Aspects of the intensity of infection of *Ascaris lumbricoides* (Nematoda)

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ABSTRACT

Various aspects of the intensity of infection of Ascaris lumbricoides were investigated, in particular trends in intensity of infection and the impact of high worm burdens on human populations. It was concluded that generative mechanisms of intensity trends cannot be determined precisely unless the role of the immune response is clearly defined. Acute pathology due to high worm burden is well documented. The public health significance of the larval migratory stage has not been fully determined due to difficulties inherent in its measurement. The role intensity of infection plays in nutritional disturbances and thus malnutrition has not been fully assessed. Transmission dynamics are affected by intensity, with those most heavily infected shedding the highest number of eggs into the environment. Chemotherapeutic control measures must also take intensity trends into account to determine the best protocol to use.

The methods used to measure Ascaris intensity were reviewed. Egg counts are the most common method being simple, inexpensive and having little impact on host lifestyle. The relationship between faecal egg count and worm burden was studied using a large data set (n = 681) of corresponding egg and worm counts from different areas of the world. It was concluded that the observed relationship between egg count and worm burden was very variable. However, egg counts may be of use as a semi-quantitative measure of intensity as after some manipulation of the data to reduce variation some correlation between egg count and worm burden could be ascertained.

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I declare this thesis to be entirely my own work apart from those who have contributed above.

This thesis is dedicated to those who still suffer Ascaris infection.

CONTENTS

CHAPTER ONE : INTRODUCTION TO ASCARIS LUMBRICOIDES

Ascaris lumbricoides is a dioeious nematode which as an adult infects the small intestine of approximately one billion people around the world (Crompton, 1988). It is an infestation common in countries where poverty, poor sanitation and poor health education prevail.

The worm has a direct life history pattern; it is transmitted from host to host without the need for an intermediate host or vector. Humans are infected by the ingestion of infective eggs containing L2 larvae. When the fully embryonated eggs of A. lumbricoides are swallowed and have hatched in the lumen of the small intestine, the larvae bore through the small intestinal wall, enter vessels of the hepatic portal system and reach the liver. After about four days, survivors move on, some reach the lungs and then after a further 10 days the larvae return to the small intestine via the bronchii, trachea and oesophagus. The larvae undergo three moults during the course of tissue migration, from the L2 larvae to the L5 (juvenile) stage in the small intestine and then develop to the sexually mature adult form (Crompton, 1989a) (see Figure 1.1).

There has been some debate regarding the relationship between Ascaris lumbricoides found in humans and Ascaris suum which commonly infects pigs. It has been suggested that both are either variants of the same species or different species (Crompton, 1989a). For the

purpose of this thesis the two are considered to be separate species.

Infection status within a host population is usually measured by two parameters; prevalence and intensity. Prevalence is defined as the proportion (usually expressed as a percentage) of people within a community infected with the worm while intensity of infection or worm burden is defined as the mean number of worms haboured per infected host. A detailed review of Ascaris prevalence has been given by Crompton (1989b).

The intensity of infection depends on one of two populations which govern the population dynamics of the infection. The first population is the number of worms haboured by the host (Anderson, 1986). This number will depend on the gain of the worms (ingestion of eggs) and the loss of worms (worm death, host immunity, removal by chemotherapy). Likewise the second population (the number of infective eggs in the environment) will rise with gains (from eggs shed by infected individuals) and will decline with losses (egg death and ingestion of eggs). The rates of gain and loss in both populations can be influenced by many factors.

The aims of the work described in this thesis are to examine trends in infection intensity of Ascaris lumbricoides in human populations and to examine the importance of studying intensity from the viewpoints of pathology, transmission and control. Methods used to measure intensity are also examined with regard to both indirect and

direct techniques. Finally the usefulness of the relationship between egg count and worm burden is examined with a view to providing practical guidance about intensity for field workers. In this context, usefulness is concerned with the application of knowledge for the prevention and control of ascariasis in human populations. Ascariasis is the name assigned by the World Health Organisation for the acute and chronic disease syndromes in humans that are initiated by infection with A. lumbricoides.

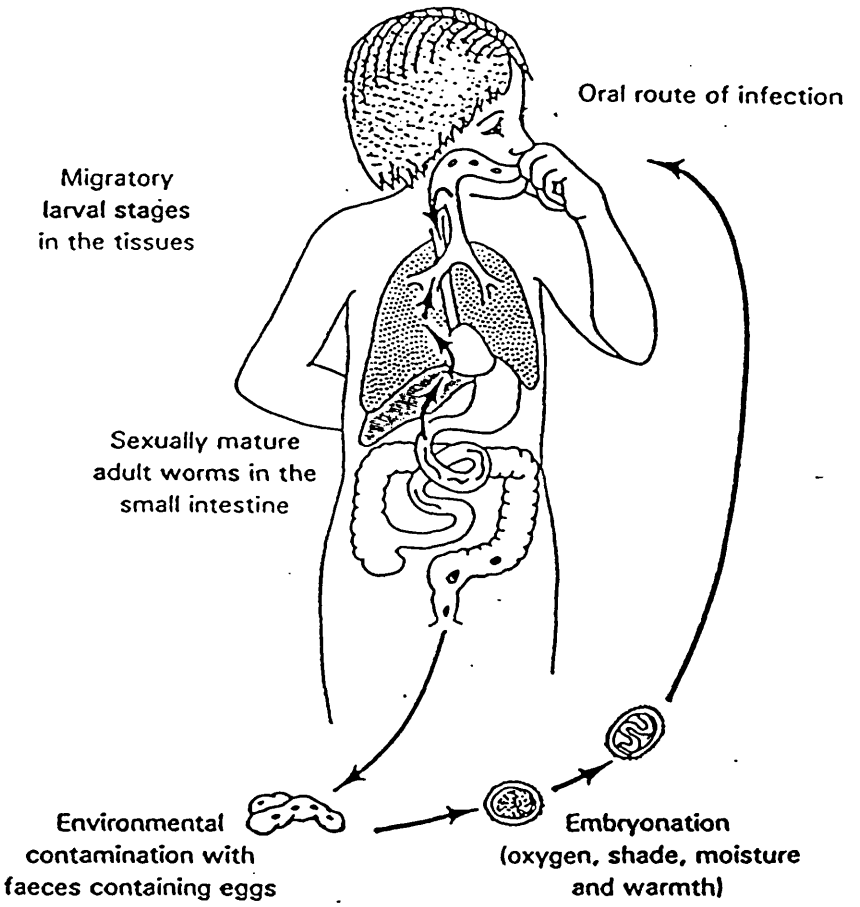


FIGURE 1.1 : Life cycle of Ascaris lumbricoides

(From Crompton 1989a)

CHAPTER TWO : TRENDS IN INTENSITY OF INFECTION OF *ASCARIS LUMBRICOIDES*

2.1 Introduction

Studying intensity of infection patterns of a helminth like *Ascaris lumbricoides* is important because it yields valuable information about the infection and its morbidity and transmission. This can then be used to identify people at risk and implement control procedures.

2.2 Frequency distribution and predisposition to infection

2.2.1 Frequency distribution of numbers of worms per host

The frequency distribution of numbers of *Ascaris lumbricoides* per host in a population tends to be highly aggregated, with few individuals harbouring high worm burdens and the majority harbouring light infections (see Figures 5.1 - 5.4, Chapter 5). It is an ubiquitous trend observed in many helminth infections in hosts where the parasites do not multiply (Anderson 1982).

This distribution has also been called overdispersed and can be described mathematically by the negative binomial distribution model (Bliss and Fisher, 1953; Crofton, 1971). The parameter k, also

known as the aggregation parameter, which can be calculated from fitting frequency distribution data to the negative binomial, gives an inverse measure of the degree of aggregation (Anderson and May, 1985). As k approaches zero, parasite aggregation within the host population is severe. A higher k value (>5) means parasites are observed to be less aggregated and tending towards a random distribution (Anderson, 1989a). Another simpler means of investigating the frequency distribution of number of worms per host is to calculate the variance to mean ratio (mean number of worms per host). Values considerably greater than one are again indicative of an overdispersed or aggregated frequency distribution (Anderson and Gordon, 1982).

The overdispersed frequency distribution is important from the perspective of an individual because of the increased risk of morbidity, mortality and nutritional disturbances associated with high worm burdens (Bundy, 1988; Croll and Ghadrian, 1981; Crompton, 1989a; see Chapter 3). From an epidemiological standpoint, parasite aggregation means that a large number of hosts should be examined in order to obtain accurate information about the infection from the study population (Anderson, 1982). The overdispersed frequency distribution is also important on theoretical grounds for the regulation and stability of the parasite population in a population of hosts. If density dependent constraints on fecundity and survival exist, it would mean that the majority of the parasite population will be under such constraints and their egg output into the environment reduced (Croll et al., 1982).

2.2.2 Epidemiological studies involving patterns of overdispersion in a Ascaris lumbricoides infected population

As overdispersion is a well known epidemiological trend in Ascaris infection, it is the object of this section to deal with the patterns the aggregation parameter k exhibits in relation to certain host variables. Some recent epidemiological studies conducted on Ascaris lumbricoides frequency distributions are summarised in Table 2.1.

A study by Croll et al., (1982) in Iran calculated k to be 0.5 - 0.8 in a population infected with A. lumbricoides, with no change in aggregation observed between different age groups. Likewise, Thein Hlaing et al. (1984) in Burma and Martin et al. (1983) in Bangladesh found k to be 0.6 and 0.44 respectively with no change in the degree of aggregation between age classes. This position was not found by Bundy et al. (1987b; 1988), Haswell-Elkins et al. (1989) and Elkins et al. (1986) who all found that the frequency distribution of worms was age-related; highest aggregation occurring in the younger age classes. Although, the degree of aggregation was observed to have decreased in older age classes, distribution still remained overdispersed within each age class (Table 2.1).

Guyatt et al. (1990) conducted a study in which values of k were

analysed from various studies over a wide geographical range. They found that the value of k remained highly conserved between studies despite coming from very disparate host populations with a wide variety of environments and cultures.

2.2.3 Predisposition

Investigation of the overdispersed frequency distribution of worm numbers per host has led to the theory that individuals may be predisposed to particular intensities of infection with A. lumbricoides. Predisposition is defined by Keymer and Pagel (1990) as the extent factors in the host (i.e. genetic/immunological determinants) or host life style (social, behavioural or enviromental factors) influence intensity of worm infection.

2.2.4 Evidence of predisposition

Most evidence for the occurrence of predisposition has been compiled from longitudinal studies measuring rate of reinfection in individuals following expulsion chemotherapy. The presence of predisposition is determined by the statistical comparison of pre-treatment and post-treatment worm and egg counts (Anderson, 1986; Haswell-Elkins et al., 1987), assuming that high egg counts reflect high worm burdens (see Chapter 5).

Haswell-Elkins et al. (1987) studied the worm counts of 174 individuals before and after chemotherapy over an 11 month study period in rural South India. They determined that those people who harboured heavy or light infections re-acquired equivalent degrees of infection intensity relative to the average level within the population. Elkins et al. (1986) measured egg counts of A. lumbricoides from a population after chemotherapy over a period of 6 months. At the time of chemotherapy the population was divided arbitrarily into "non-wormy" (those that harboured less than 17 worms) and "wormy" (those that harboured more than 16 worms). These who were "wormy" or "non-wormy" tended to re-acquire heavier or lighter infections (as measured by egg counts). It was also noted that there was a greater tendency toward predisposition in females and the younger age classes.

Greater predisposition in females was not found in a study conducted in Nigeria by Holland et al. (1989). Predisposition was observed in individuals over 3 worm counts, 6 months apart (total study period one year). Worm burdens were again divided into heavy, medium and light and the data examined using a transition matrix which recorded changes in reinfection rate between the different phases of treatment. The matrix showed that although a high proportion of the children shifted from the heavily infected category to the lighter infection categories, a sizeable proportion still remained heavily infected.

Thein Hlaing et al. (1987) working in Myanmar (formerly Burma) found

that after chemotherapy a more rapid return to pre-control levels of intensity was observed in children and "wormy" people than in adults and "non-wormy" people. In addition, predisposition on an individual basis to high or low worm loads was also observed. Likewise, Bundy et al. (1987b) found predisposition to Ascaris infection over an interval of 17 months before and after chemotherapy. The degree of the predisposition, however, was independent of host age. Hall et al, 1992 found predisposition to heavy and light infections in a study involving 880 people from Dhaka, Bangladesh.

In contrast to the findings of the above studies, Croll and Ghadrian (1981) failed to detect evidence for predisposition in a population in Iran one year after anthelmintic treatment. This was possibly due to use of small sample sizes.

Keymer and Pagel (1990) analysed the results of several predisposition studies involving different species of helminth. They noted that in many cases the statistical values measuring the extent of the predisposition were very low. This meant that only a proportion of the data was included in the analysis. This, the authors noted, coupled with an inadequate length of time allowed by observers for reinfection, may act to weaken the evidence for predisposition.

2.2.5 Generative mechanisms of predisposition and overdispersion

Investigation of generative mechanisms has concentrated on two area of research. Firstly, that predisposition is caused by some behavioural factor which consistently influences individual exposure to infective eggs. Secondly, genetic factors inherent in every individual perhaps operate through the immune response to influence worm burden.

Wong et al. (1988) discovered that the practise of geophagia (eating soil) varied in a population of children in two children's homes in Jamaica. The frequency distribution of this practice was overdispersed with 20% of individuals ingesting 60% of the total soil consumed. It is tempting to suggest that this could be a contributing factor for the generation of predisposition, but whether the same children who exhibited extreme geophagia were the same as those that had high worm burdens was not demonstrated. Why the degree of soil contamination was not compared directly with worm burden was not mentioned, it surely would have been valuable to do so.

Amount of exposure is a result not only of the quantity of soil ingested, but also of the degree and pattern of egg contamination in the soil. Wong and Bundy (1990) quantitatively assessed the degree of soil contamination by A. lumbricoides and Trichuris trichiura eggs at the same children's homes referred to above. The prevalence and intensity of infection was calculated at both homes by treating

the children with anthelmintics and counting worms passed. An examination of the soil over a two-month period showed that the home with the higher infection level (36% prevalence) had the higher soil contamination. Egg distribution in the soil around this home was overdispersed. Not surprisingly the home which had the lower infection level (8% prevalence) had a lesser degree of soil contamination and the distribution of eggs in the environment was underdispersed. Unfortunately, the authors did not give details about the frequency distributions or intensity of Ascaris infection in the children within these homes at that time. The underdispersed egg distribution was postulated to be caused by low soil contamination whereas overdispersed egg distribution was caused by eggs being deposited in clumps within the faeces; only becoming dispersed during faecal degradation. This means a child could be exposed to a high number of eggs just by playing in one area. An underdispersed worm frequency distribution pattern could therefore be caused by random exposure to widely scattered eggs in the environment.

Wong et al. (1991) followed these two studies by determining the rate of exposure of children to Ascaris eggs. This was calculated by multiplying the rate of soil ingestion by the density of eggs in the soil. The rate of exposure was estimated to average 9-20 Ascaris eggs ingested per child per year, with evidence of overdispersion, i.e. a few children ingested many more eggs than others. This overdispersed rate of exposure was significantly related to worm burden at one of the homes but not at the other.

The home without the significant relationship was the one with the overdispersed frequency distribution of eggs in the soil. The home that had the significant relationship between rate of exposure and worm burden had the more randomly dispersed egg distribution in the soil.

The authors explained the difference in relationship as being due to a number of factors. Perhaps because the eggs in the soil were clumped in the first home, rate of exposure does not only depend on rate of ingestion but whether the soil came from heavily or lightly contaminated areas. Alternatively because there was no correlation between rate of exposure and infection status in one home and a poor correlation in the other, there may be some other determining factor in operation, such as individual host differences in the effectiveness of the immune response.

Forrester et al. (1990) investigated the occurrence of predisposition involving A. lumbricoides infection within families in Mexico. They found that predisposition was only significant in families with a large number of members. This suggested that an exposure mechanism rather than a genetic/immunological one operated to cause predisposition. It is possible that differing infection intensities between families could be caused by variations in types of sanitary arrangement from household to household (Bundy, 1988). However, Feachem et al. (1983) failed to discover any positive association between different sanitary infrastructures and infection status of people with A. lumbricoides and other gastrointestinal

parasites. The authors stressed that these results could be due to low sample size and low compliance rates by the population in their study.

Genetic differences in immune responses to gastrointestinal helminth infections has been observed in a number of animal models. By using specially inbred mice, studies have demonstrated how genetic variability in the genes linked to the mouse Major Histocompatibility Complex (MHC) can affect the progress of infection (Wakelin, 1984). MHC gene products regulate T-cell action, and T-cell mediated responses are important in the generation of immunity to helminth infections (Wakelin, 1985). Wassom et al. (1987) determined that mice which express particular Class II MHC antigens (involved in the presentation of antigen to T-cells) on antigen-presenting cells are more susceptible to infection with Heligmosomoides polygrus (=Nematospiroides dubius) and Trichinella spiralis. Mice express I-A and I-E Class II antigens on antigen-presenting cells. In some inbred strains of mice, I-E molecules are not expressed but these mice still have an effective immune response. The authors determined that expression of I-E molecules increased susceptibility to infection by inducing the production of suppressor T-cells. These inhibit normal effective responses induced by the expression of I-A molecules (Wassom et al., 1987). Genetic control of immunity to helminths and nematodes has been thoroughly reviewed elsewhere (Wakelin, 1985; Kennedy, 1989).

Kennedy et al. (1986) studied the responses of mice of different inbred strains to A. suum excretory/secretory (E/S) antigens. Each strain of mouse was found to differ in their antibody recognition of A. suum antigens. Only those mice with identical MHC haplotypes had the same recognition patterns. Tomlinson et al. (1989) exposed different inbred mice strains to E/S A. suum antigens and found that no one strain recognised all the components of the E/S antigen mixture. Using congenic mice (mice which differ only at the MHC locus and have identical genotypic backgrounds or mice which have identical MHC haplotypes but different genotypes) they determined that the mice with identical MHC and different genotypes had identical recognition patterns of A. suum antigens but those mice who had different MHC backgrounds responded heterogenously. Only one strain responded to a 14 kD component of E/S antigens by producing IgG. Crossing strains and examining antigen recognition profiles of the progeny did not show predictable results. The results of this study illustrate the complexity heterozygosity imparts to the immune response; presumably similar complexity exists with human hosts.

Humans have also been shown to react heterogenously to A. suum antigens (Kennedy, 1989). E/S antigens from lung-stage larvae were immunoprecipitated with sera obtained from people infected with A. lumbricoides. Each individual exhibited a different precipitation pattern when analysed by SDS-PAGE methods (Kennedy, 1989). Ascaris lumbricoides - infected subjects in a study by Haswell-Elkins et al. (1992) also showed considerable variation between individuals in the

type and quantity of antibody response to A. suum larval antigen. These authors also demonstrated that a strong positive correlation existed between the level of antibody response and egg counts taken four months later. This result suggested that the antibody response against the A. lumbricoides infection was not protective in nature as the larval worms inducing the response had obviously gone on to be adults. This, they argued, meant that differences in infection intensities between age groups and individuals is likely to be caused mainly by the amount of exposure to infective eggs.

Bundy (1988) discussed the result of a study in which an association was sought between human HLA (MHC) antigen frequencies and intensity of infection with A. lumbricoides and T. trichiura. Comparison of intensity with the frequency of expression of Class II molecules revealed a higher frequency of antigen DQW2 in those members of the population who were infected. DQW2 antigen was postulated to play a role in the determination of susceptibility as it is considered to be the marker for coeliac disease, a disease associated with types of T-cell in the gut. A larger number of individuals were examined for association between the frequency of expression of Class I molecules and intensity of infection. A significant relationship was determined between intense infection and an uncommon antigen called B14/BW55. It is a marker for IgA deficiency and thus could result in increased susceptibility. However, Bundy (1988) stressed that the results from these studies were preliminary and further assessment was required. This study also proposed that differences in individual or familiar nutritional

status may be indirectly predisposing individuals to heavy or light infections. Bundy et al. (1985a, b) found a significant inverse relationship between plasma zinc deficiency and the intensity of T. trichiura infection. Zinc is required for thymus mediated immune responses (Golden et al., 1977).

Holland et al, 1992 selected three groups of Nigerian children infected with A. lumbricoides and classified them as uninfected, lightly infected and heavily infected. A blood sample was taken from each of these children and analysed for HLA type 1 antigens. Comparing the heavily and lightly infected groups with the non-infected group revealed a significant difference in the frequency of an antigen called A30/31. The frequency of this antigen was higher in those children infected with A. lumbricoides and never found in the uninfected children. The authors, however, pointed out that the sample size used was small (n = 82) and therefore the analysis should be conducted on a larger sample to obtain more useful results.

The mechanism by which MHC-restricted responses might act to influence resistance and susceptibility has not been determined (Kennedy, 1990). Most work has concentrated on antigen recognition profiles, little being done to determine and define the role of T-cells. As T-cells are involved to a great extent in the immune response to helminth infections like Ascaris, including the regulation of the B cell response, this balance should be rectified in future work.

2.3 Density dependence

2.3.1 Introduction

Density dependence is an ecological concept which describes the constraints imposed on population size by availability of food and resources leading to competition between individuals (Anderson, 1982). If competition becomes too intense, individual growth, longevity and fecundity will decrease and population numbers will fall. The concept can be extended to encompass intestinal helminth infections within the gut, as ultimately each worm will have to compete for space (there is only limited space in the intestine) and nutrients (which will be affected by the quality and quantity of host diet). In addition the level of response by the host's immune system to the infection might be dependent on the number of worms present (Keymer, 1982).

Density dependent constraints on worm fecundity are possibly largely responsible for observed stability in intestinal worm populations. Overdispersed frequency distributions of worm numbers/host might add to this regulatory force (Keymer, 1982). If the parasite population is under severe density dependent constraints and parasite numbers are aggregated then the proportion of infective stages entering the environment will be regulated (Croll et al., 1982).

The study of density dependence in A. lumbricoides infections has concentrated on comparison of worm load with various worm parameters, such as worm fecundity and size (Croll et al., 1982; Martin et al., 1983; Thein Hlaing et al., 1984; Elkins et al., 1986; Holland et al., 1987; 1989).

2.3.2 Epidemiological studies

Thien Hlaing et al. (1984) in Burma found that egg per gram per female worm (epg/fw) decreased with respect to worm load until the intensity reached 10-14 worms. Thereafter the per capita rate of egg production remained constant with increasing worm burden. Martin et al. (1983) discovered a similar result in Bangladesh, although they determined that there was no density dependent effect on worm size. In Panama, Holland et al. (1987) found a density dependent effect on worm fecundity although their results were less pronounced than the above. This was attributed to a scarcity of male worms in low worm burden classes masking any observed effects of density dependence. This study in Panama also determined that there was no relationship between worm burden and worm biomass.

Holland et al. (1989), working in Nigeria with large samples, found a decrease in the per capita rate of egg production as worm burden increased. The authors suggested that host age should be taken into account as they noted that different age groups had different

average egg per gram per female worm values. Elkins et al. (1986) and Croll et al. (1982) also showed density dependent decreases in worm fecundity.

There has recently been some debate regarding the validity of egg counts in studies such as these, with one review emphasising that care should be taken with the interpretation of data obtained from egg counts (Keymer and Slater, 1987). Egg counts are well known for their inconsistency and variation (see Chapter 5). The authors stressed that density dependent effects must be in operation because of observed stability in helminth populations and because the relationship between helminth fecundity and intensity is very consistent. Michael and Bundy (1989) carried out a study using the mouse-worm model for Trichuris infection in humans and investigated the validity of Trichuris muris egg counts as a measure of density dependence. They determined that egg counts, although exhibiting a degree of daily variation, were a product of density dependent effects. They also observed a relationship between intensity and female worm size; as worm numbers increased, worm biomass decreased, suggesting a density dependent effect. In addition, a relationship was observed between female worm size and egg production suggesting a possible causative effect of density dependence on egg production. The causes of density dependence was postulated to be competition for finite resources in the gut. The mice had been rendered immunotolerant to T. muris infection, minimising the potential role of the immune response in generating density dependence.

