26.8	(26)	14	1-78	60.3	(296)	265.2	1-3704
Trichuris trichiura	94.8	(110)	88	1-658	40.2	(39)	9	1-94	62.3	(306)	34.8	1-658
Hookworm	36.2	(42)	8	1-200	2.1	(2)	1·0	6-44	30.5	(150)	5.0	1-200
Hymenolepis nana	16.4	(19)	**	**	3.1	(3)	**	**	8.4	(41)	**	**
H. diminuta	2.7	(3)	**	**	—	—	—	—	0.8	(4)	**	**
Taenia spp.	1.8	(2)	**	**	—	—	—	—	0.4	(2)	**	**

% with one helminth species	22.2	(109)
% with two species	37.3	(183)
% with three species	16.5	(81)
% with four species	4.3	(21)

[1] Mean eggs per gram ($\times 10^{-2}$)
[2] % based upon samples from all five communities
** egg counts not reliable for determination of adult counts

species of helminth. Of the positive, 45·0% harboured one helminth species, 47·5% harboured two and 5·0% three species. In contrast, in the fishing community children 2·6% had one helminth species, 52·6% had two, and 30·2% and 12·1% had three or four helminth species respectively.

The high infection and density rates in the children from fishing villages was possibly due to the use of preferred defaecation sites, walking barefoot and drinking unprotected well water. Despite improved hygiene, diet and health, the elite cildren experienced a relatively high frequency of *Ascaris* and *Trichuris* infection but this was combined with lower egg counts. Exposure to faeces on the roads (and pavements) in the local neighbourhoods although not in the immediate living area may facilitate this pattern of high frequency/low density transmission.

Health education seeking to decrease helminthiases should attempt to define the epidemiology of each major population segment. Programmes could then be specifically designed for each community to target the main pathways of parasite transmission which may vary from group to group. Such a direct approach may be more successful and cost-effective.

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