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Exploring the interaction between roundworms and whipworms by analysing re-infection data from Bangladesh and South India

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Abbreviations

DALY = Disability Adjusted Life Year

EPG = Eggs per gram

MDA = Mass Drug Administration

R&D = Research and Development

STH = Soil-Transmitted Helminth

WHO = World Health Organization

Abstract

***Background**:* Co-infection by two or more of the three most significant soil-transmitted helminths – the roundworm *Ascaris lumbricoides*, the whipworm *Trichuris trichiura* and the hookworms *Necator americanus* and *Ancylostoma duodenale* – is very common in regions where infections by these worms are endemic, especially co-infections of whipworm and roundworm. Given the recent increase of mass drug administration (MDA) of benzimidazole drugs (albendazole and mebendazole) and the relatively low effectiveness of these drugs against *T. trichiura*, it is important to determine whether the expulsion of *A. lumbricoides* worms from a co-infected individual could result in the remaining *T. trichiura* worms gaining a competitive advantage or suffering a negative impact (resulting from antagonistic and synergistic interactions respectively).

***Methodology**:* Data collected on egg counts of *T. trichiura* and egg and worm counts of *A. lumbricoides* in Dhaka, Bangladesh between 1988 and 1990 and in Pulicat, India in 1984 were used to explore whether the proportional change in *T. trichiura* egg counts relative to baseline was altered in any way indicative of interspecific interactions after treatment which removed roundworm populations from infected individuals. First, descriptive statistics such as age-intensity profiles were calculated to show the burden of the worms on different host age and sex groups, then models were constructed to investigate the impact of treatment on whipworm egg counts.

***Results**:* Differences in the age-profiles and prevalences of infection of men and women were noted for *A. lumbricoides,* but not for *T. trichiura,* in the data collected from Bangladesh. No such differences were apparent in the data from South India. The models that were developed showed that whipworm seemed to gain no competitive advantage (or disadvantage) from treatment that expelled roundworms.

***Conclusions**:* Although based off the data used there seems to be no significant impact on *T. trichiura* egg counts by the removal of adult *A. lumbricoides* from an infected individual, the current treatment regimens used in attempts to control the soil-transmitted helminths are inadequate in terms of combatting whipworm infection.

1. General Introduction

It is often noted in papers discussing soil-transmitted helminthiases that 1.2 billion people are thought to be infected with *Ascaris lumbricoides* (roundworm) worldwide[1], approximately 20% of the world's population. Less mention is made however of the number of global *Trichuris trichiura* (whipworm) infections, which is estimated to be as great as 800 million[1] and consequently also represents a significant public health burden in many countries worldwide.

Though in the long term it is hoped that infrastructural and socioeconomic improvements will reduce the prevalence of soil-transmitted helminth (STH) infection in developing countries[2], in the short term community (preventative) chemotherapy is the recommended course of action by the World Health Organization (WHO) for reducing the level of morbidity and economic damage caused by these helminths; in fact, anthelmintic therapy may be the only measure available to help individuals within the near future given that findings have suggested that there are few realistic actions that individuals and single households in endemic areas can take to protect themselves from infection[3].

The WHO's current strategy for helminth control[4] is to treat individuals deemed at highest risk (such as school-age children and women of childbearing age) once or twice a year with an appropriate anthelmintic. The length of time between treatments depends on the intensity of infection and burden of disease.

Concomitant STH infections are the norm rather than the exception[5]. This is particularly true of co-infection by *A. lumbricoides* and *T. trichiura*, possibly because both of these worms tend to infect individuals in the 'domestic' or 'peri-domiciliary' domain[6] i.e. in and around the home.

Anthelmintic drugs have different efficacies against different STH species[7], meaning that after drug treatment there can be infrapopulations of certain species remaining in individuals who have been treated[8]. For example, the benzimidazole anthelmintics (such as albendazole) have a much greater efficacy against *A. lumbricoides* than against *T. trichiura*[8]. This means that when an individual suffering from co-infection with both of these helminth species is treated, the burden of *A. lumbricoides* will decrease relatively more than the burden of *T. trichiura*.

The species-specific efficacies of anthelmintics used in treatment programmes could pose problems for helminth control initiatives because the removal of one helminth species via chemotherapy could possibly provide an advantage to any other less susceptible species. Little is understood of the interactions between STH species within infected hosts, though there have been papers on concomitant infections in animal hosts[9] and mathematical models[9, 10, 11, 12] describing the possible impact of both antagonistic and synergistic interactions between helminth species in hosts and possible repercussions of this on the outcome of treatment programmes.