The relationship between worm burden and worm biomass has also been investigated in A. lumbricoides infection in humans (Elkins and Haswell-Elkins, 1989). A population was treated by anthelmintics twice, the second time 11 months after the first. A total of 3505 worms recovered after the first treatment were measured for length and weight and compared with worm burden. No inverse relationship was detected between these variables leading to the conclusion that there were no apparent density dependent constraints on worm size. This is consistent with a number of other field studies (see above). The sizes of the worms, however, showed considerable variation related to host age, with children harbouring a higher proportion of smaller worms and adults harbouring larger worms. In addition, worms collected and measured after the second round of treatment were larger and more homogeneous in size than those collected after the first, especially in the most heavily infected group including the children. The authors postulated that these results fit a hypothesis proposed by Jung (1954), where adult worms somehow (via hormonal signals or by stimulating the host immune response) prevent the establishment of younger worms. Children had a higher proportion of smaller worms because a high rate of infection made conditions unfavourable for worms to grow to full size. After treatment, however, the first worms to be established grew to their maximum size which then prevented younger worms from becoming established.

2.3.3 Causes of density dependence

Possible causes of density dependent effects on worm fecundity fall into two categories. First, intraspecific competition for limited resources of food and space within the gut impose constraints on worm fecundity. It would be interesting to determine if density dependent effects on egg production were more severe in small hosts or those hosts with inadequate diets; such work could be done with pigs harbouring A. suum.

A second possible cause of density dependence is that an acquired immune response to Ascaris could reduce or even inhibit worm fecundity, the size of the response increasing as the degree of antigenic stimulation (i.e. worm load) increases (Anderson and May, 1985; Keymer, 1982). The immune response could work against worms by triggering inflammatory responses in the intestine resulting in the expulsion of worms or, by perhaps making conditions unfavourable for nutrient uptake by the worms forcing female worms to cut egg production.

2.4 Age-intensity trends in A. lumbricoides infection

2.4.1 Introduction

Observing how the intensity of infection of A. lumbricoides changes with age is important because it allows predictions to be made about

the force or rate of transmission within a community (Anderson, 1986). It also may be able to determine whether a particular age class is more at risk from heavy infections.

2.4.2 Epidemiological studies

In most epidemiological studies the observed age-*A. lumbricoides* intensity curve shows a peak intensity in the 5-15 year old group. Haswell-Elkins *et al.* (1987) found that peak intensity was reached in the 4-8 year age class, after which intensity fell away with increasing age. Likewise, Elkins *et al.* (1986) observed that 5-9 year olds harboured the highest worm loads, intensity again falling away as age increased. Thein Hlaing *et al.* (1984) also found the highest worm burdens in 5-9 year olds. Bundy *et al.* (1987b) in St. Lucia determined an age-intensity curve with a peak intensity of 6.5 worms per person in 5-10 year olds. All these studies used worm counts following chemotherapy to determine the intensity of infection.

One study measuring intensity by egg counts showed a peak occurring in the 1-6 year old age group (Bundy *et al.*, 1988).

However, in contrast two studies in Iran show age-intensity curves in which the peaks were not found in the childhood age range. Croll *et al.* (1982) observed that the age-intensity profile rose to its peak at 10-14 years and then remained constant throughout the

remaining age-classes. Arfaa and Ghadrian (1977) discovered that in 5 of 6 villages they examined, the intensity of infection rose with increasing age.

2.4.3 Generative mechanisms of age-intensity trends

A change in the rate of parasite acquisition could result in the observed age-intensity patterns with some age-related behavioural factor influencing the amount of exposure to infective eggs with age. Children have a higher incidence of geophagia (eating soil) than adults, whereas adults have better personal hygiene habits (Halstead, 1968). Wong et al., (1988) studied patterns of geophagia and T. trichiura infection within the same community. The amount of geophagia that occurred was measured by the silica that was present in the stool. In children the amount of silica from soil in the stool exceeded the level that could be attributed to diet whereas in adults diet alone was responsible for stool silica content. Studies of T. trichiura age-intensity patterns in the same community indicated that as the incidence of geophagia increased so did intensity of infection. As geophagia decreased, however, (as in adults) so did T. trichiura infection. It was not suggested that this was wholly responsible for the observed age-related curve but it may contribute towards it (Bundy, 1988). It may be that something similar exists for A. lumbricoides especially as both helminth infections are often associated with each other and depend on essentially the same mode of transmission (Robertson, 1989; Booth

and Bundy, 1992).

Increased parasite mortality could occur in older age-classes if there was an acquired immune response, the shape of the age-intensity curves reflecting the result of a higher past experience of infection in hosts of older age classes (Bundy, 1988). However, the presence of a protective immune response has not been determined as yet. Haswell-Elkins et al. (1992) measured exposure to infection by antibody response to larval Ascaris antigens and determined that exposure to infection varied with host age. They also determined that the presence of antibodies to larval antigens did not confer effective immunity; egg counts four months after the collection of blood samples correlated significantly with the amount of antibodies produced to larval antigens. This suggested that all the larval worms in the host body achieved maturation in the small intestine.

2.5 Conclusions

1. It has been suggested that overdispersion, predisposition and age-related changes in intensity are caused by exposure rates and/or individual differences in immune response to Ascaris lumbricoides. However, studies have still not shown convincingly whether exposure or immunity cause these trends. It might be useful to conduct more work on the relationship between

overdispersion, predisposition and age-intensity patterns. For example how much does predisposition change with age? Does predisposition cause overdispersion or vice versa? If the relationship between these three variables could be addressed perhaps patterns could emerge allowing the roles immunity and exposure play to be determined.

2. Resolution of these issues is not helped by the persistent use of unreliable egg counts. Egg counts are extremely variable (see Chapter 5) and whether they can be used to compare results from different epidemiological studies must be questioned. They may be masking trends in Ascaris epidemiology because of their extreme variability.

3. Perhaps generative mechanisms for the trends discussed in Ascaris population biology could be addressed by determining the presence or absence of an effective immune response. The immune response by humans to a gastrointestinal helminth infection like A. lumbricoides seems to be characterised by a dependence on the degree of antigenic stimulation (dependent on accumulated past experience of infection), an ability to elicit only protection against reinfection and a transient efficacy (Anderson and May, 1985; Wakelin, 1984). It has been suggested that if an effective

immune response to gastrointestinal helminths like A. lumbricoides does exist, it is slow to arise and provides only partial protection (Wilkins et al., 1984). Whether such an immune response is effective enough to influence worm numbers or worm fecundity (in the case of density dependence) has not yet been determined.

TABLE 2.1 : Field data determining overdispersion in populations infected with *Ascaris lumbricoides*

COUNTRY	SAMPLE SIZE	K VALUE/ V:M* RATIO	INTENSITY MEASUREMENT	RESULTS	SOURCE
St Lucia	113	<1	Worm burden	Higher in very young children then levels out. Although discussion says overdispersion independent of age.	Bundy *et al.* (1987b)
Malaysia	1574	0.21	Egg count	Age dependent over 0-8 age group. Highest aggregation in youngest age class	Bundy *et al.* (1988)
India	224	0.8	Worm burden	Higher in young children, lower in older children and young adults.	Elkins *et al.* (1986)
Panama	203	10.2	Worm burden	Children 3-5 years	Holland *et al.* (1987)
Nigeria	808	17.13	Worm burden	See text	Holland *et al.* (1989)
Panama	661	0.015	Egg count	Apply to children	Robertson *et al.* (1989)

(* V:M - Variance : mean ratio)

CHAPTER 3: THE SIGNIFICANCE OF THE INTENSITY OF INFECTION WITH ASCARIS LUMBRICOIDES

3.1 Introduction

Aspects of the relationship between Ascaris lumbricoides and its human host are influenced by the intensity of infection (Anderson, 1986). These include morbidity or the severity of the disease and the transmission dynamics of the infection. Transmission processes have major implications for the design and implementation of control programmes.

3.2 Acute morbidity : pneumonitis and surgical complications

3.2.1 Introduction

Pathology attributed to A. lumbricoides infection can be separated into two phases reflecting the stages of the worm's life cycle within the human body. These are the larval tissue migratory phase and the adult intestinal phase. Some pathological consequences of the infection are acute and life threatening while others are chronic and debilitating (Table 3.1).

3.2.2 Tissue migratory stage

In spite of extensive tissue migration, protective immunity and pathology attributed to invading Ascaris larvae appear to be rare or have still not been detected (Pawlowski, 1982; Pawlowski and Davis, 1989). However, there have been reports of pathological effects which seem to be immune-mediated; inflammatory and hypersensitivity reactions have been observed in the liver and lungs during Ascaris tissue migration. Phills et al. (1972) detected elevated titres of both IgE and IgM in two patients, from a group of four, who had been maliciously infected with a massive number of infective A. suum eggs. Both these immunoglobulin types can mediate hypersensitivity reactions; IgE type I and IgM type III. Lungs seem to be the most seriously affected organ with symptoms that can range from a slight cough to severe Loeffler's syndrome (Type III hypersensitivity) (Pawlowski, 1978). Beaver and Danaraj (1958) reported a fatal case of pulmonary ascariasis; the individual concerned had, on post-mortem examination, massive infiltration of the lungs with eosinophils. Gelphi and Mustafa (1967), in a study concerning seasonal pneumonitis attributed to ascariasis, noted a brief history of illness with symptoms of cough, dyspnea and substernal discomfort, the latter exacerbated by deep breathing or coughing. Liver damage is observed in Ascaris suum infections in pigs the condition being known as "milk spots" (Soulsby, 1982). In humans, abdominal pathology due to migrating larvae is reported to be rare (Pawlowski, 1978), although Phills et al. (1972) detected hepatomegaly and biochemical evidence

of transient hepatocelluar damage in their Ascaris - infected patients.

The public health significance of this stage of A. lumbricoides infection has rarely been studied, quantification of symptoms is difficult and ethical considerations prevent investigative measures (Stephenson, 1987). What has been concluded from the community studies that have been conducted, is that symptomatic pulmonary ascariasis is more common when infection is seasonal (Gelphi and Mustafa, 1967). Whereas, when transmission is year-round, such as in the study of Spillman (1975) in Columbia, clear clinical signs of pulmonary ascariasis are uncommon. The seasonal effect has been postulated to be due to a "desensitisation" to larval antigens by the host (Gelphi and Mustafa, 1967).

The existence of hypersensitivity and immune-mediated pathology to invading larvae complicates any conclusion about the relationship between the numbers of invading larvae and the related pathology. Predisposition to high or low intestinal worm burdens (see Chapter 2), if generated by host genetic effects such as immune response to the invading larvae, will result in more pathology to the individual whose immune response manages to eliminate more larvae. This observation is substantiated by Phills et al. (1972), whose two patients with greatest immunopathology passed less immature A. suum worms in their stools after anthelmintic treatment. In addition, it is possible that clinically significant pulmonary ascariasis is only observed in those who are not continuously being exposed to A.

lumbricoides. Additionally, the intensity of invading larvae is presently impossible to estimate accurately, although there may be hope that serological detection of infection may lead to an answer to this (Parkhouse and Harrison, 1989). Thus, it becomes apparent that more studies will have to be conducted to assess firstly, the public health significance of larval ascariasis and, secondly, the role of the immune response in the lungs towards the aetiology of pathology.

3.2.3 Adult intestinal stage

The presence of adult A. lumbricoides in the small intestine causes the most widespread and dangerous pathological consequences of infection. These result either from the migration of adult worms into unusual places in the body or from intestinal complications due to the physical presence of a large number of worms : "The great number of worms intertwine forming a conglomerate that may cause partial or complete intestinal obstruction" (Pinus, 1982). Migration of worms to unusual places or ectopic sites is believed to be more common in single worm or single sex infections (Pawlowski, 1982). Some cases of ectopic ascariasis are given in Table 3.3. An analysis of some of the publications that have dealt with acute ascariasis caused by the presence of adult worms is shown in Table 3.2.

As might be expected, intestinal obstruction is more common in high intensity infections (Pawlowski, 1982). Surgical intervention is often necessary to alleviate the problem, the severity depending on

the number of worms present and on the extent of symptoms such as colic, nausea and vomiting. However, clinical treatment (conservative management) is usually attempted first (Pinus, 1985). Intestinal complications caused by A. lumbricoides include volvulus, and intestinal perforation often leading to peritonitis. If left untreated, such acute complications can result in death (Pinus, 1985).

There are many reports that cite A. lumbricoides as a major cause of abdominal complications leading to hospital admission (Crompton, 1986; Pawlowski and Davis, 1989). Although mortality caused by ascariasis is relatively small compared with the total number of people estimated to be infected - approximately one billion (Crompton, 1989a) - the high prevalence means that the number of people that die as a result of A. lumbricoides infection is still high. Walsh and Warren (1979) estimated the number of mortalities to be 20,000 a year, although Pawlowski and Davis (1989) reckoned the number of yearly mortalities may be as much as 100,000. These figures equal or exceed the numbers of people that die as a result of poliomyelitis, meningitis and typhoid (Pawlowski, 1984). Identification of those with high worm loads thus becomes of major importance. Hospital admission places an economic burden on health care services and, as there are safe anthelmintics to deal with Ascaris infection (see Chapter 4), this situation need not occur and can be avoided.

3.3 Chronic morbidity : impaired host nutrition

3.3.1 Introduction

Recently much attention has been focused upon the relationship between A. lumbricoides infection and its effect on human nutritional status. This is a subject under active research and involves a lively and sometimes controversial debate (Schultz, 1982; Stephenson, 1987; Crompton, 1986; Taren and Crompton, 1989; Crompton, 1992a). Malnutrition, especially in children, is probably the world's major public health problem (Crompton, 1986; Stephenson, 1987). Malnutrition and ascariasis share a common distribution and it has been proposed that ascariasis and other intestinal infections may, under certain conditions, make not an insignificant contribution to childhood malnutrition (Crompton and Nesheim, 1984). It is the purpose of this section of the chapter not only to review the suspected role of ascariasis in malnutrition, but also to relate effects of the infection to worm burden or intensity.

Nutritional status is a term of convenience which is based on the interpretation of clinical signs and symptoms, biochemical measurements and anthropometric assessments. The anthropometric measurements usually involve height, weight, head and arm circumferences and skinfold thickness which must then be related to the subject's age by means of reference standards (WHO, 1986).

Possible ways in which A. lumbricoides infection might act to affect nutritional status include depressing host appetite and disrupting nutrient uptake. Adult worms are positioned in a prime spot to cause such effects - the jejunum of the small intestine where most nutrient digestion and absorption occurs (Stephenson, 1980). The aetiology of specific effects that A. lumbricoides may have on host nutritional status has been reviewed elsewhere, (Crompton, 1986; Stephenson, 1980; 1987), and more generally for helminths in mammalian hosts by Robertson (1989), for intestinal parasites by Rosenberg and Bowman (1984) and for all types of parasites by Taren and Crompton (1989).

Investigation of interactions between A. lumbricoides and human nutrition has involved, firstly, clinical studies in which the effect of infection on a specific nutritional aspect is examined, secondly, a number of studies involving the nutritional status of Ascaris suum - infected pigs, and thirdly, field studies, in which the effects of infection are assessed on the nutritional status of populations measured by changes in growth rates.

3.3.2 Clinical studies

Tripathy et al. (1971) studied ascariasis in twelve children in a metabolic ward who were reported as having moderate to heavy infections. Nitrogen retention was seen to be disrupted in children

who had high worm burdens and low protein intakes. Subsequently, Tripathy *et al.* (1972) examined jejunal biopsies taken from five children heavily infected with *A. lumbricoides*. Mucosal abnormalities in the form of broadening and shortening of villi, crypt elongation and a reduction in the villus:crypt ratio were observed. Cellular infiltrates in the *lamina propia* were also detected which returned to normal levels after anthelmintic treatment. Another clinical study, by Brown *et al.* (1980), discovered that the changes observed in nitrogen absorption in infected children could be, after administration of an anthelmintic, statistically correlated with worm burden. Fat absorption improved significantly in those children who had had high worm burdens. However, carbohydrate absorption and D-xylose excretion did not change significantly between treated and untreated children. Venkatachalam and Patwardhan (1953) examined nine children with heavy *Ascaris* infection and detected a significant difference in the amount of faecal nitrogen excreted before and after anthelmintic treatment, although the intensity of infection was not evaluated in detail.

Studies conducted in Panama on *Ascaris* - infected children involved examination of the effects of infection on specific intestinal functions. Carrera *et al.* (1984) found a significant relationship between worm burden and amount of hydrogen exhaled after children had been given a lactose supplement. The principle behind this technique was that if any disruption of the intestinal mucosal border had occurred (especially if this affected mucosal lactase

activity) then lactose would be metabolised by the anaerobic bacteria further down the gut resulting in the production of hydrogen gas. This could be detected in the breath with the amount of hydrogen produced assumed to be proportional to the amount of lactase disruption. A similar finding was made by Taren et al. (1987), measuring breath hydrogen, who also noted a decrease in intestinal transit time in A. lumbricoides - infected Panamamian children which was inversely proportional to the log of the faecal egg count. The exact effect this would have on host nutritional status was not determined.

A study conducted on the relationship between vitamin A status and Ascaris infection indicated that serum vitamin A in infected children increased after administration of an anthelmintic, although the increase did not appear to be related to worm burden (Mahalanabis et al. 1976). Another investigation concluded that five children with ascariasis had a decreased absorption of vitamin A of the order of 20% (Sivakumar and Reddy, 1975). Mahalanabis et al. (1979) then discovered malabsorption of water soluble vitamin A in both Ascaris- and Giardia lamblia- infected children. Treatment of children to relieve ascariasis alone did not show an improvement in absorption, but treatment of those who had concurrent Ascaris and Giardia infections did. Recently, Taren et al. (1987) discovered a significant association between Ascaris infection and reduced plasma vitamin A concentration in Guayami Indians in Panama.

3.3.3 Pig - *Ascaris suum* studies under experimental conditions

The pig - *A. suum* relationship is a good model for the human - *A. lumbricoides* nutritional relationship as the nutritional needs and digestive tracts of pigs and humans are physiologically similar and the phylogenetic relationships and life histories of *A. suum* are exceedingly close to those of *A. lumbricoides* (Pond and Haupt, 1978; Crompton, 1990).

Stephenson *et al.* (1980a) found that the intensity of infection of *A. suum* in pigs could be correlated with the degree of hypertrophy of the *tunica muscularis*, although the role of this change in the nutritional status of the pigs was not ascertained. The work was developed by Forsum *et al.* (1981) who discovered that growth rate, food intake and mucosal lactase activity were depressed in *A. suum* - infected pigs and that these factors could be correlated with intensity of infections. Martin *et al.* (1984) studied the morphology of the small intestine in infected pigs by means of electron microscopy. The mucosal surface showed evidence of villous atrophy and crypt hyperplasia which was not observed in uninfected controls. It was suggested that these changes may decrease the amount of area available for absorption in the small intestine. In addition, numerous small craters were detected by scanning electron microscopy in the microvillous coat of the enterocytes. The cause of these was not determined, but they were postulated to have been a result of bacterial colonisation of the infected gut; perhaps the presence of *A. suum* renders the pig gut vulnerable to bacterial

invasion.

3.3.4 Field studies

Malnutrition in a human community is likely to be caused by a complicated array of factors whose possible interrelationships are shown in Figure 3.1. Infection with A. lumbricoides would be included in the box labelled "Diseases". The effects of ascariasis on nutritional status are usually studied in children and assessed by measuring growth rates before and after intervention with anthelmintic treatment. The growth rate of children, however, is slow and thus in order to detect a distinct and statistically significant change in growth, the period of observation has to last for an appropriate time (Nesheim, 1985). In addition, human studies cannot obviously assume the controlled environment of the laboratory and must account for heterogeneity between groups in such factors as behaviour, cultural practices and socio-economic status (Mata, 1982) and for concurrent infections or polyparasitism (Pawlowski, 1984). Also, strict ethical conditions must be observed and informed consent should be obtained from the study population. Finally, accurate epidemiological information must be sought about the infection of interest. Factors such as these, and the failure to take them into account, probably explain why contradictory conclusions exist between the accounts from different field studies. Information about the diversity of several field studies has been summarised in Table 3.4 to illustrate differences in study designs and the results obtained.

What stands out from the information in Table 3.4, is the disregard of some field workers to assess the intensity of infection and examine its role on nutritional status. Several authors have argued the importance of intensity in such studies (Latham, 1982; Pawlowski, 1984; Anderson, 1989b; Holland, 1989). In many studies, it is the presence or absence of worms which is considered to be important, with no regard to the stratification or observation of intensity groups. Thus, individuals with high and low intensities have been grouped indiscriminately together when specific consideration would have yielded very much better information.

3.3.5 Discussion

The results from clinical studies of the human-*Ascaris* relationship and the pig-*Ascaris* relationship, can be relatively simple to assess. The experimental design relieves them from many confounding variables and simplifies statistical investigation of the data. It is possible to see that intensity of infection can be correlated with a number of digestive disturbances, the degree of which increase with worm burden. These include changes in the mucosal architecture of both humans and pigs (Tripathy *et al.* 1971, 1972; Stephenson *et al.* 1980a; Martin *et al.* 1984), absorption of nutrients such as nitrogen (Brown *et al.* 1980) and change in mucosal lactase activity in humans (Carrera *et al.* 1984; Taren *et al.* 1987).

Several authors have suggested that the effects of A. lumbricoides infection on nutritional status may only have been observed in those individuals with marginally nutritious diet and with high worm loads (Tripathy et al. 1971; Brown et al. 1980). In individuals with low infections and an adequate diet, Ascaris may be expected to have little detectable effect on nutritional status. A possible mechanism for this is that the presence of worms causes change and disruption of the gastrointestinal tract. The gut may then begin to compensate as a whole organ (Castro, 1989), but as worm burden increases, the amount of damage cannot be offset and widespread disruption of nutrient digestion occurs. This will be exacerbated by the presence of a marginally nutritious diet. The situation results in a detrimental effect on nutritional status. This could also explain the anomalies observed in the results between clinical and pig studies (i.e., specific observations of nutrient disruption) and the results obtained from field studies (e.g., weight loss).

3.4 Transmission

Overall transmission success of A. lumbricoides, and other soil-transmitted helminths, is determined by interactions between the two parasite populations involved in the worm's direct life cycle. Like most other macroparasites, Ascaris does not replicate in numbers once it has established an infection. Each adult worm, therefore, represents a single infection event and each adult female worm is a unit of transmission. Transmission rate is, therefore, a

function of intensity of infection (Bundy, 1988).

The parameter that involves the adult worm population, in particular the female worms, is known as the reproductive rate (R_o). It is defined as the average number of female offspring produced by a sexually mature female worm in the absence of density dependent constraints (Anderson, 1989a). If the reproductive rate can be lowered below unity (when R_o equals unity every mature female worm will be replaced by another mature female worm), the worm population will not be able to maintain itself within the host population (Anderson and May, 1982). R_o can be roughly calculated by measuring the rapidity at which average intensity rises with age from birth, and by reinfection studies which measure the time taken for intensity to return to pre-control levels (Anderson and Medley, 1985).

Identifying those individuals with high worm burdens becomes important as they will shed the highest proportion of eggs into the environment (Croll and Ghadirian, 1981). This will in turn increase the rate of recruitment of new infections in the host population. Eliminating these heavy infections from the population would mean a large proportion of the existing reproducing worm population would be removed which would improve the chances of reducing R_o below unity.

The production of infective eggs also depends on the probability of A. lumbricoides having mated, which requires sexually mature worms

of either sex to meet in the host intestine. A critical average worm burden will exist at which the probability of mating will be too low to ensure that transmission occurs. This is termed a 'breakpoint' and is determined partly by the degree of worm aggregation within a host community (Anderson and Medley, 1985). Although Croll et al. (1982) found this break point to be very low (about 0.3 - 0.5 worms per host) and thus was thought to be of little significance in the regulation of population stability (Anderson and May, 1985). However, several studies have found by reducing prevalence (and thereby reducing worm aggregation), meant that the relative proportion of low intensity infections was raised. This resulted in an increase in the numbers of individuals excreting unfertilised eggs because the probability of successful mating had been reduced. Therefore the reproductive rate (i.e. R_o) was lowered because the release of unfertilised eggs into the environment incapable of establishing new infections was increased (Seo et al. 1979; Yokagawa, 1985).

3.5 Control measures

Control measures should aim to reduce and retain the intensity of infection at a level at which R_o will fall below unity. The types of control which will be dealt with here will be chemotherapeutic strategies.

Accurate epidemiological information about A. lumbricoides infection in a community should be collected before any control measures are implemented (Anderson, 1989a; Thein Hlaing, 1989). There are three different chemotherapeutic strategies commonly in use. These are mass chemotherapy, targeted chemotherapy and selective chemotherapy.

Mass chemotherapy is treatment of numbers on a population scale with entire communities being treated regardless of intensity of infection status, age, sex, or any other socio-biological characteristic. It can be used in areas of endemic infection and high prevalence, be repeated for several years, and ideally implemented along with improvements in sanitation, hygiene and education. The latter measures prevent reinfection occurring as a result of the persistent presence of the environmentally resistant egg and the movement of other infected hosts into the community (Anderson, 1989a). In the absence of repetitive mass treatments, worm burdens will rapidly revert to pre-treatment levels (Anderson and May, 1982). The time taken for the average intensity of infection to return to pre-control levels depends on several factors, the most important being the degree of which the average intensity was depressed (itself dependent on the efficacy of the drug, proportion of the population treated, and time interval between treatments), the magnitude of the reproductive rate and the average lifespan of the adult worms. The time taken for post-control worm burdens to reach pre-treatment levels after one mass treatment in areas of moderate to high transmission intensity, is about one year (Anderson and Medley, 1985). In the field, Thien

Hlaing *et al.* (1987) found that a two or three month gap was needed between mass treatments to suppress worm burdens. Intervals between treatment can be calculated looking at age intensity profiles, as the rate at which intensity rises with age is related to R_o (Anderson and Medley, 1985).

Targeted chemotherapy is applied to groups of individuals defined by some socio-biological characteristic that identifies them as having a greater risk of harbouring high worm loads. For *A. lumbricoides* such groups of individuals include the five - nine-year-old age group who, on average, consistently harbour the highest worm loads in age-intensity profiles observed by several authors (see Chapter 2). As these are mainly children of primary school age, they are relatively accessible for treatment. Bundy *et al.* (1990) conducted a study in which targeted treatment of two - fifteen-year-old children infected with *Trichuris trichiura* was assessed by random sequential sampling on the island of Montserrat in the Caribbean. Intensity and prevalence were observed to fall in the targeted population. Of interest, was the finding of a "subsidiary effect" on the 0 - 0.9 and 16 - 25-year-old groups. The authors postulated this effect to be due either to a reduction in transmission because the major source of environment contamination by infective eggs had been treated, or a 'knock-on' effect of increased awareness of disease by other members of the population, perhaps expressed as increased hygiene levels or self-treatment. A similar effect was noted on *A. lumbricoides* intensity and prevalence although due a low national prevalence (1.8%) it was not so marked.

Selective chemotherapy is applied on an individual basis in the community, with those who harbour high worm loads being identified and subsequently treated. However, selective control is thought to be only effective if the generation of worm aggregation within human communities is caused by long-term factors such as genetic influences rather than short-term factors, i.e., heterogeniety in exposure to infective eggs (Anderson and Gordon, 1982). If predisposition to heavy or light infection is proved to exist during Ascaris infection, and a totally reliable method of determining intensity can be developed, then it becomes an attractive strategy (Haswell-Elkins et al. 1987; Anderson and May, 1982). The costs of identifying those with heavy infections has precluded the use of selective treatment - simulation experiments suggest that for maximum effectiveness of the treatment campaign, heavily infected individuals should be detected at each round of treatment (Anderson and Medley, 1985). Haswell-Elkins et al. (1989), conducted a theoretical study on field data, the results of which suggested that in comparison to selective treatment, age-targeted treatment was most cost-effective causing maximum worm death with the minimum of cost. The targeted population was children and thus easily accessible at school whereas selective treatment required the continued identification of heavy infections at each round of treatment and a good deal of compliance from the population.

Asaolu et al. (1991) conducted a study investigating the efficacy of different control protocols in an A. lumbricoides - infected

population in Nigeria. Four villages were studied, one had mass chemotherapeutic treatment, one selective, one targeted and the fourth served as a control. They determined that mass treatment was the most effective but that targeted treatment showed a reduction in egg counts with a subsidiary effect on the adult population.

3.5 Conclusions

1. It cannot be simply concluded that acute pathology due to larval migration can be related to intensity of infection. The amount of pathology that results may be affected by the heterogeneity in host immune responsiveness, the form of exposure (i.e., whether it is seasonal or continuous) and the history of past exposure to infection. Additionally, we have no reliable means at present of measuring larval intensity, although perhaps serological techniques may solve this problem in the future.

2. Some clinical and pig studies have indicated that several damaging effects on digestive functions can be attributed to the intensity of Ascaris lumbricoides infection, but until improved field studies are undertaken that specifically address the relationship between intensity and nutrition, we cannot assess the full impact of intensity of infection on nutritional

status.

3. Those individuals with heavy infections, as well as being particularly at risk from pathology and nutritional disturbances mentioned above, will also contribute significantly to the transmission of A. lumbricoides as they will excrete the highest proportion of eggs into the community.

4. The main disadvantage of selective chemotherapy is the cost incurred in identifying those with heavy infections. Mass chemotherapy, although wasteful in the sense that even those not infected will receive treatment, does not require the identification of any heavy infections. Targeted chemotherapy for Ascaris has an advantage, because age-intensity curves consistently show that children have the highest infection and are also conveniently placed at school for treatment. It is not surprising that several studies (Bundy et al. 1990; Haswell-Elkins et al. 1989) have concluded that targeted chemotherapy on children is efficient and causes the maximum amount of worm death with minimal cost. However, it should be emphasised that chemotherapy, regardless of treatment tactics, is only a short-term solution and must be supported with improvements in sanitation and health education.

TABLE 3.1 : Manifestations of Ascariasis
(From WHO, 1967)

ALLERGIC ACTION OF THE ADULT AND LARVA :

(a) Substances from adult: - allergic phenomena among laboratory workers

(b) Substances from larva: - cutaneous signs : urticaria, erythematous lesions
- blood eosinophilia
- Loffler's syndrome

(c) Associated infections - complications due to Strongyloides stercoralis and Escherichia coli
- cryptogenetic and malignant eosinophilia

ACTION OF THE ADULT ON THE INTESTINAL TRACT :

(a) Nutritional disorders and enterocolitis of the diarrhoeal type

(b) Surgical forms :

Intestinal subocclusions and occlusions caused by mass of Ascaris - intussusception (in children), volvulus, hernial strangulation (penetration of Ascaris into the loop involved)

Acute mesenteric adenitis

Penetration into appendiceal lumen or into intestinal diverticulum

Postoperative troubles due to movement of Ascaris (colic, peritonitis, fistula)

WANDERING OF THE ADULT :

(a) From mouth, nose, lacrimal fossa or through Eustachian tube to the middle ear

(b) Through glottis (glottal oedema) to trachea or bronchi

(c) Into bile ducts : obstructive jaundice, gall-stones, cholangitis, liver abscess

(d) Into pancreatic duct : acute haemorrhagic or purulent pancreatitis

(e) Migration across tissue walls from intestine to peritoneal cavity and elsewhere

TABLE 3.2 : Analysis of publications dealing with acute Ascariasis (adult worms) mainly during the last decade (Crompton, 1992b)

1. Total number of publications: 258 (21 reporting fatalities).

2. Biliary ascariasis: 99

2.1 Intrusion and obstruction:	60
2.2 Hepatic abscess:	16
2.3 Pancreatitis:	12
2.4 Gall bladder:	7
2.5 Haemobilia:	2
2.6 Gall stones:	2

3. Intestinal complications: 60

3.1 Obstruction:	28
3.2 Appendicitis:	12
3.3 Volvulus:	9
3.4 Intussusception:	6
3.5 Haemorrhage:	5

4. Perforations and complications: 48

4.1 Perforations:	24
4.2 Granuloma	14
4.3 Peritonitis:	7
4.4 Meckel's diverticulum:	2
4.5 Umbilicus:	1

5. Ectopic ascariasis: 26

Blood (eggs)	Encephalopathy	Kidney
Eustachian tube	Lacrimal duct	Urethra
Abdominal skin	Heart	Uterus
Eye	Thoracic cavity	Vagina
Middle ear	Spleen	

6. Acute abdomen: 12

7. Surgical interference: 8

8. Respiratory complications: 5

TABLE 3.3 : Reported cases of ectopic migration of adult *A. lumbricoides*
(Adapted from Pawlowski and Davis, 1989)

Site of migration	No. of cases
Head and neck:	32
Brain (lateral ventricle, pituitary fossa)	2
Vertebral canal	1
Eye (orbit, conjunctiva)	4
Ear (external ear, middle ear, eustachain tube, mastoid)	19
Nose	4
Tonsil	2
Breast:	23
Chest wall	1
Pulmonary artery	2
Trachea, bronchia	10
Lung	2
Cavity of empyema	8
Abdomen:	1307
Liver-biliary tract system and pancreas	861
Liver	788
Pancreas	73
Appendix	356
Others	90
Abscess of abdominal wall, umbilicus, retroperitoneal cavity	16
Operation wound on abdominal region including nephrectomy wound	19
Subphrenic abscess	3
Perforation of stomach or intestine including sutural wound	26
Abdominal abscess, abdominal cavity (site of perforation obscure)	18
Intestinal wall (larvae)	8
Genito-urinary system:	24
Urinary organs (kidney, bladder, prostrate, uretha)	16
Genital organs (adnexa, uterus, vagina)	8
Others:	12
Abscess in other than abdomen	12
TOTAL OF CASES	1398

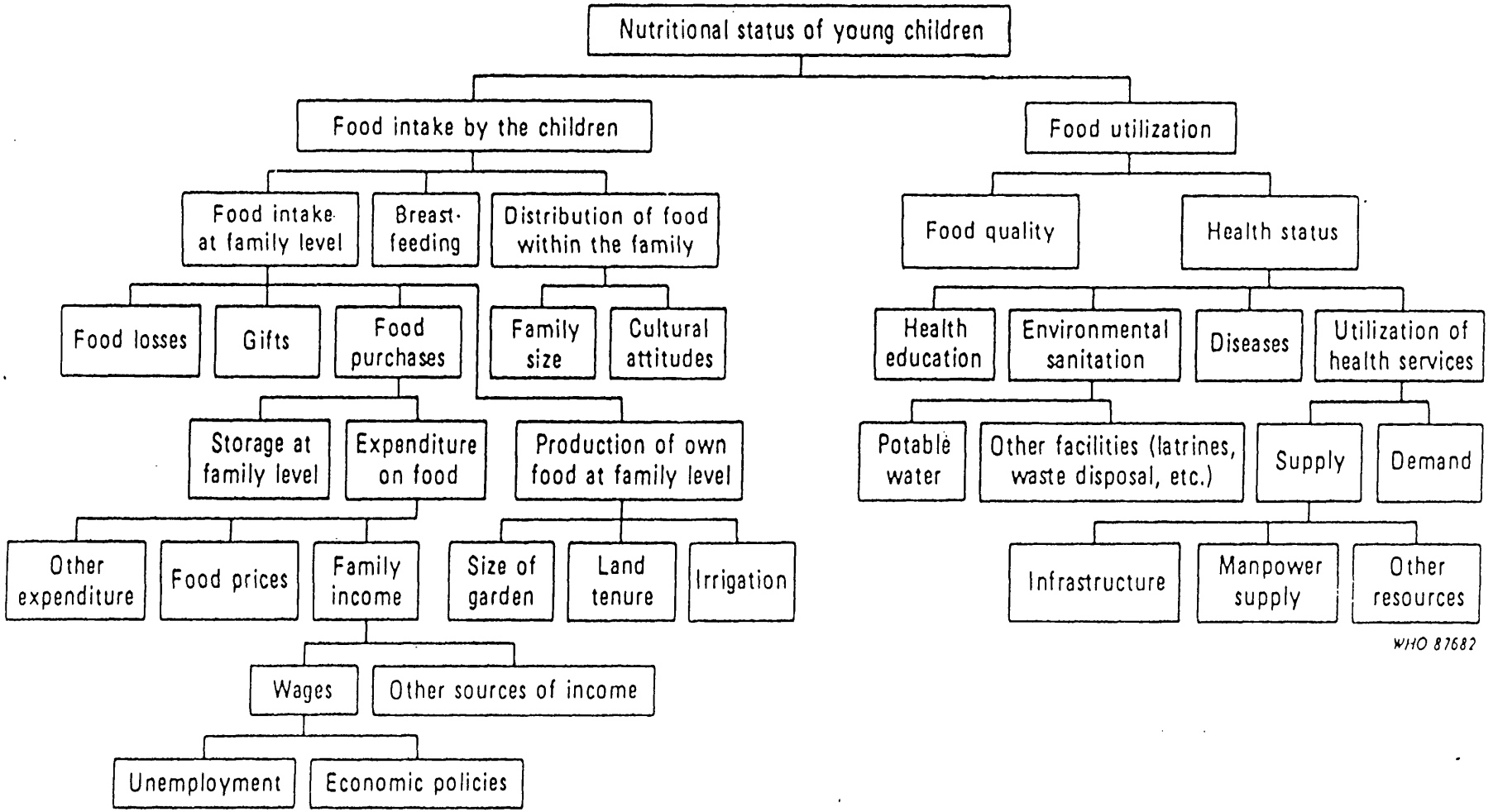
TABLE 3.4 : THE NUTRITIONAL EFFECT OF ASCARIS INFECTION IN FIELD STUDIES (1)

COUNTRY	NO. PEOPLE EXAMINED	HOW WAS NUTRITIONAL STATUS ASSESSED?	WAS A RELATIONSHIP BETWEEN ASCARIS AND NUTRITIONAL STATUS FOUND?	WAS INTENSITY OF INFECTION EVALUATED?	LENGTH OF STUDY	REFERENCE
Tanzania	273 (weight) 263 (height)	Comparison of weight and length gain between treated and non-treated children.	Yes	No	12 months	Willett et al (1979)
Kenya	136	Changes in various anthropological measurement, weight and height, before and after deworming infected children and comparing with controls (uninfected children).	Yes	No	28 weeks	Stephenson et al (1980b)
Guatemala	159	Comparison of changes in weight and height between treated and untreated children.	No	No	1 year	Gupta and Urrita (1982)
Brazil	337	Comparison of increase in weight between treated and untreated children.	No	Yes	10 months	Kloetzel et al (1982)

TABLE 3.4 : THE NUTRITIONAL EFFECT OF ASCARIS INFECTION IN FIELD STUDIES (2)

COUNTRY	NO. PEOPLE EXAMINED	HOW WAS NUTRITIONAL STATUS ASSESSED?	WAS A RELATIONSHIP BETWEEN ASCARIS AND NUTRITIONAL STATUS FOUND?	WAS INTENSITY OF INFECTION EVALUATED?	LENGTH OF STUDY	REFERENCE
Ethiopia	97	Changes in various anthropological and clinical findings after anthelmintic treatment.	No	No	1 year	Freij et al (1979)
Bali	56	Difference in weight for age between infected and uninfected children.	Yes	Yes	1 year	Cerf et al (1981)
Bangladesh	185	Differences between measurement of various anthrometric indicators of growth before and after chemotherapy.	No	Yes	11 months	Greenberg et al (1981)
India	154	Comparison of weight expressed as a percentage of the reference weight for age before and after treatment.	Yes	No	1 year	Gupta et al (1977)
Papua New Guinea	113	Difference in anthrometric haemotological and biochemical findings between uninfected and infected groups before and after deworming.	No	Yes	1 year	Ostwald et al (1984)

FIGURE 3.1 : Malnutrition and Ascaris lumbricoides in a human community (From Beghim et al., 1988)



CHAPTER 4 : THE MEASUREMENT OF INTENSITY OF ASCARIS LUMBRICOIDES INFECTION IN HUMAN HOSTS

4.1 Introduction

The accurate measurement of the intensity of infection of Ascaris lumbricoides in human hosts is important for the reasons discussed in Chapter 3. The direct and indirect methods used to achieve accurate enumeration of worm load encompass faecal egg counts, chemotherapeutic worm expulsion and the latest advances in molecular biological techniques used in the development of seroepidemiological tests.

4.2 Indirect methods : faecal egg counts

4.2.1 Introduction

A number of techniques are in use for the quantitative detection of helminth eggs in human stools (Muller, 1975; Theinpoint et al., 1979). It becomes apparent when reviewing the literature that each research worker prefers a certain technique and will often modify that technique to suit particular requirements. These requirements could include the assessment of epidemiological variables, such as prevalence and intensity, and the worm species under investigation. Of additional importance are the conditions under which the study

will be conducted and the resources and equipment available.

4.2.2 Practical considerations for choice of egg count technique

The measurement of both qualitative (detection and diagnosis of infection) and quantitative (intensity of infection) variables require the satisfaction of slightly different criteria. Measuring qualitatively requires that the eggs are identified only, whereas when calculating the intensity of infection, the technique must be good enough to enable the eggs to be identified and then counted. In addition, the technique must sample a precise quantity of stool so that a comparable figure can be given to the egg count.

As this thesis deals primarily with aspects of the epidemiology of Ascaris lumbricoides, it is the aim of this section to discuss the best stool sampling technique for this species of helminth. Accordingly, the choice of sampling technique should take into account the biology of A. lumbricoides and the host factors that influence the parasite and the release of eggs in the stools.

There are two morphologically distinct forms of Ascaris eggs which can be observed from a stool sample. These are, firstly, the fertilised egg which is oval in shape, and measures 50-70 x 40-50 µm. The second type is the unfertilised egg which is often detected in single sex infections, when young females are present or where there is a biased female:male sex ratio in favour of females. This

is slightly larger that the fertilised egg measuring 60-100 x 40-60 µm, and is more ellipsoid in shape (Crompton 1989a). Both types of egg are usually brown, the colour often thought to be due to the presence of bile pigments in the host's intestine (Crompton, 1989a). However, phenols (which have been identified as the precursors of tanning agents) have been detected in eggs of A. lumbricoides and may be responsible for the colour (Wharton, 1980). Often Ascaris eggs are covered by a mucopolysaccharride coat which is probably responsible for their tenacious adhesiveness (Kagei, 1983).

The number of eggs a mature Ascaris lumbricoides female produces in a day has been calculated by Brown and Cort (1927) to be 200,000 or to average 240,000 (Sinniah, 1982). The number of eggs a female worm produces per gram of faeces has been calculated as 2,000 (Brown and Cort, 1927) and 3,000 (Mello, 1974), although this figure will vary with differing amounts of stool.

Production of such a vast number of eggs will ultimately affect the ability to detect and quantify Ascaris eggs in the faeces. Large numbers of eggs in stools increase the probability of detection - even a single infection of one sexually mature Ascaris female is thought theoretically to produce enough eggs to be detected by one direct smear (WHO, 1967). Since even light Ascaris infections produce many eggs, in heavy infections manual counting of eggs in a faecal sample can become tedious and laborious. This can contribute to human error (WHO, 1967).

Egg counts are usually expressed as egg per gram of faeces (epg) and occasionally measured as eggs per day (epd). The daily count has the disadvantage of requiring another measurement to be taken; the quantity of stool passed in a known amount of time. This is a difficult measurement to make reliably in the field or on a larger scale as the subjects must return all faeces passed over a known period of time to be weighed. This requires extensive compliance from the study population (Crompton, 1989a).

Choice of examination technique should reflect the aims and the conditions of the study for which it is being used (WHO, 1963; WHO, 1964). In a field study, the examination of many stool samples, coupled with small resources and a shortage of technical staff (who may have had little training or access to poor equipment) will necessitate the use of a simple and quick examination technique. A well equipped laboratory with well trained staff may have the resources and expertise for the use of a more involved technique. As conditions and circumstances vary it is impossible to expect results that are uniform for any technique between different studies. A study by Melvin *et al.* (1956) showed that workers examining the same stool samples, produced consistently high or low egg counts.

Theinpoint *et al.* (1979) stress that simplicity of technique is of great importance, and state that absolute accuracy is unobtainable because of the many variables that affect the numbers of eggs in the faeces. This issue is also discussed by Beaver and Yokogawa (1980),

who stated that the chosen technique does not have to be the most accurate but there should be an appreciation of its reliability. They also stress that the ultimate choice of technique should lie with the laboratory that would conduct the examination.

4.2.3 Stool sampling techniques commonly used to study *Ascaris lumbricoides*

Techniques originally developed for one type of measurement are often modified and used for the other. This means it is impossible to define stool sampling techniques as being qualitative or quantitative. For example, Suzuki (1980) describes the direct smear method, the Kato technique and concentration techniques as those to be useful in qualitative study. Conversely Stevens (1978) considered the Kato method as a quantitative technique and WHO (1964) suggested that the direct smear could be used to give an approximation of worm burden. Therefore, in this review there has been no attempt to classify different techniques as quantitative or qualitative tools but to assess each one with respect to the study of ascariasis.

Step by step descriptions of faecal examination techniques are extremely well covered in other work and the reader is referred to accounts given in more detail by Muller (1975), Cheeseborough and McArthur (1976), Theinpoint *et al.* (1979) and WHO (1967). The advantages and disadvantages of various methods have been summarised in Table 4.1.

From the information in Table 4.1, it can be seen that stool sampling techniques can be roughly divided into two types - direct sampling from the stool (direct smear, Beaver's method, the Kato technique and its derivatives) and indirect sampling which include the concentration and flotation techniques, Bell's techniques and Stoll's dilution egg count. The indirect methods involve modifying the stool sample in order to increase sensitivity and reproducibility of egg counts. The more complicated the technique becomes, the greater the likelihood that error will compound on itself to influence the final egg count (Martin and Beaver, 1968). Therefore, there is a distinct advantage in direct measurement of the faecal sample itself.

Although WHO (1967) state that the direct smear may be used for imprecise and relative calculations of intensity, it is obvious that it is not appropriate for a study that requires more accurate measurement of egg counts. This limitation occurs because the exact amount of stool sampled is not measured. Beaver's method utilises photoelectric measurement to determine a uniform smear thickness thus quantifying the stool sample. However, the method requires special equipment and trained personnel (see Table 4.1). It is for these reasons that the Beaver technique is considered impractical for mass surveys (WHO, 1963) where its speed and simplicity would be of greatest use.

The Kato technique and its derivatives - the modified Kato,

Kato-Katz and Quick Kato - are simple, quick and inexpensive on time and apparatus. Review of the literature shows that they are readily used for epidemiological and field surveys for these very reasons. The main advantage of these methods is that they sample direct from the stool and thus are not susceptible to the variations caused by modifying the stool sample (Martin and Beaver, 1968). The Kato techniques assume that eggs in the faeces are evenly distributed (Muller, 1975), but there is still a debate as to whether this is the case. It is prudent to mix stools thoroughly before sampling to ensure a suitably representative sample (Hall, 1982). Hegazi and Abdel-Magied (1980) stated that the Kato technique was not suitable for stools containing large amounts of fibre in them as coarse particles may interfere with the examination. Both these problems can be circumvented by sieving the stool sample through a coarse grid, a procedure that has been adopted for the modified Kato and Kato-Katz techniques. The sieving step acts, in principle, to concentrate the stool sample and certain authors have suggested that the Kato-Katz technique be considered as a concentration method.

Sometimes the amount of stool sampled by these techniques can result in too many eggs to count accurately and the work becomes very tedious and time consuming. This is especially relevant when quantifying a fecund helminth infection like Ascaris. To reduce the counting time, Stephenson (1987) suggested that the number of eggs should be counted for a row of the sample (or a number of microscopic fields) and a multiplication factor applied to determine eggs per gram. From a practical viewpoint, when diagnosing Ascaris

infection, the high number of eggs produced by adult female worms render the concentration techniques redundant, especially when they are seen to be complicated and expensive.

The Stoll (1923) dilution egg count technique, originally devised for counting hookworm eggs, has a long history of wide acceptance (WHO, 1967). It is also useful for quantifying Ascaris eggs because, as a dilution technique, the number of eggs to be counted is much more manageable. It is not suitable for light infections as it does not allow detection of anything less than 200 eggs per gram of faeces (Muller, 1975). The Stoll technique uses a good deal of glassware and is quite involved. However, an advantage of the technique, is that it samples a relatively large amount of stool perhaps compensating for any aggregated distribution of eggs. The technique assumes that eggs are randomly distributed in the dilution flask. Whether eggs are randomly distributed in the stool is a subject that has not been resolved (Martin, 1965).