This project's primary objective is to explore possible interactions between STH's in a human host, specifically any conceivable interactions between roundworms and whipworms (because they have similar modes of transmission). To achieve this objective, two sets of data, collected in Bangladesh by Andrew Hall and co-workers[13] and South India by David Elkins and Melissa Haswell-Elkins and co-workers[14], to first create some descriptive statistics of helminth prevalence and intensity in these regions, and then to formulate a statistical model to investigate the possible effect on *T. trichiura* of the removal of *A. lumbricoides* worms from a co-infected host.

1.1. The lifecycles of *Trichuris trichiura* and *Ascaris lumbricoides*

Infections by *T. trichiura* and *A. lumbricoides* occur when individuals consume infective eggs containing L2 larvae. The *T. trichiura* larvae that hatch from the ingested eggs then moult into L3 larvae and penetrate the caecum or colon epithelia, where they take approximately 12 weeks to develop into adults[15] (*Figure 1a*). The *A. lumbricoides* larvae on the other hand pierce the mucosa of the infected individuals' intestine and migrate first to the liver and then to the lungs. The larvae then go over the epiglottis in order to once more enter the gastrointestinal tract, where they develop into adult worms; the whole cycle takes between 9 and 11 weeks[15] (*Figure 1b*).

Whipworms have a life expectancy of between 18 and 24 months[15], though there are some estimates of up to 48 months[16, 17]. Adult female whipworms produce up to 5000 eggs per female per day[15] which are then excreted in faeces by the host. *A. lumbricoides* worms have a shorter lifespan of only a year, but the daily egg output of an adult female worm can be as high as 200,000 (*Table 1*). Roundworm eggs can survive in the soil for up to seven years[18] and are typically resistant to many climatic fluctuations. These qualities are a major reason why *A. lumbricoides* infection is so prevalent worldwide.

The posterior end of the whipworm sticks out into the intestinal lumen, whilst the anterior stays in an epithelial tunnel[19]. In contrast to this *A. lumbricoides* worms swim freely in the small intestine against the peristaltic movement and are therefore more vulnerable to worm paralysing drug treatments than *T. trichiura*[8].

As the manner in which roundworms and whipworms infect individuals is thought to be similar (i.e. ingestion of infective, embryonated eggs), they are often lumped together when considering treatments or preventative measures. This manner of infection is used by nearly all STH species, though their modes of transmission and epidemiology can vary substantially. Whilst it is certainly true that both *A. lumbricoides* and *T. trichiura* thrive in areas with poor sanitation, it may not be entirely correct to amalgamate all control efforts into combatting the two species together before considering differences between them.

In addition to whipworms and roundworms, hookworms (*Necator americanus* and *Ancylostoma duodenale*) are also a significant cause of morbidity worldwide and these three different worm types are often referred to as the most important of the STHs, though infections with threadworm (*Strongyloides stercoralis*) or pinworm (*Enterobius vermicularis*) are of considerable significance as well. Although some information on hookworms is included in *Table 1*, this is purely for comparative purposes and further exploration of hookworm will not play a significant part in this project. This is not only because hookworms are considered to have a different mode of transmission than roundworms and whipworms (as while infection can occur by consuming *A. duodenale* larvae, it is much more common for individuals to be infected by penetration of the skin by infective, L3 larvae, for example when walking barefoot where there are contaminated faeces, meaning that hookworm infection tends to predominately occur in the 'public' domain[6]) and so should possibly be treated separately but also because the hookworm egg count data is not as reliable as the egg count data for the other two species. This is because the eggs are less resistant to the reagents used for the diagnostic procedures, and they may also start hatching in the faecal preparation if the specimen is not observed soon enough.