4.2.4 Studies conducted to assess each technique with relation to A. lumbricoides infection

The prolific egg production of a sexually mature female Ascaris means that detection of even an infection with a single female worm may be relatively easy. Seghal et al. (1977) compared the diagnostic efficiency of the Kato technique against the direct smear, formol-ether concentration and the nigrosine-methylene blue

methods. The authors determined that the Kato technique could detect very light infections and gave the most reliable results. A study conducted by Zamon and Cheong (1967) concluded that the Kato method was superior to the zinc-sulphate flotation method for the detection of Ascaris and Trichuris eggs and the study highlighted the simplicity and low resources required for its use. Pamba and Mulega (1981) concluded that the Kato method was superior to the formol-ether concentration method for detecting Ascaris eggs, but that the reverse was true for infection with hookworm, Hymenolepis nana and Strongyloides stercoralis. Kobayshi (1980) cited a number of studies which found the Kato technique to be suitably sensitive for the detection of Ascaris eggs. However, in comparison, Hall (1982) found the Kato thick smear to be less efficient at detecting hookworm, Trichuris and Ascaris infection. As the results of these studies were not consistent, Hall (1982) questioned the value of any conclusions made from comparative studies outside the circumstances in which they were conducted and emphasised that results may reflect the skill of individual observers.

There appears to be no need to concentrate stool specimens to increase the chances of qualitative diagnosis for A. lumbricoides. Therefore, there is good justification for using the direct smear and Kato methods.

Some workers have conducted studies concluding that the best egg count method to estimate intensity is that which produces the highest egg per gram figure. This may not, however, always give the

most accurate enumeration of egg count. Although Martin (1965) demonstrated that particle distribution in faeces was random, he emphasised that a random distribution is not the same as an even or uniform distribution. Randomness means that there is an equal probability that one egg will come to rest in any given part of the stool, not that eggs are distributed at regular intervals. Sinniah (1982) determined that eggs are not distributed randomly in the stool, and Hall (1981) showed that egg counts from three samples taken from the same stool exhibited a good deal of variation. Thus it is perfectly possible that workers may be examining a sample of stool which contains a disproportionate high or low egg count.

Results from studies using this principle show contradicting results. For example, Vinayak et al. (1978) found the Kato-Katz technique to be superior when compared to the Stoll technique for quantifying infections of various helminths, whereas Massoud et al. (1978) found the Stoll technique to be better than the Kato-Katz in a study involving Ascaris eggs. Nasr et al. (1979) compared the use of one direct smear, three direct smears, one brine flotation concentration method, the Stoll technique and the cleared thick smear (Katz technique). They found that the Stoll technique gave the highest egg count, followed by the thick smear.

Sinniah et al. (1981) conducted a study in which an attempt was made to assess the reliability of the Beaver, Katz and Stoll methods. This was achieved by the comparison of actual worm load against estimated worm load calculated from both the value of the egg counts

obtained from each of the three methods and from an estimation of egg production per day. The results indicated that the Beaver technique gave consistently higher egg counts than the Stoll technique, which in turn gave higher values than the Katz technique. The Katz technique was shown to give the most accurate estimation of worm burden. The study continued to calculate a standardisation factor which could be applied to the egg counts from the various methods and determined whether application of this factor would make the estimated values more accurate. This standardisation factor was calculated from the measurements that Brown and Cort (1927) used to estimate the number of eggs a female worm produced in a day (200,000). In addition, Sinniah et al. (1981) stated that this correction factor relied heavily on the value of the egg count.

A study by Katz et al. (1970) involved the adding of a known quantity of Schistosoma mansoni eggs to uninfected human faeces and then counting the number of eggs by the modified Kato technique. The results showed that the estimated eggs per gram figure was slightly higher than the actual egg count. It was suggested this was due to the slight concentration effect caused by sieving the sample. This experimental procedure would be of interest with relation to A. lumbricoides.

Determining, which sampling technique gives the most accurate estimation of worm burden is a difficult task. Simplicity, accuracy, expense and field conditions are all factors which need to

be addressed. Stephenson (1987) recommended the Kato-Katz technique as the method to use for all soil-transmitted helminths including Ascaris.

4.3 Direct methods - worm expulsion chemotherapy

4.3.1 Introduction

Another technique used to estimate intensity is to count the number of A. lumbricoides expelled from the intestine of the host after anthelmintic chemotherapy. Usually the study population is treated with a drug and then requested to collect all faeces and worms passed in the following 48 hours (Crompton, 1989a). The faeces can then be collected and sieved by research workers to recover the worms. The worms are counted and the numbers of male, female and immature worms recorded.

4.3.2 Common anthelmintics

Common drugs that are used to treat A. lumbricoides infection, and therefore of use in epidemiological studies, are listed in Table 4.2. Obviously a major consideration when choosing the drug is its demonstrated safety; modern anthelmintics have minimal side effects, toxicity having been vastly reduced during the last 30 years (Davis, 1985). An additional consideration is that the drug should have a

high efficacy to ensure that as many worms as possible are expelled for counting.

4.3.3 Advantages and disadvantages

One of the main advantages of counting worms following chemotherapy is that the number of male, female and sexually immature worms can be determined. Worm expulsion results in a more accurate estimation of intensity not being subject to the type of variation observed in egg counts. However, disadvantages arise (particularly in field studies) because extensive participation on the part of the subjects under study is required to ensure that all worms expelled have been collected (Crompton, 1989a). Worms can be lost during the collection and sieving of the faeces (WHO, 1985), and this coupled with the actual treatment also causes a major intrusion in the life style of the population under study.

4.4 Seroepidemiology

4.4.1 Introduction

The use of serological techniques in epidemiological studies of *A. lumbricoides* are uncommon and very much in their infancy. However, they have an important advantage over egg and worms counts. If they could be directed against pre-patent, larval infections they would

yield valuable information about the public health significance of this stage of Ascaris infection.

4.4.2 The immunological host-parasite interface : antibody detection versus antigen detection

There are two approaches to detecting a parasite infection using serology, the first is to detect host antibodies that have arisen as a result of infection and the second is to detect parasite antigens.

The immunological host-parasite interface in helminth infections such as ascariasis, is extremely complex. The host is exposed to a multitude of different antigens (see Figure 4.1). Parkhouse and Harrison (1989) classified the antigens into four groups: developmental stage; antigen compartment within a stage (surface, secreted or somatic antigens); antigenic components within a compartment and epitopes of an antigenic component. The serological response to each epitope will vary in terms of quantity, iso-type affinity, class and kinetics of antibody produced (Parkhouse and Harrison, 1989).

Antibody responses will differ from host to host due to heterogenetic effects on immune responsiveness within a population (see Chapter 2). Consequently, the probability a given parasite antigen will be recognised by all sera obtained from a population of individual hosts, all of which may be at different stages of

infection, will be low (Parkhouse and Harrison, 1989). To circumvent this lack of specificity a mixture of cloned antigens could be used in the assay (Parkhouse and Harrison, 1989), perhaps differentiating between different stages of infection.

The diverse antibody response to parasite antigens observed in and between individual hosts precludes the use of such an assay to estimate the intensity of infection, unless the antibody responses were profiled so that their characteristics were clearly defined throughout the course of infection. Antibody responses are dynamic events; different immunoglobulin types at different stages of the infection being present (Parkhouse and Harrison, 1989). An exhaustive knowledge of the immunoglobulin profiles produced in an infected population might facilitate the production of an assay system which could dissociate between late and early infections. This could result in the construction of a infection profile for an individual which would have important consequences for the epidemiological picture for whole populations of hosts and worms. It could also aid in the determination of immunological predisposition to the parasite both in terms of the resistance to infection and risk of pathology (Parkhouse and Harrison, 1989). For example, if an individual was suffering from severe hepatomegaly, and the host had a particular antibody profile, this information could be used to identify others who might be at risk from this particular pathological symptom.

The use of immunochemical techniques to detect antibodies raised

against the parasite cannot indicate the presence of an ongoing infection. Antibody titres may remain high after a helminth infection has been removed (Parkhouse and Harrison, 1989). Thus detection of host antibodies cannot accurately diagnose infection but can be of use to determine exposure rates to the infection.

The alternative method to detecting antibodies raised against the parasite is to detect directly the presence of parasite antigens within the body. The presence of parasite antigens within the bloodstream of a host would denote an ongoing infection. An immunoassay would probably involve monoclonal antibody technology to produce the antibodies which would detect the parasite antigens. Choice of which parasite antigens to be detected in a serodiagnostic assay for Ascaris could depend on the following:

1. Antigen should be expressed by all worms. Work conducted by Fraser and Kennedy (1991) revealed heterogenetic ES antigen expression in a population of A. lumbricoides worms, 48 hours after hatching and thus at identical stages of development. Earlier, Kennedy et al. (1987), in an experiment designed to examine the antigen characteristics of A. lumbricoides and A. suum, recognised the possibility of differing antigen expression between strains of worms from geographically distinct areas of the world.

2 Antigen should be species specific to avoid cross

reactions with other species of helminths. Some studies have shown that there is significant antibody cross reactions between certain Ascaris and Toxocara canis antigens (Kennedy et al., 1987; Kennedy et al., 1989). Cross reactivity has important implications for the development of specific serodiagnostic tests for Ascaris and Toxocara.

3. Kennedy and Querishi (1986) and Kennedy et al. (1987) concluded that the expression of Ascaris antigens was stage specific, with antigens expressed on infective stage (L2) larvae differing from those expressed on lung stage (L4) larvae. There would be a great advantage having a stage specific serodiagnostic assay. It would help to determine the public health significance of the tissue migratory stage of infection.

4. Antigens to be detected should only be present when the worms are viable, although it might be useful to detect somatic antigens to determine whether a drug treatment was effective (Kennedy, 1989). Somatic antigens include a high proportion of metabolic and structural proteins which are likely to be highly conserved between worm species. Thus there would be a good chance that cross reactions could occur with other species (Parkhouse and Harrison, 1989).

5. Antigens should not be too immunogenic or allergenic as they will be removed by the immune response too quickly to be detected. The relevant monoclonal antibodies to be used in the assay could be produced by presenting the antigen to the antibody producing cell with an adjuvant. Such an antigen (perhaps also stage specific) would be the ideal target for a diagnostic assay (Parkhouse and Clark, 1983). In addition, heterogeneticity in immune responsiveness will mean that some individuals remove antigens (and infections) more efficiently than others, making the accurate quantification of infection difficult and impeding comparability between individuals. However, using a parasite product which is a poor immunogen may alleviate this problem.

If the nature of the host-parasite interface were better defined, if the rate at which a helminth such as Ascaris secreted a particular antigen were known, and if complimentary antibodies could be developed, then it might be possible to construct a quantitative serological test (Parkhouse and Harrison, 1989).

For any diagnostic test to be of use as an epidemiological tool in the study of ascariasis, it should be sensitive, specific, robust enough to survive field conditions, simple to use and able to give

quantitative results. Modern immunochemical assays such as the ELISA (Enzyme linked immunosorbent assay) and techniques involving monoclonal antibodies might be able to meet these criteria (Parkhouse and Harrison, 1989) once more is known about the human immune response to A. lumbricoides.

4.4.3 Serological field studies involving Ascaris lumbricoides

Field studies conducted on epidemiological aspects of A. lumbricoides using serology have concentrated on diagnosis rather than the the intensity of infection. In the absence of detailed characterisation of Ascaris antigens, the materials used in the assay have been crude extracts of homogenised worms.

Jones (1977) used an indirect haemagglutination test to study Ascaris infection in 810 people in Papua New Guinea and Timor, and compared the results of the stool examination with serum tested for the presence of anti-Ascaris antibodies. Although the author stressed at the outset of the paper that the test had little value as a diagnostic aid (the study involved the detection antibodies to the parasite in serum), he found that although the egg count declined with age, the level of response to parasite antigens by the patients' sera did not. This suggested that antibody challenge to infection was continuing with age whereas intensity of infection appeared to have fallen. Prakash et al. (1980) used a test on children to determine the antibody response to Ascaris antigens

using antigen injected into the skin. Distinguishing between different levels of response was done by measuring the area of skin weal just after injection of the antigen and then 15 minutes later. If the ratio between these two areas was more than two, then the reaction was judged positive for Ascaris infection. They discovered that of the children found positive for infection by faecal sampling, 97.3% were judged to have a positive skin test. Of the children who had negative egg counts, 95.4% were found to be negative by the skin test.

Tanaka et al. (1983) used an antigen extracted from the body fluid of Ascaris suum and created a radioimmunoassay (RIA) to detect this antigen in the sera of infected patients. By aiming to detect a parasite antigen, this assay revealed the presence of an ongoing parasite infection. However, the test showed cross reactivity in individuals infected with Toxocara sp., Anisakis sp., Schistosoma japonicum and Taenia saginata, probably because of the use of crude, homogenised worm extracts in the construction of the radioimmunoassay.

4.4.5 Future work

The recent application of molecular biological techniques, (e.g., monoclonal antibody production and recombinant DNA cloning of antigens) will greatly aid work conducted to characterise parasite antigens and the host immune response (Kennedy, 1989; Ogilvie et

al., 1990), leading perhaps to the production of a suitably specific diagnostic (and thereafter quantifiable) test. The production of highly purified antigens is expensive by conventional means, but, by using genetic engineering techniques, adequate quantities of defined antigens may eventually be produced cheaply (Almond and Parkhouse, 1985). Karlenga and Gamble (1991) constructed a complementary DNA library for mRNA Trichnella spiralis larval muscle. The expression library obtained was immunologically screened for diagnostic ES antigens. They discovered a cDNA transcript, 539 base pairs long which coded for a 123 kDalton betagalactosidase fusion protein that did not cross react with sera from host with Trichuris suis or Ascaris suum. The prospect of the use of such a technique for A. lumbricoides would be very exciting. Firstly, it would aid the characterisation of antigens and, secondly, it would facilitate the inexpensive production of a large amount of antigen, should it be needed.

An alternative immunochemical approach could be to measure parasite antigen in the faeces. This could detect and perhaps quantify intestinal Ascaris lumbricoides infection. Provided the production of antigen was not density dependent and was therefore directly proportional to the number of worms present, the amount of antigen detected could give an estimation of worm burden. Green (1986) has invented a diagnostic ELISA for the detection of Giardia lamblia using this approach.

4.5 Conclusions

1. The validity of egg counts to obtain an estimate of intensity of infection has often been challenged due to the many variations intrinsic to the host/parasite relationship. Additional variation exists due to errors that result whilst conducting the sampling method (Anderson and Schad, 1985). This is exacerbated by the lack of consistency observed from worker to worker as regards the choice of technique. Despite this, the use of egg counts as a measure of intensity persists, although little attempt has been made to determine sources of variation. Surely it must be of importance to assess different sampling techniques to determine their limits and accuracy. Beaver and Yokogawa (1980) stated that although choice of the most accurate technique is not necessary its accuracy should be known. It is perhaps difficult, or practically impossible, to assess variation of egg count caused by host and parasite, but to know the limits of different techniques would contribute greatly to the use of egg counts as an epidemiological tool. There has been little in the literature to suggest that this has been adequately investigated.

2. Chemotherapeutic worm expulsion, as a method of measuring infection intensity, is expensive in time and intrusive for hosts although the drugs used have a high efficacy and are safe. However, worm counts measure intensity directly and are therefore much more accurate than egg counts.

3. The use of serological techniques to measure epidemiological parameters such as prevalence and intensity are in their infancy. The host-parasite immunological interface is complex in helminth infections because a vast array of antigens is presented to a host, each of which elicits an antibody response. Heterogenetity also exists in both host population - varying immune response (Chapter 2) and in the worm population antigen expression appears to vary between Ascaris worms even if the worms are at identical stages in development (Fraser and Kennedy, 1991). Characterisation of the parasite antigen interface is needed (Parkhouse and Harrison, 1989) to determine which antigen/antibody system would be relevant for use in diagnosis and thereafter determination of intensity of infection. Detection of parasite antigens enables the assay to determine a current infection; detection of antibodies produced in response to the parasite does not. Ascaris lumbricoides has been shown to exhibit stage specific

antigen expression (Kennedy and Querishi, 1986) which if a suitable test was developed could determine the stages of the infection present in the host. Recent advances in molecular biological techniques, may facilitate development of such tests (Kennedy, 1989; Ogilvie et al. 1990).

Most work in the field of serodiagnosis has been conducted on Trichinella spiralis and on filarial worms. Little work beyond the use of crude assays has been done with A. lumbricoides. There is no obvious reason to suggest that the type of work that has been done with filarial worms cannot be duplicated for Ascaris lumbricoides. Although it is not surprising that research priorities should concentrate on filarial worms as conventional diagnosis is difficult (Almond and Parkhouse, 1985), the pathology caused by the migrating larvae of Ascaris (see Chapter 3), plus the sheer numbers of people infected with Ascaris, would seem to make this a valid area of research too.

TABLE 4.1 : STOOL SAMPLING TECHNIQUES COMMONLY USED IN EPIDEMIOLOGICAL STUDIES OF ASCARIS LUMBRICOIDES (1)

TECHNIQUE	PRINCIPLE INVOLVED	APPROX AMOUNT STOOL	ADVANTAGES	DISADVANTAGES	NOTES
DIRECT SMEAR	Small piece of stool is mixed with a drop of saline and examined. (14)	2 - 5 mg (14)	Very quick and simple allows stool and thus eggs to be examined in unaltered state. (3)	Small amount examined means light infections are often missed. Not quantitative enough to measure intensity (except approximately). (11) (30)	Theorectically one female Ascaris produces so many eggs that infection can be detected by one smear. (18) (30)
BEAVER'S DIRECT EGG COUNT	Direct smear is standardised using photoelectric measurement to determin density (thickness). (2)	Same as the direct smear	Examines stool directly and quickly Requires no correction for consistency or size of stool. (2)	Requires special apparatus and accurate photoelectric measurements - makes it impractical for mass surveys. Should have experienced operators. Routine photoelectric calibration is laborious and time consuming. (28) (6)	Requires correction for colour of faeces.
FLOTATION eg BRINE, MgSO4	The different specific gravities between eggs and faecal material mean eggs float to the top of highly concentrated solutions. (14)	0.5 g -10 g (3) (7) (14) (24) (26) (29)	Good for detecting very light infections as a comparitively large amount of faeces are examined. (10)	If eggs are kept too long in highly concentrated solutions they may become distorted and cannot be recognised. (26)	Female Ascaris worms produce so many eggs that these techniques are not really required for detection and counting eggs becomes difficult (14) Brine flotation does not detect infertile Ascaris eggs (15)
SEDIMENTATION eg FORMOL-ETHER TELEMAN	Eggs are deposited at the bottom of a sample tube by centrifugation.	0.5 - 10 g		Both sedimentation and concentration techniques require more apparatus and are more complicated than direct methods. Thus they are more time consuming and expensive (11)	

TABLE 4.1 : STOOL SAMPLING TECHNIQUES COMMONLY USED IN EPIDEMIOLOGICAL STUDIES OF ASCARIS LUMBRICOIDES (2)

TECHNIQUE	PRINCIPLE INVOLVED	APPROX AMOUNT STOOL	ADVANTAGES	DISADVANTAGES	NOTES
KATO THICK SMEAR	Exploits the property that a thin smear of faeces will clear after being in contact with cellophane soaked in glycerin leaving eggs visible. (22)	50 - 200 mg (3) (10) (13) (14) (19) (22) (25) (29) (31)	Rapid, simple, incur little expense and do not need special technical expertise.	Largeness of samples means eggs can be blocked from view (3) (22) (24) Kato can be unrealible if samples are full of fermentation gas and fibrous material. Hook-worm eggs can become distorted and may clarify through time. For heavy infections counting of eggs may become tedious and be potentially inaccurate. (20) Can be messy and if stools are old there may be difficulties in reading preps. (8)	Assumes eggs are distributed randomly in the stool. (11) Good for the detection of Ascaris ova which can be seen at any time. Reported the most sensitive diagnostic technique for Ascaris. (1) (13) (9) Perhaps best to count number of Ascaris ova in a sample of fields or rows and then multiply accordingly. (21)
MODIFIED KATO	Stool sample is quantified by weight then sieved to remove large amounts of fibre and cleared using glycerin soaked cellophane. (12)	50 - 100 mg (12)	Larger samples mean light infections are not as easily overlooked as the direct smear. (13) (19) (24) Estimated to be able to detect as low as 40 - 100 epg. (27) Considered suitable for epidemiological use. (10)		
KATO-KATZ	Stool is quantified by volume using a template after being sieved. (8)	Approx 50 mg (8) (21)			
QUICK KATO	A smaller amount of stool is quantified and examined. (22)	20 mg (22)	Takes less time than above (2 minutes as opposed to 5 minutes). (22)	Will detect only heavy infections and counts average 35% higher than those using the standard Kato method.	

TABLE 4.1 : STOOL SAMPLING TECHNIQUES COMMONLY USED IN EPIDEMIOLOGICAL STUDIES OF ASCARIS LUMBRICOIDES (3)

TECHNIQUE	PRINCIPLE INVOLVED	APPROX AMOUNT STOOL	ADVANTAGES	DISADVANTAGES	NOTES
STOLL DILUTION EGG COUNT	A weighed amount of stool is diluted in sodium hydroxide solution, homogenised and then a drop of the resulting solution is examined. (23)	Approx 3 - 4g (23) (28)	Good for quantifying heavy infections as counting becomes less tedious. Eggs are visible as faecal debris is removed. (28)	Not suitable for light helminth infections as it will not detect less than 200 epg (1) (14) (27) Is a complicated technique which is time consuming and requires a lot of glassware	As Ascaris worms produce so many eggs it is good for quantification. Has been well used and tested for years. (30) Ascaris eggs have a characteristic appearence due to loss of their outer coat. (28) Compensation for amount of fluid in stool sampled is required. This is done by multiplying egg count by consistency conversion factors. (28)
BELL'S TECHNIQUE	Stools collected over 24 hours are weighed, diluted to a known volume and then homogenised One ml of the suspension is concentrated onto filter paper through a sieve column. Eggs are stained with nihydrin and counted on the paper. The mean counts from three papers are used to estimate daily egg production (4).	24 hour specimen (4)	Very sensitive - from 200 g of stool it will detect 5 eggs and it is reckoned that an egg production of 1000 per day can be detected. Large faecal debris is also removed. Stained filter paper is semi-permanent. (4)	Complicated technique which is time consuming and uses a lot of apparatus. Also stools over a 24 hour period have to be collected.	This technique is most frequently used for Schistosoma mansoni studies. (22)

Reference list for Table 4.1

1 Bailey (1987)

2 Beaver (1949)

3 Beaver and Yokogawa (1980)

4 Bell (1963)

5 Cheeseborough and McArthur (1976)

6 Dunn (1968)

7 Faust et el. (1939)

8 Jordan et al. (1981)

9 Kobayshi (1980)

10 Komiya and Kobayshi (1980)

11 Margono et al (1980)

12 Martin and Beaver (1968)

13 Massoud et al. (1978)

14 Muller (1975)

15 Nasr et al. (1979)

16 Pamba and Mulega (1981)

17 Ridely and Hawgood (1955)

18 Rijpstra (1975)

19 Sehgal et al. (1977)

20 Sinniah (1981)

21 Stephenson (1987)

22 Stevens (1978)

23 Stoll (1923)

24 Suzuki (1980)

25 Teesdale and Amin (1976)

26 Theinpoint et al. (1979)

27 Vinayak et al. (1978)

28 WHO (1963)

29 WHO 1964)

30 WHO (1967)

31 Zaman and Cheong (1967)

TABLE 4.2 : Anthelmintic activity of selected drugs in frequent use
(Adapted from Gustafsson et al., 1987; McIntosh et al., 1985, 1989)

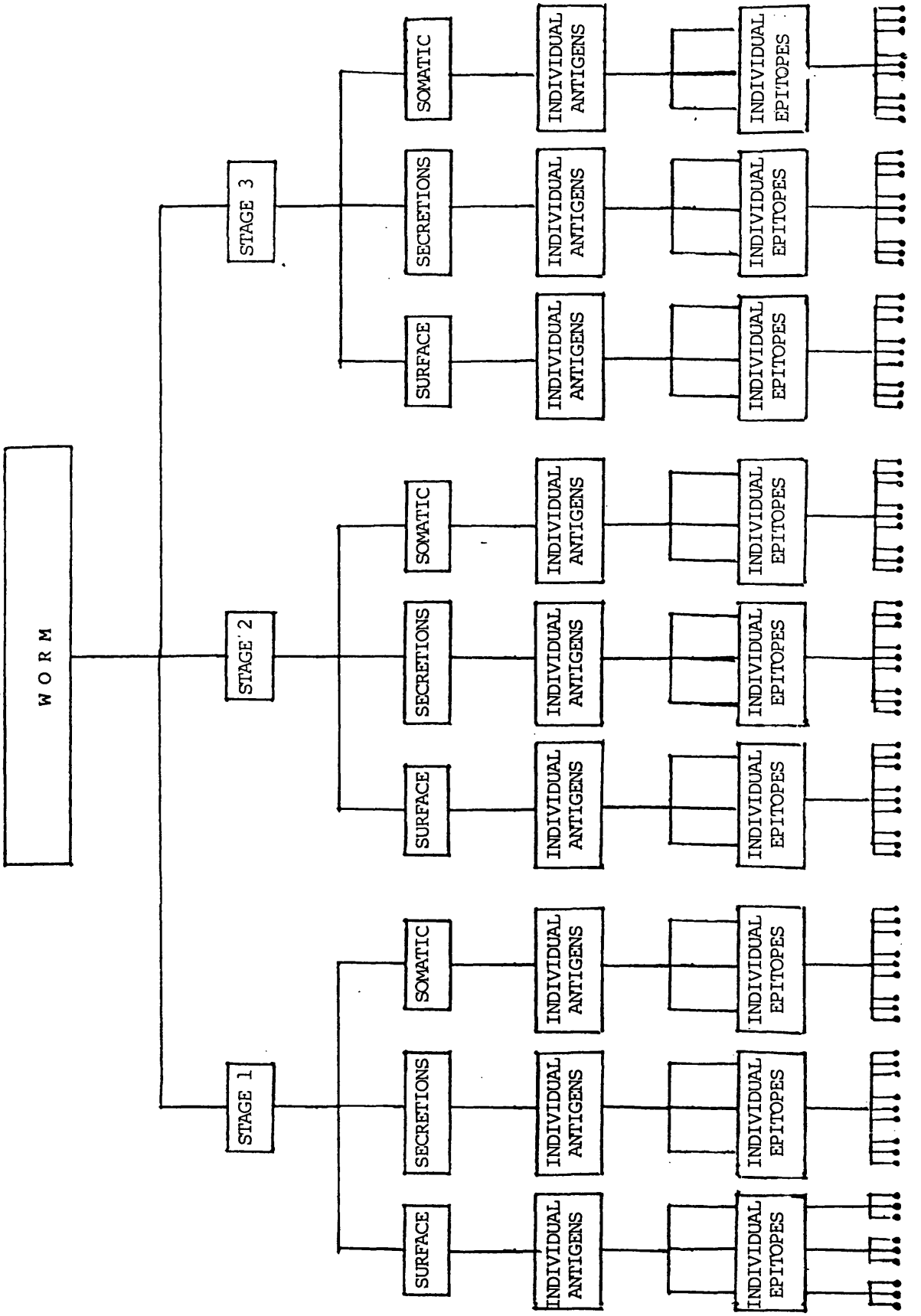
Drug (Manufacturer)	Trade name	Approx date Introduced	Chemical type	% Efficacy * against Ascaris
Levamisole (ICI : Janssen)	Ketrax Decaris	1968	Imidazothiazole	> 90% Very good
Pyrantel (Pfizer)	Combantrin Antiminth	1966	Tetrahydropyrimidine	> 90% Very good
Mebendazole (Janssen)	Vermox Pantelmin	1971	Benzimidazole Carbamate	> 90% Very good
Albendazole (Smith, Kline & French)	Zentel	1979	Benzimidazole Carbamate	> 90% Very good
Piperazine salts (many manufacturers)	Antepar	1953	Diethylenediamine	60-100% Good - Very good

ACTION OF DRUGS (Source Glover, 1983)

Levamisole inhibits the fumarate reductase enzyme system interfering with anaerobic glycolysis.
Pyrantel pamoate depolarises the worm causing spastic paralysis.
Mebendazole impairs helminth mitochondrial phosphorylation and uptake of glucose.
Albendazole blocks helminth glucose uptake.
Piperazine causes flaccid paralysis of worm muscle allowing perastalic action of gut to remove worms.

(* - Efficacy is defined as 90% of individuals egg positive before treatment are egg negative a certain time after treatment)

FIGURE 4.1 : Representation of antigens present during a helminth infection (Adapted from Parkhouse and Harrison, 1989)



CHAPTER 5: THE RELATIONSHIP BETWEEN ASCARIS LUMBRICOIDES EGG COUNT AND WORM BURDEN

5.1 Introduction

Faecal egg counts are the most common method used to measure the intensity of infection of Ascaris lumbricoides. This is because they are relatively inexpensive to carry out, do not cause extensive disruption to host lifestyle and are not too dependent on compliance and co-operation from the population under study in contrast to worm expulsion chemotherapy (see Chapter 4).

However, egg counts are no more than an indirect method of estimating intensity whereas worm counts are direct; this means that worm counts are much more likely to be reliable assuming that all the worms in the stools are collected. Reliability in this context is important because intensity of infection has significant implications for host and worm populations (see Chapter 3). Therefore, it would be very useful to ascertain how much confidence can be afforded to egg counts as a measure of intensity.

A set of pooled data consisting of A. lumbricoides faecal egg counts and corresponding worm burdens has been investigated. Preliminary observations of the data set were made first and variation in egg counts examined. Finally, the relationship between egg count and

worm burden was studied and an attempt was made to determine the degree of reliability that could be afforded to egg counts as a measure of worm burden. A table was then constructed with the aim of providing a practical guide for field workers.

5.2 Material and methods

Data from various sources which listed egg counts and worm burdens from the same individuals were pooled and examined. A total of 681 observations were used. The data originated from different countries: Panama (Holland et al., 1987) (see Appendix), Nigeria (Holland et al., 1989), Egypt (Farid et al., 1966), Brazil (Mello, 1974) and Malaysia (Sinniah, 1982). Most data were obtained from field studies (Holland et al., 1987; 1989). Other workers (Farid et al., 1966; Sinniah, 1982; Mello, 1974) had used individuals selected for infectivity and closely monitored for several days after anthelmintic treatment. The data was examined using the Minitab statistical software package.

5.2.1 Investigation of the data set

The data set was first examined for general observations and trends, namely means, standard deviations, ranges, male:female worm sex ratio and the variation observed in egg counts. Sex ratio was calculated by summing the total male and female populations and

comparing the resulting values.

5.2.2 Variation observed in egg counts

A "Box and Whisker" plot of egg count for each female worm burden (up to 21) was constructed to give a pictorial representation of the variation observed in the egg count.

5.2.3 The relationship between egg count and worm burden

Regression and correlation analyses were conducted on log (x + 1) transformed egg counts, female worm burdens and total worm burdens. Egg counts were then divided into classes or intervals of 5,000 up to 30,000 eggs per gram (epg) and egg counts over 30,000 (see p.91). The log mean egg count and log mean female/total worm burdens from each class were calculated, and regression and correlation analyses were conducted on these mean values.

Raw data was split into egg count classes again (see p. 92) and the number of individuals in particular female worm burden classes for each egg count class was calculated.

5.3 Results

5.3.1 Investigation of the data set

The results of the preliminary investigation of the data set can be seen as Table 5.1. The variance/mean ratio for egg counts, female worm burden, male worm burden and total worm burden, were all greater than one; indicative of overdispersed frequency distributions. These are illustrated in Figures 5.1 - 5.4.

5.3.2 Variation observed in egg counts

The extent of the variation observed in egg counts is illustrated in the "Box and Whisker" plot shown in Figure 5.5. Each female worm burden has its corresponding range of egg counts next to it. The range is indicated by the "whiskers", the boxes are the inter-quartile ranges, covering 50% of the observations and the horizontal lines across the boxes represent the median egg counts. The range of egg counts observed is high indicating the presence of considerable variation. However, the whiskers indicate extreme results which usually encompass only a small number of values. The boxes indicate a trend toward greater variability as female worm burden increases. The medians also show a slight upward trend with egg count increasing for female worm burden of less than 10. It should be noted that the number of observations in each class in the

higher female worm burdens is relatively small as would be expected in view of the observed overdispersed frequency distribution of number of female worms per host.

5.3.3 Relationships between egg count and worm burden

Regression analysis of the log-transformed egg counts versus log-transformed female worm burden is shown in Figure 5.6. A significant relationship was detected (F-ratio = 447.93, $p<0.05$, degrees of freedom 1, 680, $r^2 = 0.397$). The correlation coefficient was found to be 0.630 ($p<0.05$). Regression between transformed egg counts and total worm burden was also seen to be significant, F-ratio = 585.77, ($p<0.05$, degrees of freedom 1, 680) although R^2 was fairly low with a value of 0.463. The correlation coefficient was 0.680 ($p<0.05$)(see Figure 5.7). The low correlation coefficient and R^2 value resulted from the omission in the construction of the regression line of a good deal of the data due to variation. The Minitab statistic package is programmed to construct the best regression line by selecting the most appropriate data.

In an attempt to counteract the effect of this variation in egg counts regression analysis was also conducted on data first sorted into egg count classes. The classes chosen arbitarily were; 0 - 4,999, 5,000 - 9,999, 10,000 - 14,999, 15,000 - 19,999, 20,000 - 24,999, 25,000 - 29,999 and over 30,000. Analysis of the mean log-transformed egg counts for each of the classes (see above) versus mean log female worm burden for each egg count class showed a

significant relationship (F-ratio = 147.89, $p<0.05$, degrees of freedom 1,5). In this case more of the natural variation had been taken into account in the construction of the regression line since R^2 was seen to have risen to 0.96. The correlation coefficient was 0.984 ($p<0.05$) (Figure 5.8). A similar analysis of mean log egg count for each of the egg classes versus mean log total worm burden for each each egg count class also showed a significant relationship (F-ratio = 598.41, $p<0.05$, degrees of freedom 1,5) with the R^2 value being equal to 0.99. The correlation coefficient was also significant, $r=0.996$ ($p<0.05$). The graph for this relationship is shown in Figure 5.9.

Egg counts were divided into classes of a larger range that those above and the number of values in female worm burden classes of 0 - 10, 11 - 20 and > 20 for each egg count class calculated. This was done to ascertain whether egg counts could determine female worm burden accurately. These were 0 - 4,999, 5,000 - 14,999, 15,000 - 29,999 and more than 30,000 epg. Not surprisingly the lowest female worm burdens dominate the lower egg count classes and the highest female worm burdens occur more frequently in association with the highest egg count classes (Table 5.2, Figure 5.10). In the third egg count class, which encompasses a wide range of egg counts (15,000 - 29,999 epg), the number in each female worm burden class is nearly identical. Clearly it would be very difficult to estimate the size of the female worm burden in infections if egg counts were in this range (Table 5.10).

5.4 Conclusions

1. On the basis of the data available for study, Ascaris egg counts were found to vary considerably with respect to worm burden. This is quite clearly shown by Figure 5.5, and Figures 5.6 and 5.7. There are many possible causes for such variation (Hall, 1982). These include sampling errors; uneven distribution of the eggs in the faeces will lead to an unrepresentative egg count if the stool is not mixed thoroughly before sampling (Hall, 1981; Martin, 1965; Martin and Beaver, 1968; Melvin et al., 1956). Variation caused by the use of different stool sampling methods is especially relevant if the limitations of the technique are not known making comparison between different studies difficult (see Chapter 4). In this study no attempt was made to account for whether use of different egg count methods and variation between type of study affected the relationship between egg count and worm burden. Thus the use of different egg count techniques could contribute to variation. Lastly, enumeration errors could cause variation in egg count (Melvin et al., 1956; Markell et al., 1978).

   The number of eggs a female worm produces could also cause variation. This number could depend on age of

female worms (Hall, 1982), daily variation in egg output (Croll et al., 1982), and number of worms present through density dependence - it is well known that as number of worms increases the per capita rate of egg production decreases (see Chapter 2).

The amount and nature of faeces produced will also affect the egg per gram figure. For example dietary fibre could affect egg count in three ways. By increasing faecal bulk and diluting the number of eggs the egg per gram figure could be lowered. Fibre also decreases gut transit time which could also lower epg (Hall, 1982). The presence of copious amounts of fibrous material can interfere with the practical reading of the stool sample; especially when using the direct smear and Kato methods (see Chapter 4).

Consistency of stools should also be taken into account. The amount of water in the stool will affect weight, making comparison difficult between individuals and will influence epg either by increasing it (hard stool) or decreasing it (watery stool). Nawlinski et al. (1978) and Scott and Headlee (1938) have suggested classifying stool samples into different consistencies and applying multiplication factors to the egg count to counteract the amount of water in the stool. It should also be borne in mind that diarrhoeal diseases also reduce gut transit

times which will also affect egg count (Hall, 1982).

The age of the host will also affect faecal output. Children who so often have maximum worm intensities will also produce small stools, this will increase the epg figure. Likewise, individuals who have low dietary intakes will produce smaller stools.

2. The egg count variation tends to reduce the value of egg counts as a quantitative measure of worm burden. Regression and correlation analyses on log-transformed data show significant relationship between worm burden and egg count, but much of the variation in the data has not been taken into account (small R^2 values). In order to stabilise the variation, the data was split into egg count classes and the mean log egg counts correlated and regressed against mean log worm burdens. This gives stronger correlation and regression values, but also serves to emphasise the problems for using egg counts as a quantitative tool. The strong correlation and regression relationships are based on mean values of the egg count rather than on single observations.

3. It may be possible to use egg counts as a semi-quantitative measure of intensity perhaps to discriminate between very light and very heavy infections. This is similar to the conclusions reached

by Anderson and Schad (1985) in a study involving hookworm. There are important implications for Ascaris epidemiology since many studies must rely on egg counts as the sole measure of infection intensity. This is understandable for reasons of study design in many cases, but in areas of active debate (such as the presence of predisposition and density dependence) the resolution of such issues will not be aided by the use of egg counts unless the limitations of different techniques are understood.

Forrester and Scott (1990) concluded that worm burden could be reliably predicted from egg counts. Although they detected a significant correlation, only 59% of the variation of the data appeared to have been accommodated in the test. Although, this correlation value is higher than in this study (Figures 5.8 and 5.9) it still means that 40% of the variation was ignored.

TABLE 5.1 : Investigation of data set (n = 681)

	MEAN ± S.D	RANGE
Egg count (e.p.g.)	14880 ± 20521	1 - 195394
Female worm burden	6.4 ± 7.09	1 - 95
Male worm burden	5.34 ± 6.41	1 - 86
Total worm burden	11.69 ± 12.95	1 - 181

SEX RATIO MALE:FEMALE WORMS = 1 : 1.19 (3639 males : 4332 females)

TABLE 5.2: Distribution of female worm burdens in egg count classes

EGG COUNT	TOTAL NO. IN EGG COUNT CLASS	NO IN WORM BURDEN CLASS FOR EACH EGG COUNT CLASS 1 to 5	6 to 10	>10
0 - 4999	278	219(78.7%)	38(13.6%)	21(5.7%)
5000 - 14999	192	114(59.3%)	51(26.5%)	27(14%)
15000 - 29999	117	46(39.3%)	39(33.3%)	32(27.3%)
> 30000	94	21(22.3%)	30(31.9%)	44(46.8%)

Figure 5.1 Frequency distribution of egg count

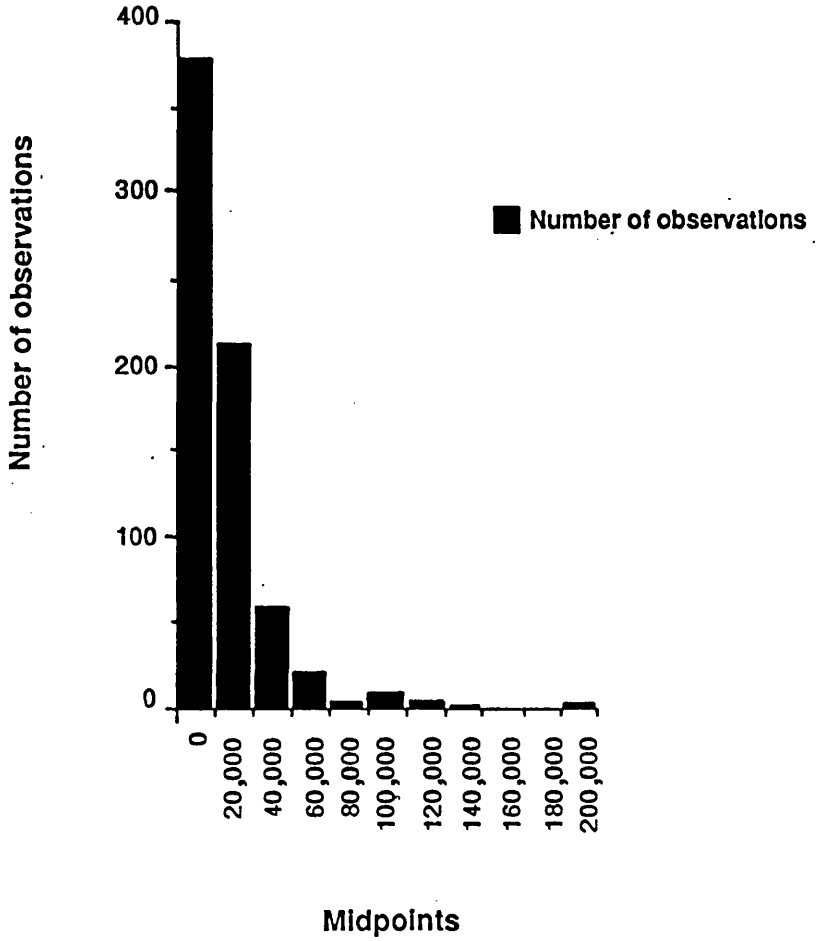


Figure 5.2 Frequency distribution of female worm burden

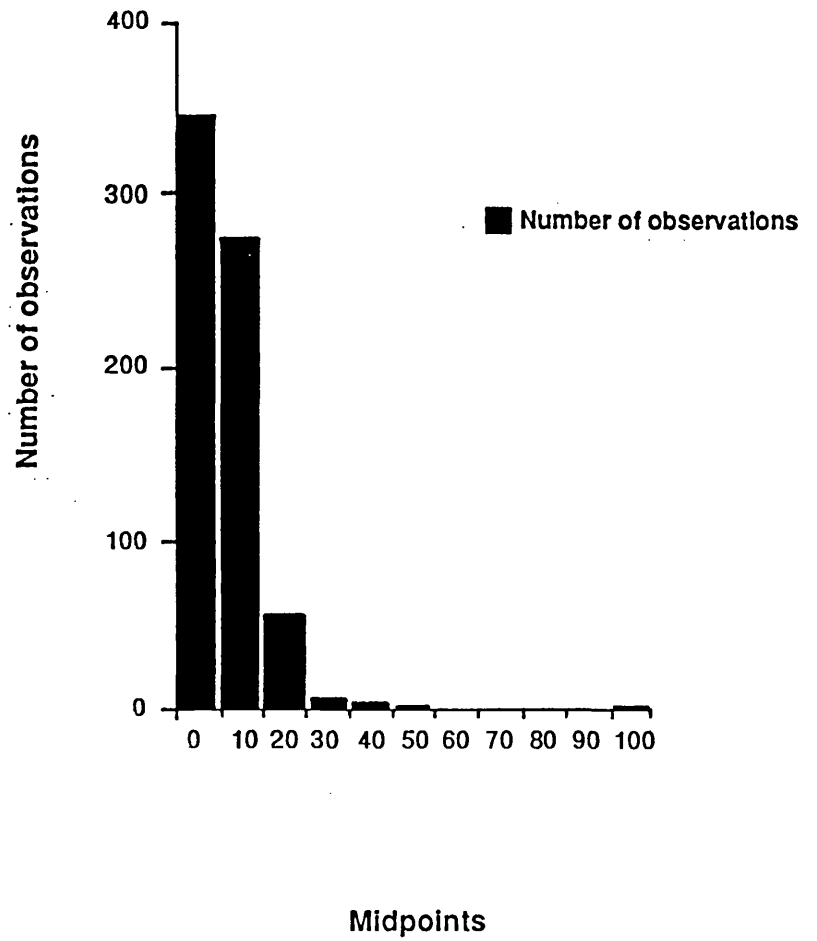


Figure 5.3 Frequency distribution of male worm burden

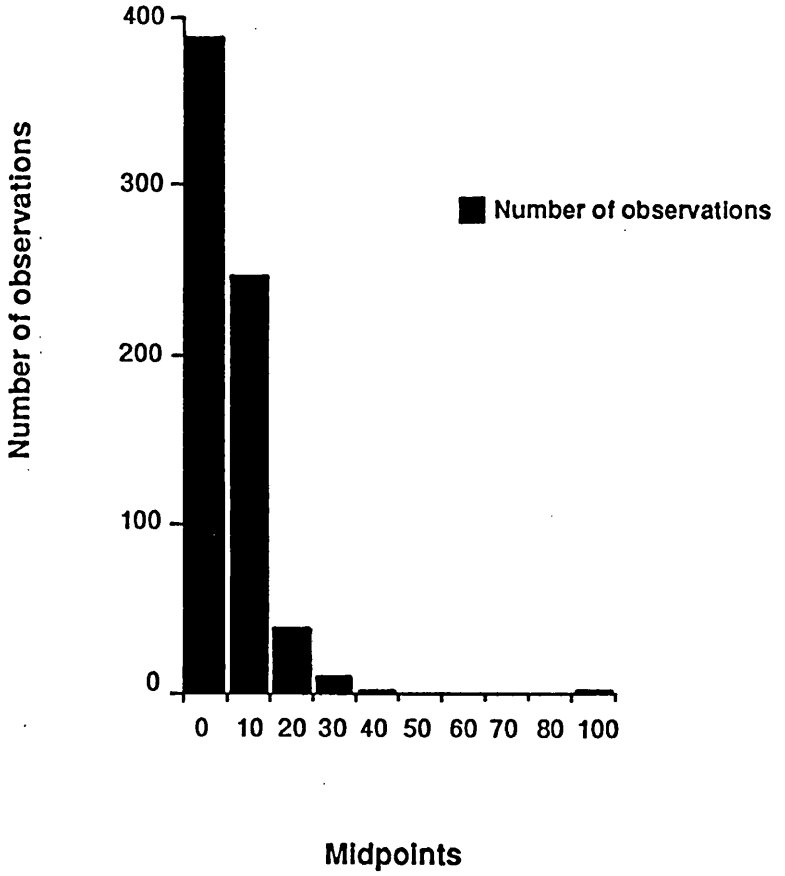


Figure 5.4 Frequency distribution of total worm burden

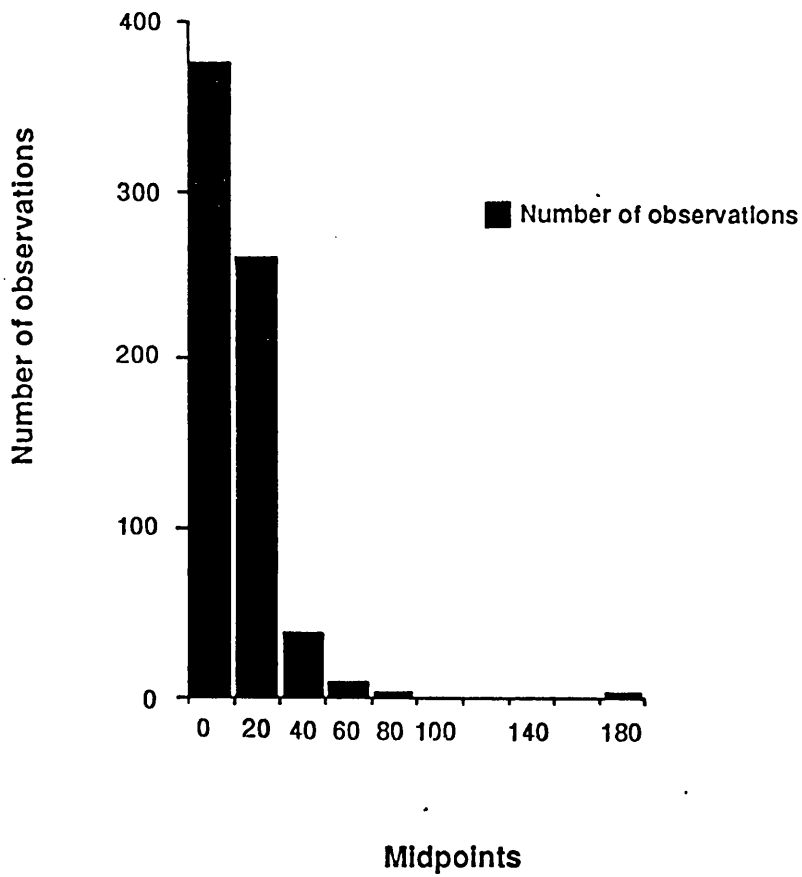


Figure 5.5. "BOX AND WHISKER" PLOTS FOR E.P.G. VERSUS FEMALE WORM BURDEN

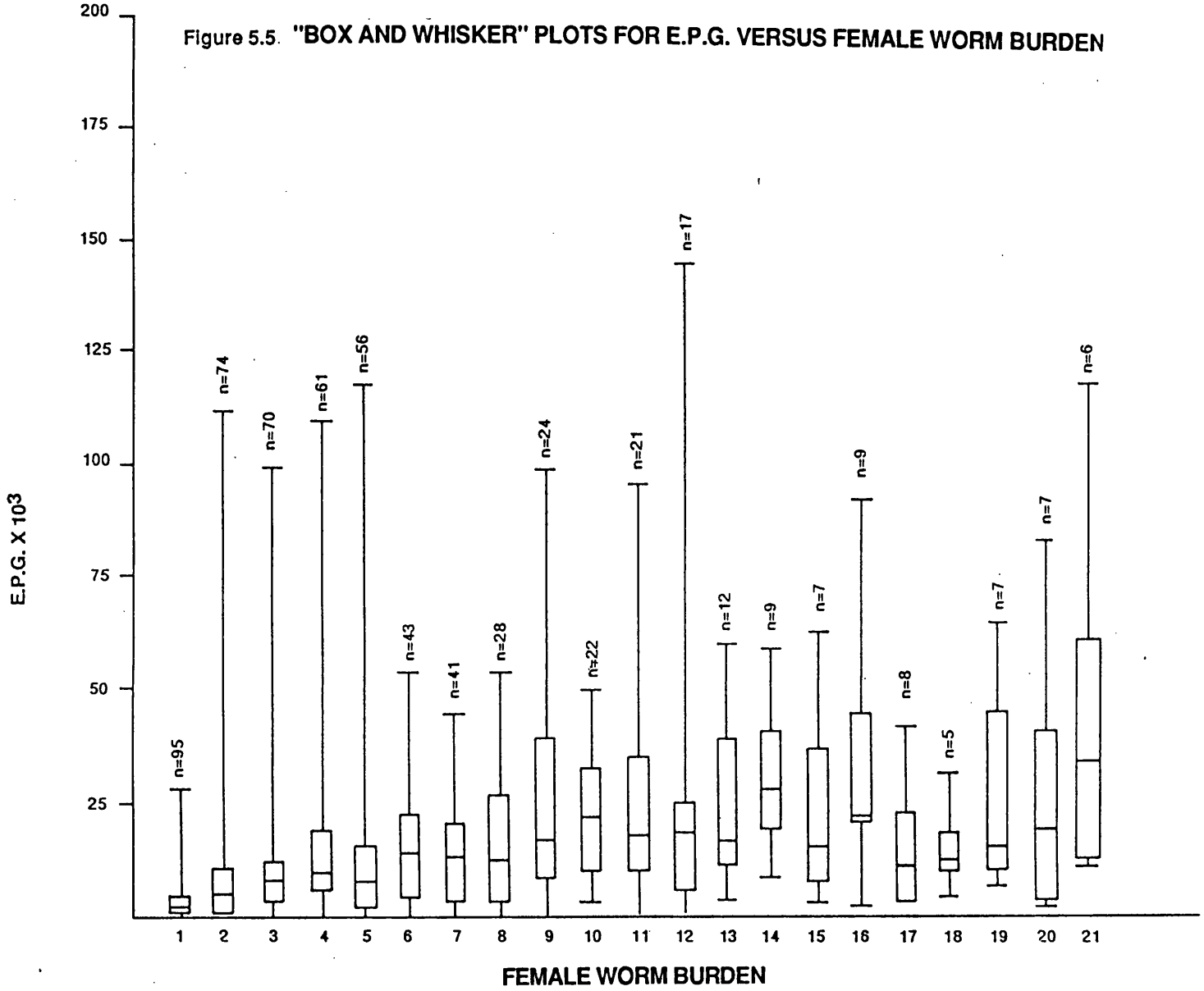


Figure 5.6

LOG EGG COUNTS VERSUS LOG FEMALE WORM BURDEN

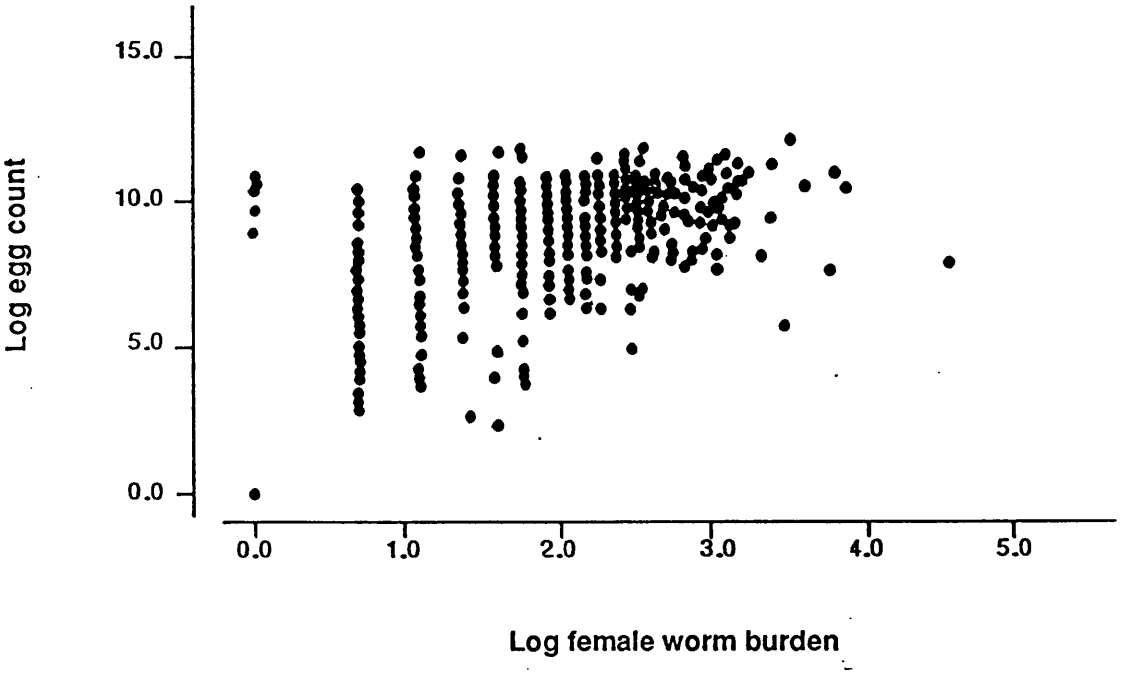


Figure 5.7

LOG EGG COUNTS VERSUS LOG WORM BURDEN

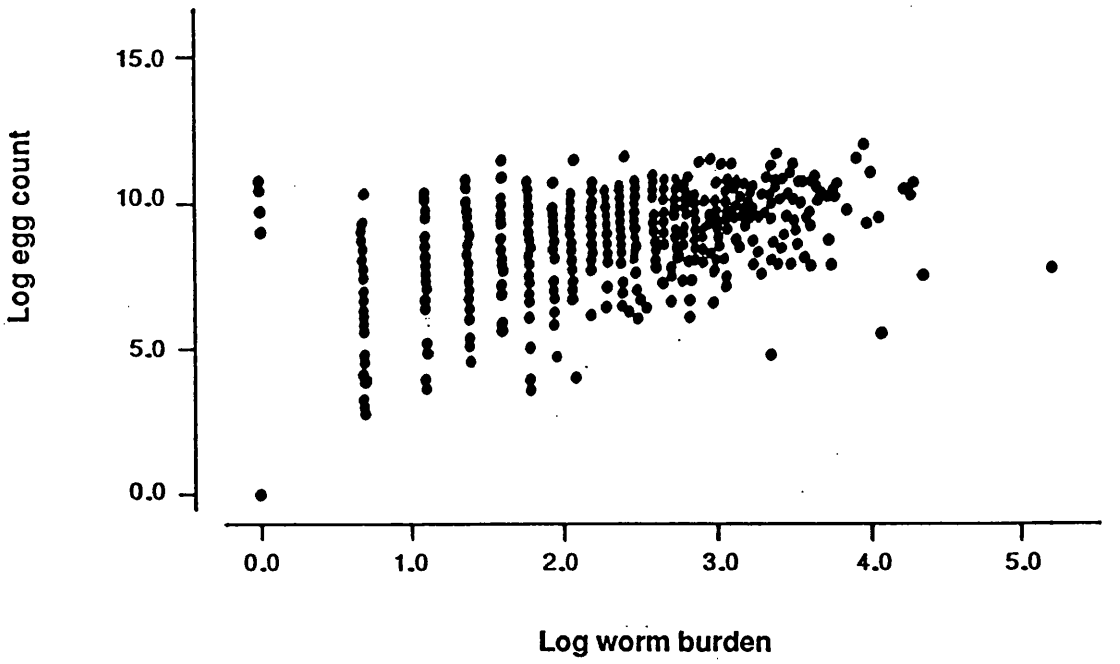


Figure 5.8

MEAN LOG EGG COUNT VERSUS MEAN LOG FEMALE WORM BURDEN

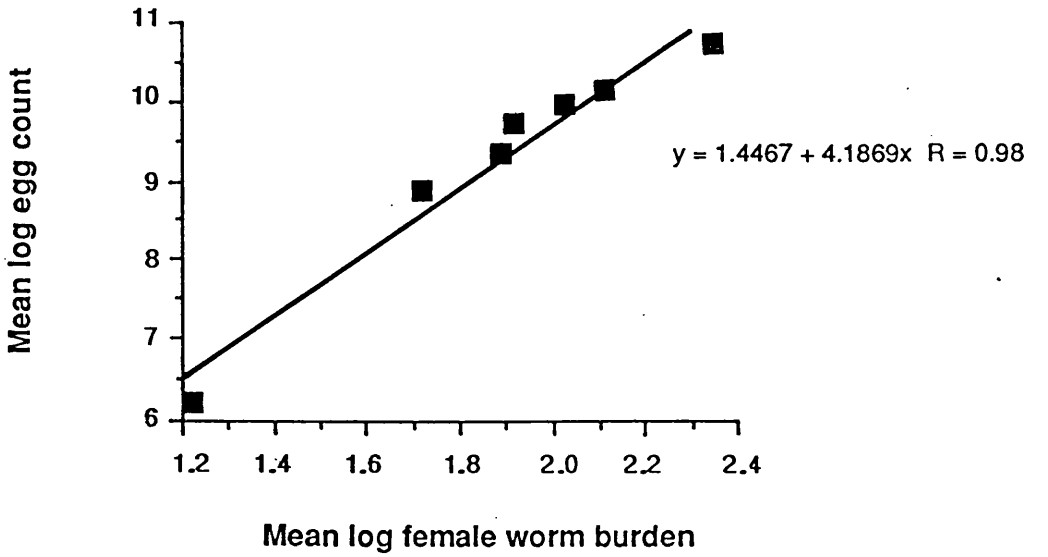


Figure 5.9

MEAN LOG EGG COUNT VERSUS MEAN LOG WORM BURDEN

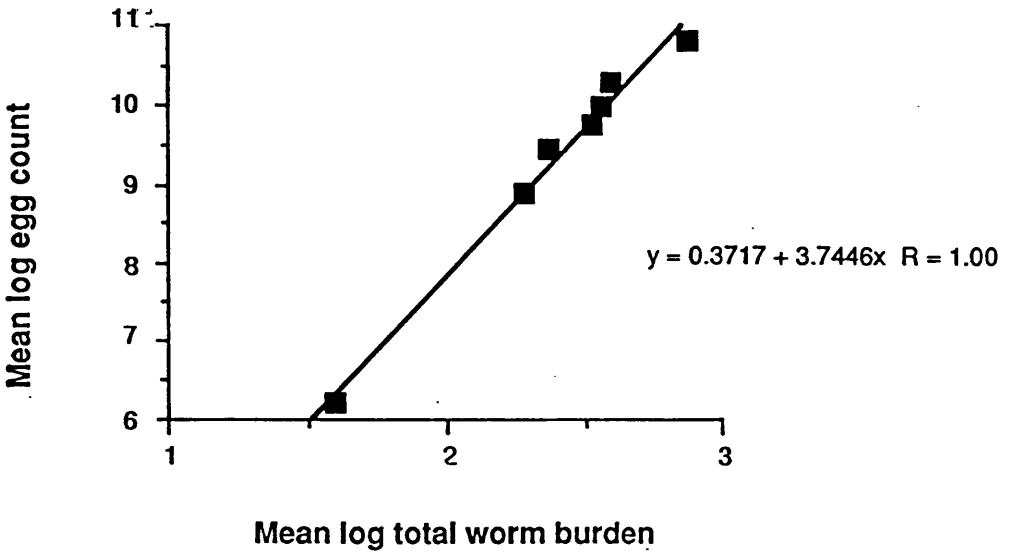
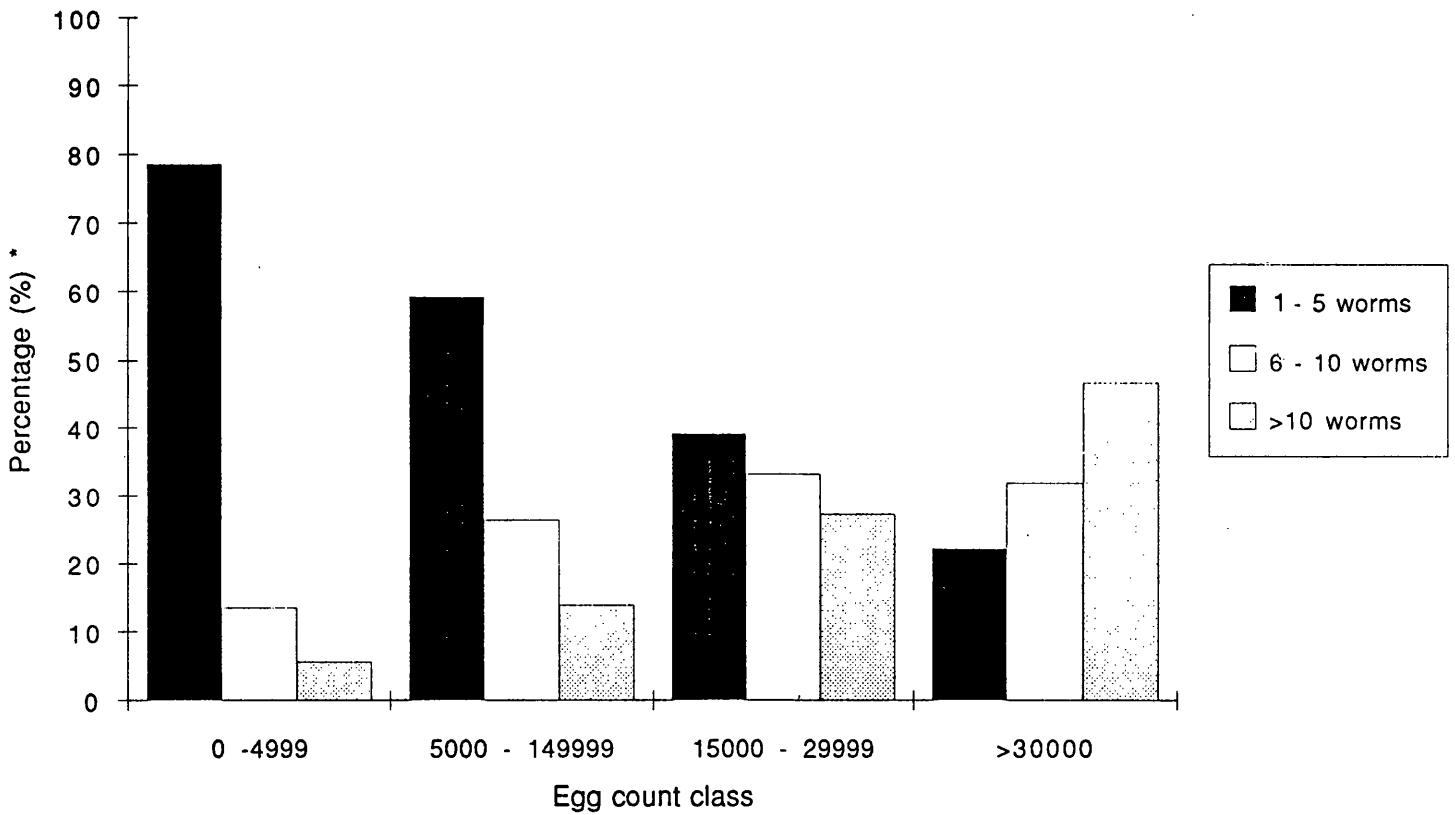


Figure 5.10 Distribution of female worm burdens in egg count classes



* Percentage of egg counts which fall into each female worm burden class

CHAPTER 6 : SUMMARY

The investigation of trends of Ascaris lumbricoides infection intensity is important because it allows identification of those individuals at risk from pathology and nutritional disturbances and yields valuable background information, essential for the implementation of control procedures and study of transmission dynamics.

Generative mechanisms of trends such as overdispersion, predisposition to infection, density dependence and age-intensity curves have not been convincingly determined. This is hampered by the lack of knowledge surrounding the exact role of the immune response to Ascaris infection in humans. Clarification of the nature of the immune response not only would address the question of its involvement in the cause of intensity trends but would aid the development of serological tests and the possibility of vaccination against disease.

The risk of acute pathology, e.g. intestinal obstruction is well documented, increasing as A. lumbricoides infection intensity increases. The public health significance of the tissue migratory stage has been inadequately assessed because of the difficulties in measuring the intensity of migrating larvae. The relationship between intensity and nutritional disturbance has not been fully defined, impeded by the failure in many studies to take intensity into account.

Assessment of pathology, control measures and examination of intensity trends are meaningless if accurate measurement of intensity is not conducted. Techniques to measure intensity include faecal egg counts, chemotherapeutic worm expulsion and perhaps in the future serological tests. If serological tests could be developed they may be able to assess the public health significance of the larval migratory stage. The advancement of seroepidemiology should be aided by recent developments in molecular and immunological technology, eg gene cloning and monoclonal antibody production.

The most common method to measure intensity of infection is based on faecal egg counts probably due to their speed, inexpense, simplicity and their low impact on host lifestyle. However, egg counts are persistently variable and it becomes difficult to extrapolate the count into a direct assessment of worm burden. The nature of the relationship between faecal egg counts and worm burdens was investigated using a large data set of egg counts and corresponding worm burdens. Given that worm burden measurements were accuarate it was determined that egg counts were extremely variable with respect to worm burden. This decreases the usefulness of egg counts as measures of infection intensity. It may be possible to use egg counts as a semi-quantitative measure of intensity as after some manipulation of the data to reduce variation a correlation could be observed between grouped egg counts and worm burdens. Extent of egg count variation should be more fully assessed as it would increase their usefulness.

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A P P E N D I X

APPENDIX : Data set of egg counts and worm burdens

(Raw data as received - Holland et al, 1989)

ROW	OBS NO C1	EPG C2	NO ♀ WORMS C3	NO ♀ WORMS C4	NO ♂ WORMS C5	NO ♂ WORMS C6	NO IMM WORMS C7	NO IMM WORMS C8	TOT ♀ WORMS C9	TOT ♂ WORMS C10	TOTAL ADULT WORMS C11
1	1	3772	1	0	6	0	0	0	1	6	7
2	2	7889	0	0	0	0	0	0	0	0	0
3	3	8716	4	0	2	0	0	0	4	2	6
4	4	20393	0	0	0	0	1	0	0	0	0
5	5	7388	9	0	3	0	0	0	9	3	12
6	6	13275	4	0	2	0	0	0	4	2	6
7	7	0	0	0	1	0	3	0	0	1	1
8	8	*	*	*	*	*	*	*	*	*	*
9	9	*	*	*	*	*	*	*	*	*	*
10	10	4472	2	0	3	0	0	0	2	3	5
11	11	887	2	0	0	0	0	1	2	0	2
12	12	1547	0	3	1	0	2	0	3	1	4
13	13	0	0	0	0	0	0	0	0	0	0
14	14	56306	0	0	0	0	1	0	0	0	0
15	15	7821	4	2	1	0	0	0	6	1	7
16	16	*	*	*	*	*	*	*	*	*	*
17	17	9829	0	0	0	1	0	0	0	1	1
18	18	16521	11	0	6	0	5	2	11	6	17
19	19	27170	3	0	2	0	1	0	3	2	5
20	20	0	0	0	0	0	0	0	0	0	0
21	21	3724	1	10	3	5	0	0	11	8	19
22	22	0	0	1	0	3	0	0	1	3	4
23	23	192	0	0	0	0	0	0	0	0	0
24	24	0	0	0	0	0	2	0	0	0	0
25	25	3812	2	0	4	0	0	0	2	4	6
26	26	3962	5	2	7	2	0	1	7	9	16
27	27	12000	3	16	6	10	1	3	19	16	35
28	28	51242	2	0	2	0	0	0	2	2	4
29	29	15390	4	0	4	0	0	0	4	4	8
30	30	0	0	0	0	0	2	0	0	0	0
31	31	1290	5	0	2	2	0	0	5	4	9
32	32	3629	2	1	8	0	0	0	3	8	11
33	33	0	0	0	0	0	0	0	0	0	0
34	34	7962	1	0	0	0	1	0	1	0	1
35	35	16200	0	0	0	0	0	0	0	0	0
36	36	0	0	0	0	0	0	0	0	0	0
37	37	7714	2	2	4	3	0	0	4	7	11
38	38	5749	7	5	7	4	0	1	12	11	23
39	39	32133	10	0	1	0	3	0	10	1	11
40	40	2047	2	0	3	0	1	0	2	3	5
41	41	110174	3	1	5	1	0	0	4	6	10
42	42	478	0	0	0	1	0	0	0	1	1
43	43	2968	0	3	0	0	0	0	3	0	3
44	44	27947	8	15	6	5	3	2	23	11	34
45	45	6087	0	5	0	5	0	0	5	5	10
46	46	21207	2	18	13	1	1	1	20	14	34
47	47	1043	1	16	1	5	0	0	11	6	17

48	48	1843	0	2	0	3	0	0	2	3	5
49	49	5736	4	0	6	0	0	0	4	6	10
50	50	3461	0	0	0	0	0	0	0	0	0
51	51	34313	21	0	11	0	5	0	21	11	32
52	52	0	9	0	4	0	1	0	9	4	13
53	53	49	4	0	1	0	0	0	4	1	5
54	54	896	0	1	0	2	0	0	1	2	3
55	55	7375	*	*	*	*	*	*	*	*	*
56	56	0	0	0	0	0	0	0	0	0	0
57	57	155	1	0	2	0	1	0	1	2	3
58	58	0	0	0	0	0	0	0	0	0	0
59	59	22338	1	0	2	0	0	0	1	2	3
60	60	973	1	0	1	0	7	0	1	1	2
61	61	1304	*	*	0	*	*	0	*	*	*
62	62	0	0	0	0	0	0	0	0	0	0
63	63	0	0	0	0	0	0	0	0	0	0
64	64	2396	2	*	*	4	*	*	*	*	*
65	65	*	*	*	*	*	*	*	*	*	*
66	66	0	0	0	0	0	0	0	0	0	0
67	67	9965	0	4	4	0	0	0	4	4	8
68	68	0	*	*	0	*	*	0	*	*	*
69	69	8556	6	0	5	0	0	0	6	5	11
70	70	59	0	0	0	0	0	0	0	0	0
71	71	560	8	0	4	0	1	0	8	4	12
72	72	261	0	0	0	0	0	0	0	0	0
73	73	1853	2	1	0	0	0	0	3	0	3
74	74	2892	*	*	*	*	*	*	*	*	*
75	75	16	1	0	0	0	2	0	1	0	1
76	76	20970	4	2	1	1	0	0	6	2	8
77	77	0	0	0	9	0	0	0	0	9	9
78	78	4403	7	0	2	0	1	0	7	2	9
79	79	0	*	*	0	*	*	0	*	*	*
80	80	8	0	0	0	0	1	0	0	0	0
81	81	1174	2	0	1	0	0	0	2	1	3
82	82	0	0	0	0	0	0	0	0	0	0
83	83	20400	2	0	3	0	0	0	2	3	5
84	84	4766	5	0	3	0	1	0	5	3	8
85	85	5405	2	3	1	0	2	0	5	1	6
86	86	11250	0	1	0	1	0	1	1	1	2
87	87	13182	*	*	*	*	*	*	*	*	*
88	88	49720	0	0	0	0	0	0	0	0	0
89	89	796	0	1	0	1	0	3	1	1	2
90	90	1050	0	2	0	2	0	0	2	2	4
91	91	14030	1	7	0	5	0	0	8	5	13
92	92	31217	0	0	0	0	0	0	0	0	0
93	93	12286	1	0	1	0	1	0	1	1	2
94	94	6807	5	0	3	0	0	0	5	3	8
95	95	*	*	*	*	*	*	*	*	*	*
96	96	3666	3	0	2	0	2	0	3	2	5
97	97	*	*	*	*	*	5	0	*	*	*
98	98	91604	10	6	11	6	3	3	16	17	33
99	99	0	0	0	0	0	4	0	0	0	0
100	100	7321	0	4	0	4	0	0	4	4	8
101	101	3406	9	0	9	0	1	0	9	9	18
102	102	3616	13	0	13	0	1	0	13	13	26
103	103	4274	*	*	12	*	*	15	*	*	*
104	104	17696	17	1	17	0	1	0	18	17	35
105	105	7631	5	0	3	0	0	0	5	3	8
106	106	*	*	*	*	*	6	*	*	*	*
107	107	20784	*	*	2	*	*	0	*	*	*
108	108	19226	4	0	6	0	0	0	4	6	10
109	109	*	*	*	*	*	*	*	*	*	*
110	110	*	*	*	*	*	0	1	*	*	*
111	111	11539	*	*	*	*	*	*	*	*	*
112	112	*	*	*	*	*	0	0	*	*	*
113	113	1584	2	1	2	1	0	0	3	3	6

114	114	*	*	*	*	*	*	*	*	*	*
115	115	14	*	*	*	*	*	*	*	*	*
116	116	8979	*	*	*	*	*	*	*	*	*
117	117	8	*	*	0	*	*	0	*	*	*
118	118	0	0	0	0	0	0	0	0	0	0
119	119	1438	5	0	5	0	0	0	5	5	10
120	120	3471	0	1	0	0	0	1	1	0	1
121	121	1508	*	*	4	*	*	6	*	*	*
122	122	3150	15	0	14	0	2	0	15	14	29
123	123	0	1	0	1	0	0	0	1	1	2
124	124	127	0	0	0	0	0	0	0	0	0
125	125	5946	0	19	0	15	0	1	19	15	34
126	126	13757	13	0	13	0	4	0	13	13	26
127	127	4496	3	4	6	2	5	3	7	8	15
128	128	*	*	*	*	*	*	*	*	*	*
129	129	2189	4	1	2	0	0	0	5	2	7
130	130	4054	*	*	*	*	*	*	*	*	*
131	131	329	0	0	0	0	0	0	0	0	0
132	132	2000	8	0	12	0	2	0	8	12	20
133	133	60	5	0	2	0	0	0	5	2	7
134	134	156	0	0	0	0	0	0	0	0	0
135	135	989	2	0	3	0	0	0	2	3	5
136	136	0	1	0	0	0	0	0	1	0	1
137	137	*	*	*	*	0	7	0	*	*	*
138	138	12829	8	0	11	0	0	0	8	11	19
139	139	0	0	0	0	0	0	0	0	0	0
140	140	*	*	*	*	*	0	0	*	*	*
141	141	0	0	0	1	0	0	0	0	1	1
142	142	2720	0	0	0	0	0	1	0	0	0
143	143	3350	1	0	5	0	4	0	1	5	6
144	144	11604	2	0	3	0	1	0	2	3	5
145	145	0	0	0	1	0	1	0	0	1	1
146	146	4738	*	*	5	*	*	5	*	*	*
147	147	17369	0	0	0	0	0	0	0	0	0
148	148	5915	7	0	1	0	0	0	7	1	8
149	149	*	*	*	*	1	0	1	*	*	*
150	150	1188	0	0	0	0	0	0	0	0	0
151	151	1195	1	0	1	0	0	0	1	1	2
152	152	3306	5	0	5	0	0	0	5	5	10
153	153	11772	0	21	0	16	0	0	21	16	37
154	154	5457	1	0	5	0	1	0	1	5	6
155	155	10871	3	0	1	0	0	0	3	1	4
156	156	2810	0	6	0	4	0	1	6	4	10
157	157	904	1	0	2	0	0	0	1	2	3
158	158	*	*	*	*	0	0	0	*	*	*
159	159	*	*	*	*	0	0	0	*	*	*
160	160	*	*	*	*	0	4	0	*	*	*
161	161	9636	4	7	7	8	0	2	11	15	26
162	162	10883	9	3	15	0	1	1	12	15	27
163	163	13612	8	0	5	0	3	0	8	5	13
164	164	40546	18	1	16	0	2	0	19	16	35
165	165	11672	13	0	12	0	0	0	13	12	25
166	166	4161	0	2	1	1	0	3	2	2	4
167	167	13127	0	0	0	0	0	0	0	0	0
168	168	3195	3	0	2	0	1	0	3	2	5
169	169	0	0	0	0	0	0	0	0	0	0
170	170	0	0	0	0	0	2	0	0	0	0
171	171	6181	22	0	12	0	4	0	22	12	34
172	172	719	0	0	2	0	0	0	0	2	2
173	173	2740	5	0	8	0	1	0	5	8	13
174	174	9963	10	0	4	0	0	0	10	4	14
175	175	13749	17	0	6	0	0	0	17	6	23
176	176	8700	2	3	1	3	0	0	5	4	9
177	177	11223	4	6	6	6	0	0	4	6	10

178	178	20747	0	10	0	8	1	6	10	8	19
179	179	24238	0	5	0	3	0	4	5	3	8
180	180	1524	0	0	0	0	0	0	0	0	0
181	181	18967	1	13	0	6	2	0	14	6	20
182	182	4986	1	0	0	0	0	0	1	0	1
183	183	19531	12	0	11	0	0	0	12	11	23
184	184	9664	1	2	1	0	0	0	3	1	4
185	185	968	7	0	6	0	2	0	7	6	13
186	186	10639	0	0	0	0	0	0	0	0	0
187	187	10959	*	*	1	*	*	1	*	*	*
188	188	4016	10	*	*	11	*	*	*	*	*
189	189	10268	13	6	3	9	2	5	19	12	31
190	190	1027	0	3	0	0	0	1	3	0	3
191	191	486	5	0	0	0	0	0	5	0	5
192	192	0	*	*	0	*	*	0	*	*	*
193	193	22085	*	*	*	*	*	*	*	*	*
194	194	3878	0	1	0	1	0	0	1	1	2
195	195	*	*	*	*	*	0	0	*	*	*
196	196	7970	9	0	8	0	1	0	9	8	17
197	197	121290	0	0	1	0	0	0	0	1	1
198	198	0	0	0	1	0	1	0	0	1	1
199	199	2309	6	0	1	0	0	0	6	1	7
200	200	2706	0	2	0	2	0	2	2	2	4
201	201	17500	4	0	6	0	1	0	4	6	10
202	202	4246	6	0	5	0	3	0	6	5	11
203	203	22623	12	4	9	3	5	3	16	12	28
204	204	2979	10	0	7	0	0	0	10	7	17
205	205	19284	0	14	0	6	0	0	14	6	20
206	206	14856	3	0	1	0	2	0	3	1	4
207	207	0	0	0	0	0	0	0	0	0	0
208	208	*	*	*	*	*	3	0	*	*	*
209	209	23055	2	0	7	0	2	0	2	7	9
210	210	3413	3	0	1	0	0	0	3	1	4
211	211	0	0	0	0	0	0	0	0	0	0
212	212	0	0	*	*	0	*	*	*	*	*
213	213	3033	4	0	8	0	1	0	4	8	12
214	214	39694	12	0	8	0	0	0	12	8	20
215	215	4061	0	1	0	6	0	0	1	6	7
216	216	0	0	0	0	0	0	0	0	0	0
217	217	4030	17	1	2	0	1	0	18	2	20
218	218	0	0	0	0	0	0	0	0	0	0
219	219	12382	6	0	6	0	0	0	6	6	12
220	220	1193	0	2	0	5	0	0	2	5	7
221	221	3746	0	0	0	0	0	0	0	0	0
222	222	0	0	0	0	0	1	0	0	0	0
223	223	47	2	0	3	0	8	0	2	3	5
224	224	12689	5	3	14	0	1	0	8	14	22
225	225	3067	2	18	2	14	2	1	20	16	36
226	226	7687	3	0	3	0	1	0	3	3	6
227	227	0	0	0	0	0	0	0	0	0	0
228	228	5435	7	1	8	0	0	0	8	8	16
229	229	1589	5	2	1	2	0	0	7	3	10
230	230	4081	0	4	0	6	0	1	4	6	10
231	231	17972	3	0	1	0	3	0	3	1	4
232	232	2085	1	0	3	0	2	0	1	3	4
233	233	10839	3	0	4	0	1	1	3	4	7
234	234	792	0	3	0	0	0	0	3	0	3
235	235	2139	2	0	5	0	0	0	2	5	7
236	236	536	1	0	1	0	2	0	1	1	2
237	237	13646	7	0	12	0	0	0	7	12	19
238	238	6123	6	1	5	0	0	0	7	5	12
239	239	15134	3	16	11	27	2	5	19	38	57
240	240	11537	0	0	0	4	0	0	0	4	4
241	241	2812	95	0	86	0	15	0	95	56	151

242	242	67	0	0	0	0	0	0	0	0	0
243	243	5252	0	*	*	0	*	*	*	*	*
244	244	867	2	10	1	5	2	3	12	6	18
245	245	*	*	*	*	*	*	*	*	*	*
246	246	0	0	3	0	0	0	0	3	0	3
247	247	21634	45	4	44	2	8	0	49	46	95
248	248	*	*	*	*	*	*	*	*	*	*
249	249	1957	3	0	0	0	1	0	3	0	3
250	250	12675	7	0	7	0	0	0	7	7	14
251	251	0	0	0	0	0	0	0	0	0	0
252	252	18467	5	2	6	3	2	1	7	9	16
253	253	458	6	0	5	0	1	0	6	5	11
254	254	3797	17	0	18	0	8	0	17	18	35
255	255	320	0	0	0	0	0	0	0	0	0
256	256	1333	1	0	4	0	0	0	1	4	5
257	257	*	*	*	*	*	*	*	*	*	*
258	258	10533	6	*	*	13	*	*	*	*	*
259	259	28394	14	0	16	0	2	0	14	16	30
260	260	7264	8	0	11	0	1	0	8	11	19
261	261	8286	5	0	5	0	0	0	5	5	10
262	262	*	*	*	*	10	0	7	*	*	*
263	263	85	1	0	1	0	3	1	1	1	2
264	264	2308	0	0	0	0	0	0	0	0	0
265	265	37624	6	0	5	0	0	0	6	5	11
266	266	10858	7	0	13	1	0	1	7	14	21
267	267	725	2	0	8	0	0	0	2	8	10
268	268	29	0	0	4	0	0	0	0	4	4
269	269	5113	10	*	*	17	*	*	*	*	*
270	270	0	0	0	0	0	0	0	0	0	0
271	271	9753	23	0	10	0	4	0	23	10	33
272	272	32883	14	0	13	0	0	0	14	13	27
273	273	*	*	*	*	0	0	0	*	*	*
274	274	0	0	0	0	0	0	0	0	0	0
275	275	44139	7	3	9	0	1	0	10	9	19
276	276	47815	9	0	4	0	9	0	9	4	13
277	277	*	*	*	*	*	*	*	*	*	*
278	278	22052	13	0	6	0	3	0	13	6	19
279	279	41250	0	8	0	11	0	3	8	11	19
280	280	5550	4	0	2	9	0	2	4	11	15
281	281	839	7	0	9	0	2	0	7	9	16
282	282	390	2	0	0	0	0	0	2	0	2
283	283	*	*	*	*	0	0	0	*	*	*
284	284	13975	5	0	6	0	1	0	5	6	11
285	285	5151	5	0	7	0	0	0	5	7	12
286	286	38305	9	0	7	0	0	0	9	7	16
287	287	42675	0	0	0	0	0	0	0	0	0
288	288	0	0	0	2	0	0	0	0	2	2
289	289	11060	2	0	3	0	1	0	2	3	5
290	290	5495	0	0	0	0	0	0	0	0	0
291	291	29567	22	0	18	0	2	0	22	18	40
292	292	*	*	*	*	*	0	0	*	*	*
293	293	20627	5	0	2	0	0	0	5	2	7
294	294	17151	9	*	*	7	*	*	*	*	*
295	295	49193	6	17	3	18	0	1	23	21	44
296	296	19911	6	0	10	0	6	0	6	10	16
297	297	36087	0	5	5	2	2	0	5	7	12
298	298	13305	14	0	11	0	0	0	14	11	25
299	299	3268	0	2	0	2	0	1	2	2	4
300	300	7637	0	1	0	2	0	0	1	2	3
301	301	*	*	*	*	1	0	0	*	*	*
302	302	8668	14	0	8	0	2	0	14	8	22
303	303	8040	*	*	0	*	*	0	*	*	*
304	304	0	0	0	0	0	0	0	0	0	0
305	305	8478	0	5	0	7	0	0	5	7	0

306	306	6503	0	0	0	0	0	0	0	0	0
307	307	24477	11	0	5	0	5	0	11	5	16
308	308	*	*	*	*	*	*	*	*	*	*
309	309	14879	4	3	3	6	0	3	7	9	16
310	310	44533	0	0	0	0	0	0	0	0	0
311	311	9083	0	0	0	0	0	0	0	0	0
312	312	4431	2	0	1	0	3	0	2	1	3
313	313	42	0	0	0	0	0	0	0	0	0
314	314	3295	7	0	2	0	2	0	7	2	9
315	315	9205	7	2	4	0	0	3	9	4	13
316	316	6361	10	0	9	0	0	0	10	9	19
317	317	15008	0	2	0	5	0	0	2	5	7
318	318	18878	5	0	5	0	1	0	5	5	10
319	319	11468	2	1	2	0	2	0	3	2	5
320	320	2842	0	0	0	0	0	0	0	0	0
321	321	5682	4	1	5	1	0	0	5	6	11
322	322	16757	12	0	13	1	0	0	12	14	26
323	323	0	0	0	0	0	0	0	0	0	0
324	324	7200	0	3	0	2	0	0	3	2	5
325	325	*	*	*	*	*	2	0	*	*	*
326	326	93699	0	0	0	0	0	0	0	0	0
327	327	9750	11	0	11	0	1	0	11	11	22
328	328	10028	2	4	0	2	0	0	6	2	8
329	329	20773	0	0	1	0	0	0	0	1	1
330	330	*	*	*	*	5	0	3	*	*	*
331	331	21120	7	3	12	1	3	1	10	13	23
332	332	7328	0	5	0	5	0	6	5	5	10
333	333	8168	1	0	1	0	0	0	1	1	2
334	334	8498	4	0	8	0	4	0	4	8	12
335	335	714	6	0	3	0	0	0	6	3	9
336	336	4432	0	0	0	0	0	0	0	0	0
337	337	3600	8	0	12	0	4	0	8	12	20
338	338	5303	0	0	0	0	0	0	0	0	0
339	339	11967	*	*	*	*	*	*	*	*	*
340	340	7355	0	8	0	9	0	0	8	9	17
341	341	4667	3	0	2	0	1	0	3	2	5
342	342	28644	0	7	0	4	0	0	7	4	11
343	343	0	0	1	0	1	0	0	1	1	2
344	344	*	*	*	*	*	14	2	*	*	*
345	345	502	1	*	*	4	*	*	*	*	*
346	346	12053	10	3	8	4	5	1	13	12	25
347	347	13200	5	0	1	0	0	0	5	1	6
348	348	22053	11	1	7	0	0	0	12	7	19
349	349	12182	6	0	6	0	0	0	6	6	12
350	350	*	*	*	*	*	6	0	*	*	*
351	351	36624	9	27	7	26	2	10	36	33	69
352	352	2103	1	0	3	0	0	0	1	3	4
353	353	12115	2	0	0	0	0	0	2	0	2
354	354	15255	15	0	14	0	1	0	15	14	29
355	355	6804	2	0	6	0	0	0	2	6	8
356	356	3200	0	4	0	4	0	0	4	4	8
357	357	7522	0	0	0	0	0	0	0	0	0
358	358	12720	3	0	3	0	0	0	3	3	6
359	359	4947	0	1	6	0	0	0	1	6	7
360	360	28904	5	4	5	1	0	1	9	6	15
361	361	9092	2	7	0	8	0	2	9	8	17
362	362	2472	4	0	7	0	2	0	4	7	11
363	363	22378	7	0	3	0	1	0	7	3	10
364	364	0	0	0	0	0	0	0	0	0	0
365	365	1681	6	1	8	0	1	0	7	8	15
366	366	24670	8	0	11	0	0	0	8	11	19
367	367	3346	2	0	3	0	1	0	2	3	5
368	368	48	0	0	0	0	0	0	0	0	0
369	369	4615	7	0	3	0	0	0	7	3	10

370	370	3912	0	4	0	3	0	2	4	3	7
371	371	44080	4	11	8	8	0	3	15	16	31
372	372	4126	8	*	*	5	*	*	*	*	*
373	373	26954	5	0	4	0	0	0	5	4	9
374	374	22425	6	0	7	0	1	0	6	7	13
375	375	22033	0	0	0	0	0	0	0	0	0
376	376	25225	4	0	5	0	1	0	4	5	9
377	377	5686	0	0	0	0	0	0	0	0	0
378	378	*	*	*	*	*	*	*	*	*	*
379	379	3895	5	1	3	0	0	0	6	3	9
380	380	12315	3	0	7	0	1	0	3	7	10
381	381	21000	5	0	10	0	2	0	5	10	15
382	382	*	*	*	*	*	*	*	*	*	*
383	383	24138	2	0	1	0	0	0	2	1	3
384	384	825	0	0	0	0	0	0	0	0	0
385	385	*	*	*	*	*	*	*	*	*	*
386	386	5123	7	0	2	0	4	0	7	2	9
387	387	1781	0	0	0	0	0	0	0	0	0
388	388	4100	0	0	0	0	0	0	0	0	0
389	389	14034	5	0	4	0	1	0	5	4	9
390	390	15807	4	0	3	0	1	0	4	3	7
391	391	18923	6	0	8	0	1	0	6	8	14
392	392	13792	0	2	0	6	0	0	2	6	8
393	393	2817	0	17	0	7	0	0	17	7	24
394	394	15881	0	11	0	7	0	2	11	7	18
395	395	0	0	0	4	0	1	0	0	4	4
396	396	7019	4	0	3	0	0	0	4	3	7
397	397	866	8	0	6	0	0	0	8	6	14
398	398	10343	9	0	6	1	2	0	9	7	16
399	399	0	0	0	1	0	1	0	0	1	1
400	400	34510	0	0	0	0	0	0	0	0	0
401	410	770	0	*	*	0	*	*	*	*	*
402	402	26177	1	3	0	0	0	0	4	0	4
403	403	45349	4	0	4	0	1	0	4	4	8
404	404	14400	7	0	3	0	0	0	7	3	10
405	405	310	0	0	1	0	2	0	0	1	1
406	406	2296	0	0	0	0	0	0	0	0	0
407	407	5365	0	0	0	0	0	0	0	0	0
408	408	*	*	*	*	*	1	2	*	*	*
409	409	28963	8	2	13	0	1	1	10	13	23
410	410	3245	0	0	0	0	0	0	0	0	0
411	411	285	5	1	6	0	0	0	6	6	12
412	412	1385	0	0	1	0	0	0	0	1	1
413	413	0	0	0	0	0	0	0	0	0	0
414	414	16474	0	4	0	10	0	0	4	10	14
415	415	5809	0	1	0	3	0	0	1	3	4
416	416	*	*	*	*	15	0	16	*	*	*
417	417	27785	9	0	16	0	1	1	9	16	25
418	418	8853	4	3	0	9	0	1	7	9	16
419	419	*	*	*	*	*	3	4	*	*	*
420	420	62457	15	0	15	0	0	0	15	15	30
421	421	4941	0	0	0	0	0	0	0	0	0
422	422	27600	0	1	0	0	0	0	1	0	1
423	423	0	0	0	1	0	0	0	0	1	1
424	424	15235	4	0	1	0	0	0	4	1	5
425	425	97183	5	0	2	0	0	0	5	2	7
426	426	*	*	*	*	*	*	*	*	*	*
427	427	72055	0	0	0	0	0	0	0	0	0
428	428	31646	18	0	24	0	4	0	18	24	42
429	429	25978	1	0	4	0	3	0	1	4	5
430	430	39170	0	5	0	3	0	1	5	3	8
431	431	13362	0	2	0	2	0	0	2	2	4
432	432	32016	9	2	6	2	2	0	11	8	19
433	433	*	*	*	*	*	2	1	*	*	*
434	434	49463	0	2	1	0	2	0	2	1	3
435	435	4985	7	0	11	0	4	0	7	11	18

436	436	13227	6	*	*	7	*	*	*	*	*
437	437	59341	17	3	15	1	1	0	20	16	36
438	438	7583	0	0	0	0	0	0	0	0	0
439	439	13904	7	0	3	0	1	0	7	3	10
440	440	16200	0	*	*	0	*	*	*	*	*
441	441	1233	2	0	1	0	1	0	2	1	3
442	442	197	3	0	0	0	1	0	3	0	3
443	443	4407	8	0	5	0	0	0	8	5	13
444	444	6268	0	*	*	0	*	*	*	*	*
445	445	21302	8	1	5	2	1	0	9	7	16
446	446	39044	7	0	3	0	0	0	7	3	10
447	447	0	0	0	0	0	0	0	0	0	0
448	448	811	2	0	1	0	1	0	2	1	3
449	449	153	0	0	0	0	0	0	0	0	0
450	450	0	0	0	1	0	1	0	0	1	1
451	451	31687	7	0	16	0	1	0	7	16	23
452	452	2412	2	*	*	6	*	*	*	*	*
453	453	96298	0	3	0	1	0	0	3	1	4
454	454	337	0	*	*	0	*	*	*	*	*
455	455	4381	0	1	0	0	0	0	1	0	1
456	456	*	*	*	*	1	0	1	*	*	*
457	457	2559	2	0	6	0	0	0	2	6	8
458	458	6063	8	4	17	1	2	1	12	18	30
459	459	4989	0	3	0	1	0	1	3	1	4
460	460	4791	1	0	2	0	0	0	1	2	3
461	461	7577	5	*	*	2	*	*	*	*	*
462	462	19684	3	0	4	0	1	0	3	4	7
463	463	3214	*	*	*	*	*	*	*	*	*
464	464	28933	15	0	6	0	2	0	15	6	21
465	465	11317	0	5	0	3	0	2	5	3	8
466	466	19370	0	2	0	0	0	0	2	0	2
467	467	18857	0	0	0	0	0	0	0	0	0
468	468	*	*	*	*	4	2	0	*	*	*
469	469	*	*	*	*	*	*	*	*	*	*
470	470	13138	1	0	3	0	2	0	1	3	4
471	471	7008	1	2	2	4	0	1	3	6	9
472	472	3500	0	0	0	0	0	0	0	0	0
473	473	5628	0	0	13	0	5	0	0	13	13
474	474	*	*	*	*	*	7	0	*	*	*
475	475	7067	2	0	1	0	0	0	2	1	3
476	476	2483	2	0	3	0	0	0	2	3	5
477	477	1588	1	6	0	9	0	0	7	9	16
478	478	*	*	*	*	*	3	0	*	*	*
479	479	9850	6	0	1	0	0	0	6	1	7
480	480	3941	10	2	6	6	4	0	12	12	24
481	481	562	0	0	0	0	0	1	0	0	0
482	482	3750	7	0	1	0	0	0	7	1	8
483	483	*	*	*	*	*	*	*	*	*	*
484	484	10964	4	0	1	0	1	0	4	1	5
485	485	4773	7	1	6	0	0	0	8	6	14
486	486	4311	2	0	0	0	0	0	2	0	2
487	487	0	0	0	0	0	0	0	0	0	0
488	488	3485	3	0	4	0	2	0	3	4	7
489	489	50759	2	2	1	0	0	0	4	1	5
490	490	117106	21	0	29	0	0	0	21	29	50
491	491	*	*	*	*	*	*	0	*	*	*
492	492	*	*	*	*	*	0	0	*	*	*
493	493	*	*	*	*	*	7	*	*	*	*
494	494	12911	4	0	6	1	3	0	4	7	11
495	495	44441	10	0	6	0	0	6	10	6	16
496	496	*	*	*	*	*	1	0	*	*	*
497	497	0	0	0	0	0	0	0	0	0	0
498	498	50444	6	0	2	0	0	0	6	2	8
499	499	16868	9	0	5	0	2	0	9	5	14

500	500	1115	8	0	12	6	1	0	8	12	20
501	501	2462	0	0	1	0	1	0	0	1	1
502	502	11938	11	0	15	0	3	0	11	15	26
503	503	0	0	0	0	0	0	0	0	0	0
504	504	16580	11	0	8	0	1	0	11	8	19
505	505	18250	1	0	2	0	0	0	1	2	3
506	506	11339	5	0	6	0	1	0	5	6	11
507	507	0	0	0	0	0	1	0	0	0	0
508	508	27150	6	0	9	0	1	0	6	9	15
509	509	2133	1	*	*	4	*	*	*	*	*
510	510	8400	0	3	0	1	0	0	3	1	4
511	511	9845	3	0	8	0	3	0	3	8	11
512	512	5139	9	0	8	0	3	0	9	8	17
513	513	112436	2	0	5	0	0	0	2	5	7
514	514	3200	27	0	14	0	4	0	27	14	41
515	515	14078	0	9	0	8	0	0	9	8	17
516	516	13200	5	0	4	0	3	0	5	4	9
517	517	*	*	*	*	*	*	*	*	*	*
518	518	10800	17	0	9	0	1	0	17	9	26
519	519	17750	7	0	9	0	0	0	7	9	16
520	520	1615	3	0	2	0	1	0	3	2	5
521	521	11020	0	1	0	0	0	0	1	0	1
522	522	0	0	0	0	0	0	0	0	0	0
523	523	2249	1	0	2	0	0	0	1	2	3
524	524	*	*	*	*	*	*	*	*	*	*
525	525	885	1	0	4	0	0	0	1	4	5
526	526	0	0	0	0	0	0	0	0	0	0
527	527	7956	5	0	0	0	0	0	5	0	5
528	528	3483	1	0	2	0	2	0	1	2	3
529	529	31974	4	0	7	0	1	0	4	7	11
530	530	6067	3	0	0	0	1	0	3	0	3
531	531	27074	*	*	*	*	*	*	*	*	*
532	532	*	*	*	*	*	*	*	*	*	*
533	533	25748	10	0	5	0	1	0	10	5	15
534	534	4873	4	*	*	3	*	*	*	*	*
535	535	33827	0	0	15	0	3	0	0	15	15
536	536	10043	2	0	0	0	0	0	2	0	2
537	537	*	*	*	*	*	*	*	*	*	*
538	538	43321	5	1	3	0	0	0	6	3	9
539	539	*	*	*	*	*	*	*	*	*	*
540	540	2090	0	0	0	0	0	0	0	0	0
541	541	*	*	*	*	*	*	*	*	*	*
542	542	24811	10	0	13	0	2	0	10	13	23
543	543	9800	13	0	20	0	1	0	13	20	33
544	544	*	*	*	*	*	*	*	*	*	*
545	545	0	0	*	*	0	*	*	*	*	*
546	546	0	0	2	0	1	1	0	2	1	3
547	547	33636	1	*	*	1	*	*	*	*	*
548	548	2587	4	0	7	0	1	0	4	7	11
549	549	*	*	*	*	*	*	*	*	*	*
550	550	9814	10	0	5	0	0	0	10	5	15
551	551	5000	0	0	0	0	0	0	0	0	0
552	552	14261	2	3	0	1	0	0	5	1	6
553	553	37936	7	0	6	0	0	0	7	6	13
554	554	678	2	0	1	0	2	0	2	1	3
555	555	6130	4	0	10	0	1	0	4	10	14
556	556	21600	4	0	5	0	3	0	4	5	9
557	557	*	*	*	*	*	1	9	*	*	*
558	558	64440	0	9	0	7	0	0	9	7	16
559	559	*	*	*	*	*	*	*	*	*	*
560	560	9200	0	0	0	0	0	0	0	0	0
561	561	29374	7	0	6	0	0	0	7	6	13
562	562	0	0	0	3	0	0	0	0	3	3
563	563	7585	0	4	0	1	0	0	4	1	5

564	564	5610	3	0	0	0	0	0	3	0	3
565	565	2943	4	0	5	0	0	0	4	5	9
566	566	5670	1	0	4	0	0	0	1	4	5
567	567	4835	1	0	8	0	0	0	1	8	9
568	568	*	*	*	2	*	*	1	*	*	*
569	569	29889	10	0	7	0	2	0	10	7	17
570	570	*	*	*	*	0	*	*	*	*	*
571	571	12152	5	0	12	0	0	0	5	12	17
572	572	14740	11	0	13	0	0	0	11	13	24
573	573	0	1	0	0	0	0	0	1	0	1
574	574	24608	14	2	8	0	0	0	16	8	24
575	575	1316	0	0	0	0	0	0	0	0	0
576	576	*	*	*	*	8	*	*	*	*	*
577	577	16875	10	0	10	0	4	0	10	10	20
578	578	1141	6	0	4	0	0	0	6	4	10
579	579	11467	0	3	0	4	0	0	3	4	7
580	580	*	*	*	*	5	0	1	*	*	*
581	581	5207	*	*	*	*	*	*	*	*	*
582	582	7639	6	2	5	0	0	0	8	5	13
583	583	15716	4	0	3	0	0	0	4	3	7
584	584	10730	0	4	0	6	0	0	4	6	10
585	585	3260	3	0	4	0	0	0	3	4	7
586	586	54455	4	0	4	0	1	0	4	4	8
587	587	10555	2	0	0	0	0	0	2	0	2
588	588	19379	0	9	0	9	0	0	9	9	18
589	589	3214	5	0	3	0	0	0	5	3	8
590	590	*	*	*	*	*	2	0	*	*	*
591	591	3648	0	0	0	0	0	0	0	0	0
592	592	13328	4	0	5	0	0	0	4	5	9
593	593	53186	39	5	28	2	9	2	44	30	74
594	594	0	0	0	0	0	0	0	0	0	0
595	595	35525	8	0	4	0	0	0	8	4	12
596	596	6480	0	3	0	4	0	1	3	4	7
597	597	10136	8	4	6	0	3	2	12	6	18
598	598	*	*	*	*	*	0	2	*	*	*
599	599	0	0	1	0	0	0	0	1	0	1
600	600	30227	5	0	0	0	1	0	5	0	5
601	601	1371	1	0	3	0	0	0	1	3	4
602	602	4059	4	0	2	0	2	0	4	2	6
603	603	1550	6	0	0	1	0	0	6	1	7
604	604	11576	0	5	0	6	0	0	5	6	11
605	605	*	*	*	*	*	*	*	*	*	*
606	606	0	0	0	0	0	0	0	0	0	0
607	607	400	0	*	*	0	*	*	*	*	*
608	608	31033	0	13	0	24	0	0	13	24	37
609	609	*	*	*	*	*	*	*	*	*	*
610	610	15088	0	10	0	15	0	2	10	15	25
611	611	5204	1	1	1	0	1	1	2	1	3
612	612	49593	7	3	3	0	3	0	10	3	13
613	613	54194	6	0	7	0	0	0	6	7	13
614	614	0	0	0	0	0	0	0	0	0	0
615	615	0	1	0	0	0	0	0	1	0	1
616	616	7800	4	0	6	0	1	0	4	6	10
617	617	21152	3	0	2	0	1	1	3	2	5
618	618	17044	4	0	4	0	2	0	4	4	8
619	619	*	*	*	*	*	*	*	*	*	*
620	620	197	2	0	0	0	1	0	2	0	2
621	621	2190	*	*	0	*	*	0	*	*	*
622	622	*	*	*	*	*	*	*	*	*	*
623	623	21097	0	6	0	16	0	1	6	10	16
624	624	9206	4	0	3	0	0	0	4	3	7
625	625	95	0	0	0	0	1	0	0	0	0
626	626	2250	0	0	0	0	0	0	0	0	0
627	627	13500	6	0	3	0	1	0	6	3	9

628	628	7308	0	2	0	3	0	0	2	3	5
629	629	*	*	*	*	*	0	0	*	*	*
630	630	11200	0	3	0	2	0	0	3	2	5
631	631	10750	3	0	3	0	0	0	3	3	6
632	632	28953	7	0	3	0	0	0	7	3	10
633	633	270	0	0	0	0	0	0	0	0	0
634	634	2667	0	1	0	0	0	0	1	0	1
635	635	1612	7	1	5	0	1	0	8	5	13
636	636	30952	14	3	19	1	4	0	17	20	37
637	637	*	*	*	*	*	*	*	*	*	*
638	638	40079	14	0	14	1	5	0	14	15	29
639	639	6095	0	6	0	1	0	1	6	1	7
640	640	9653	4	0	2	0	3	0	4	2	6
641	641	0	0	0	0	0	0	0	0	0	0
642	642	4480	0	*	*	0	*	*	*	*	*
643	643	*	*	*	*	*	6	0	*	*	*
644	644	13722	7	0	8	1	0	0	7	9	16
645	645	48141	2	0	1	0	4	0	2	1	3
646	646	21333	12	0	6	1	3	0	12	7	19
647	647	14072	0	6	1	1	1	0	6	2	8
648	648	7100	0	2	0	3	0	0	2	3	5
649	649	0	0	0	0	0	3	0	0	0	0
650	650	0	*	*	0	*	*	0	*	*	*
651	651	42400	*	*	17	*	*	8	*	*	*
652	652	*	*	*	*	*	*	*	*	*	*
653	653	1380	0	0	0	0	0	0	0	0	0
654	654	3273	0	6	0	10	0	0	6	10	16
655	655	*	*	*	*	*	*	*	*	*	*
656	656	27306	15	*	*	7	*	*	*	*	*
657	657	5682	1	3	2	1	0	3	4	3	7
658	658	7889	0	0	3	0	2	0	0	3	3
659	659	16052	6	0	6	0	2	0	6	6	12
660	660	8417	2	0	2	0	1	0	2	2	4
661	661	0	0	0	0	0	0	0	0	0	0
662	662	6538	10	0	18	0	0	0	10	18	28
663	663	10619	0	3	0	6	0	0	3	6	9
664	664	1400	0	0	0	0	0	0	0	0	0
665	665	33600	1	10	0	16	0	1	11	16	27
666	666	16380	0	0	0	0	0	0	0	0	0
667	667	44	0	0	0	0	0	0	0	0	0
668	668	554	3	0	1	2	1	0	3	3	6
669	669	19671	3	0	7	0	0	0	3	7	10
670	670	7500	5	0	7	0	2	1	5	7	12
671	671	24701	8	0	5	1	0	2	8	6	14
672	672	296	32	0	27	0	1	0	32	27	59
673	673	118578	5	0	5	0	1	0	5	5	10
674	674	0	0	0	0	0	0	0	0	0	0
675	675	0	0	0	0	0	0	0	0	0	0
676	676	28069	8	0	11	0	2	0	8	11	19
677	677	3654	3	0	5	0	1	0	3	5	8
678	678	12060	5	0	10	0	0	0	5	10	15
679	679	11043	4	0	7	1	3	0	4	8	12
680	680	1336	1	1	1	0	0	0	2	1	3
681	681	0	0	0	0	0	1	0	0	0	0
682	682	*	*	*	*	0	0	3	*	*	*
683	683	43200	11	*	*	5	*	*	*	*	*
684	684	45430	6	*	*	2	*	*	*	*	*
685	685	31445	0	0	0	0	0	0	0	0	0
686	686	91	0	0	0	0	0	0	0	0	0
687	687	5839	3	0	0	0	0	0	3	0	3
688	688	3033	2	0	1	0	1	0	2	1	3
689	689	3126	1	0	2	0	2	0	1	2	3
690	690	38647	9	0	2	0	4	0	9	2	11
691	691	25324	12	6	11	0	7	0	12	11	23

692	692	5987	2	0	1	0	0	0	2	1	3
693	693	46306	1	2	0	3	0	0	3	3	6
694	694	35259	0	0	0	0	0	0	0	0	0
695	695	13587	6	0	1	0	7	0	6	1	7
696	696	0	0	0	0	0	5	0	0	0	0
697	697	4888	0	1	0	3	0	0	1	3	4
698	698	38700	11	0	12	0	1	0	11	12	23
699	699	42806	3	7	2	2	1	0	10	4	14
700	700	19080	3	0	5	0	4	0	3	5	8
701	701	4725	5	0	1	0	1	0	5	1	6
702	702	16596	5	0	9	0	0	0	5	9	14
703	703	10129	1	0	0	0	0	0	1	0	1
704	704	15694	6	0	7	0	1	0	6	7	13
705	705	12274	0	3	0	9	0	0	3	9	12
706	706	33456	19	2	20	0	19	1	21	20	41
707	707	0	12	0	11	0	1	0	12	11	23
708	708	*	*	*	*	*	0	*	*	*	*
709	709	0	0	0	0	0	1	0	0	0	0
710	710	35683	10	0	11	0	1	0	10	11	21
711	711	0	0	0	0	0	0	0	0	0	0
712	712	4868	2	1	2	1	0	1	3	3	6
713	713	21000	7	3	0	0	20	1	10	0	10
714	714	4240	2	0	5	0	0	0	2	5	7
715	715	138	11	0	17	0	1	0	11	17	28
716	716	*	*	*	*	*	0	0	*	*	*
717	717	24072	4	0	3	0	3	0	4	3	7
718	718	7870	6	0	10	0	3	0	6	10	16
719	719	14385	3	0	0	0	0	1	3	0	3
720	720	0	0	0	2	0	0	0	0	2	2
721	721	1681	2	0	0	0	0	0	2	0	2
722	722	7192	1	1	4	0	0	0	2	4	6
723	723	13333	2	0	3	0	8	0	2	3	5
724	724	39555	3	3	6	4	0	0	6	10	16
725	725	6811	4	3	6	0	2	0	7	6	13
726	726	6518	1	0	3	0	1	0	1	3	4
727	727	13612	6	0	2	0	1	0	6	2	8
728	728	10442	18	0	10	0	0	0	18	10	28
729	729	43724	*	*	*	*	*	*	*	*	*
730	730	*	*	*	*	*	2	5	*	*	*
731	731	12914	10	0	9	0	0	0	10	9	19
732	732	7853	2	0	1	0	0	0	2	1	3
733	733	0	0	0	0	0	0	0	0	0	0
734	734	51579	6	0	9	0	2	0	6	9	15
735	735	19705	0	3	0	2	0	0	3	2	5
736	736	3148	17	0	14	1	1	0	17	15	32
737	737	3558	0	4	0	1	0	0	4	1	5
738	738	28190	2	0	0	0	0	0	2	0	2
739	739	45050	13	0	7	0	4	0	13	7	20
740	740	0	0	0	0	0	1	0	0	0	0
741	741	1087	0	0	0	1	0	0	0	1	1
742	742	623	9	0	3	0	0	0	9	3	12
743	743	7819	13	0	28	0	2	0	13	28	41
744	744	12750	7	0	6	0	0	0	7	6	13
745	745	12179	0	0	0	0	0	0	0	0	0
746	746	8875	8	0	11	0	0	0	8	11	19
747	747	36480	8	0	7	0	1	0	8	7	15
748	748	11538	5	0	3	0	0	1	5	3	8
749	749	17167	0	1	0	3	1	3	1	3	4
750	750	12355	14	1	14	0	0	0	15	14	29
751	751	8828	2	0	6	3	1	0	2	9	11
752	752	57295	0	0	0	0	0	0	0	0	0
753	753	*	*	*	*	*	16	0	*	*	*
754	754	0	0	0	0	0	2	0	0	0	0
755	755	19327	17	3	25	1	3	3	20	26	46
756	756	16452	6	0	7	0	1	0	6	7	13
757	757	10138	0	0	0	0	0	0	0	0	0

758	758	*	*	*	*	*	*	*	*	*	*
759	759	17313	0	0	0	0	0	0	0	0	0
760	760	12033	18	0	16	0	5	1	18	16	34
761	761	1751	0	0	0	0	0	0	0	0	0
762	762	2188	*	*	*	*	*	*	*	*	*
763	763	3750	0	0	0	0	0	0	0	0	0
764	764	46200	0	9	0	6	0	0	9	6	15
765	765	0	0	0	0	0	1	0	0	0	0
766	766	*	*	*	*	*	1	*	*	*	*
767	767	18560	2	1	5	1	1	0	3	6	9
768	768	10000	9	0	8	0	2	0	9	8	17
769	769	10528	5	0	2	0	0	0	5	2	7
770	770	19128	7	5	6	1	2	0	12	7	19
771	771	4856	4	0	6	0	0	0	4	6	10
772	772	5423	2	0	3	0	4	0	2	3	5
773	773	22829	5	0	9	0	1	0	5	9	14
774	774	4229	3	0	1	0	1	0	3	1	4
775	775	10018	7	0	1	0	1	0	7	1	8
776	776	12420	9	0	6	0	2	0	9	6	15
777	777	1315	1	0	2	0	0	1	1	2	3
778	778	98479	5	4	5	3	0	1	9	8	17
779	779	0	0	0	1	0	2	0	0	1	1
780	780	10704	0	*	*	0	*	*	*	*	*
781	781	0	0	0	0	0	2	0	0	0	0
782	782	4431	1	0	1	0	1	0	1	1	2
783	783	0	0	0	0	0	0	0	0	0	0
784	784	23658	2	0	2	0	3	0	2	2	4
785	785	13247	1	3	1	1	0	0	4	2	6
786	786	4170	1	0	1	0	1	0	1	1	2
787	787	*	*	*	*	21	*	*	*	*	*
788	788	41073	12	5	8	6	20	2	17	14	31
789	789	17279	2	2	3	2	0	1	4	5	9
790	790	3785	2	0	2	0	0	0	2	2	4
791	791	8259	4	0	7	0	5	0	4	7	11
792	792	90941	21	*	*	7	*	*	*	*	*
793	793	8706	1	*	*	1	*	*	*	*	*
794	794	*	*	*	*	*	*	*	*	*	*
795	795	12847	2	0	2	0	0	0	2	2	4
796	796	910	5	0	1	0	3	0	5	1	6
797	797	17261	13	0	9	0	2	0	13	9	22
798	798	300	0	0	0	0	1	0	0	0	0
799	799	1248	2	*	*	2	*	*	*	*	*
800	800	13541	2	5	1	5	1	0	7	6	13
801	801	*	*	*	*	0	0	0	*	*	*
802	802	*	*	*	*	*	0	5	*	*	*
803	803	12160	4	0	3	0	0	0	4	3	7
804	804	22428	4	0	2	0	3	0	4	2	6
805	805	62182	14	8	9	1	0	0	22	10	32
806	806	16200	0	2	0	2	0	2	2	2	4
807	807	36196	0	7	0	3	0	0	7	3	10
808	808	47667	*	*	*	*	*	*	*	*	*
809	809	17839	2	9	0	13	1	0	11	13	24
810	810	*	*	*	*	*	2	0	*	*	*
811	811	12000	3	0	2	0	3	0	3	2	5
812	812	27225	11	0	6	0	0	0	11	6	17
813	813	40127	0	12	0	12	0	1	12	12	24
814	814	13600	9	0	4	0	9	0	9	4	13
815	815	50434	11	0	10	0	0	0	11	10	21
816	816	9085	1	0	1	0	2	0	1	1	2
817	817	28280	2	5	4	2	2	1	7	6	13
818	818	*	*	*	*	*	11	0	*	*	*
819	819	143433	12	0	17	0	0	0	12	17	29
820	820	29516	11	0	11	0	1	0	11	11	22
821	821	3368	44	3	24	1	20	3	47	25	72

822	822	6536	0	3	0	0	0	0	0	3	3
823	823	16043	4	0	5	0	1	0	4	5	9
824	824	16800	6	10	8	7	0	1	16	15	31
825	825	12347	29	0	22	1	9	1	29	23	52
826	826	2542	3	0	4	0	2	0	3	4	7
827	827	44056	6	0	8	0	2	0	6	8	14
828	828	59	0	0	0	0	0	0	0	0	0
829	829	*	*	*	*	8	0	4	*	*	*
830	830	0	1	0	0	0	0	0	1	0	1
831	831	3714	0	4	0	1	0	1	4	1	5
832	832	24897	5	1	3	0	0	0	6	3	9
833	833	1273	6	0	5	0	0	0	6	5	11
834	834	313	1	0	0	0	0	0	1	0	1
835	835	9375	5	0	2	0	0	0	5	2	7
836	836	10489	5	0	4	0	0	0	5	4	9
837	837	2720	3	0	2	0	2	0	3	2	5
838	838	3653	0	0	0	0	0	0	0	0	0
839	839	2241	3	0	0	0	0	0	3	0	3
840	840	29684	6	5	3	1	0	0	11	4	15
841	841	9396	0	2	0	1	0	0	2	1	3
842	842	45024	7	0	4	0	1	0	7	4	11
843	843	4038	0	0	0	0	0	0	0	0	0
844	844	28812	*	*	*	*	*	*	*	*	*
845	845	20916	7	1	0	9	0	1	8	9	17
846	846	18726	2	2	0	1	0	2	4	1	5
847	847	10543	8	0	4	0	0	1	8	4	12
848	848	0	0	0	0	0	0	0	0	0	0
849	849	14449	11	1	3	0	6	0	12	3	15
835	835	9375	5	0	2	0	0	0	5	2	7
836	836	10489	5	0	4	0	0	0	5	4	9
837	837	2720	3	0	2	0	2	0	3	2	5
838	838	3653	0	0	0	0	0	0	0	0	0
839	839	2241	3	0	0	0	0	0	3	0	3
840	840	29684	6	5	3	1	0	0	11	4	15
841	841	9396	0	2	0	1	0	0	2	1	3
842	842	45024	7	0	4	0	1	0	7	4	11
843	843	4038	0	0	0	0	0	0	0	0	0
844	844	28812	*	*	*	*	*	*	*	*	*
845	845	20916	7	1	0	9	0	1	8	9	17
846	846	18726	2	2	0	1	0	2	4	1	5
847	847	10543	8	0	4	0	0	1	8	4	12
848	848	0	0	0	0	0	0	0	0	0	0
849	849	14449	11	1	3	0	6	0	12	3	15
850	850	1576	1	0	1	0	0	0	1	1	2
851	852	740	1	0	1	0	0	0	1	1	2
852	853	58594	14	0	13	1	0	0	14	14	28
853	854	12653	3	0	4	0	0	0	3	4	7
854	855	18332	6	*	*	2	*	*	*	*	*
855	856	28600	13	*	*	10	*	*	*	*	*
856	857	3935	1	1	2	0	0	0	2	2	4