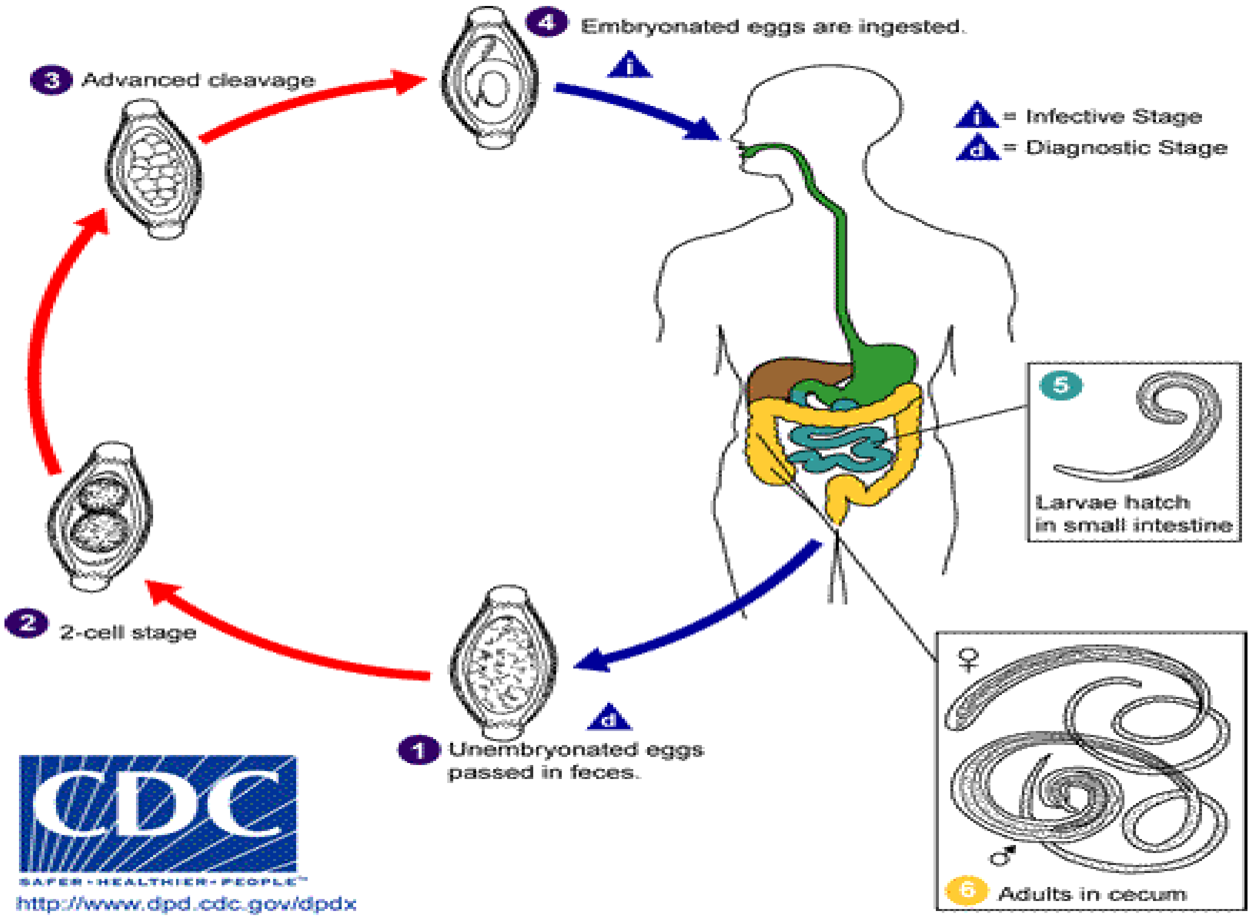
Table 1: Some descriptive statistics of the soil-transmitted helminths: information taken from [19, 20] unless otherwise noted

Species	Length (mm)	Location in host	Lifespan (years)	Daily egg output per female	Egg size (µm)	Estimated no. infected worldwide (millions)	Estimated DALY's loss caused (millions)
Roundworm							
Ascaris lumbricoides	**150-400**	**Small intestine**	**1**	**200,000**	**75 x 50[22]**	**1,221 – 1472[25]**	**1.8 – 10.5[25]**
Whipworm							
Trichuris trichiura	**30-50**	**Caecum and colon (large intestine)**	**1.5-4.0[16, 17]**	**3,000-5,000**	**57 x 26 – 78 x 30[23]**	**759 – 1050[25]**	**1.0 – 6.4[25]**
Hookworm							
Necator americanus	**7-13**	**Upper small intestine**	**3-10[21]**	**9,000-10,000**	**60 x 40[24]**	**740 - 1300*[25]**	**0.1 – 22.1*[25]**
Ancylostoma duodenale	**8-13**	**Upper small intestine**	**1-3[21]**	**25,000-30,000**	**60 x 40[24]**	**740 - 1300*[25]**	**0.1 – 22.1*[25]**

*estimate given is a combined total for all hookworm infections worldwide

DALY's = Disability Adjusted Life Years

a)



Life cycle of *Trichuris trichiura*:

1) An infected individual excretes faeces containing unembryonated *T. trichiura* eggs

2) Eggs in the soil then progress into a 2-cell stage

3) The 2-cell stage eggs mature further into an advanced cleavage stage

4) An embryonation period of between two and four weeks then takes place

5) After being consumed, the eggs hatch to release larvae in the small intestine

6) Adult worms then enter the caecum, where females can start producing eggs approximately 10 weeks after infection

b)

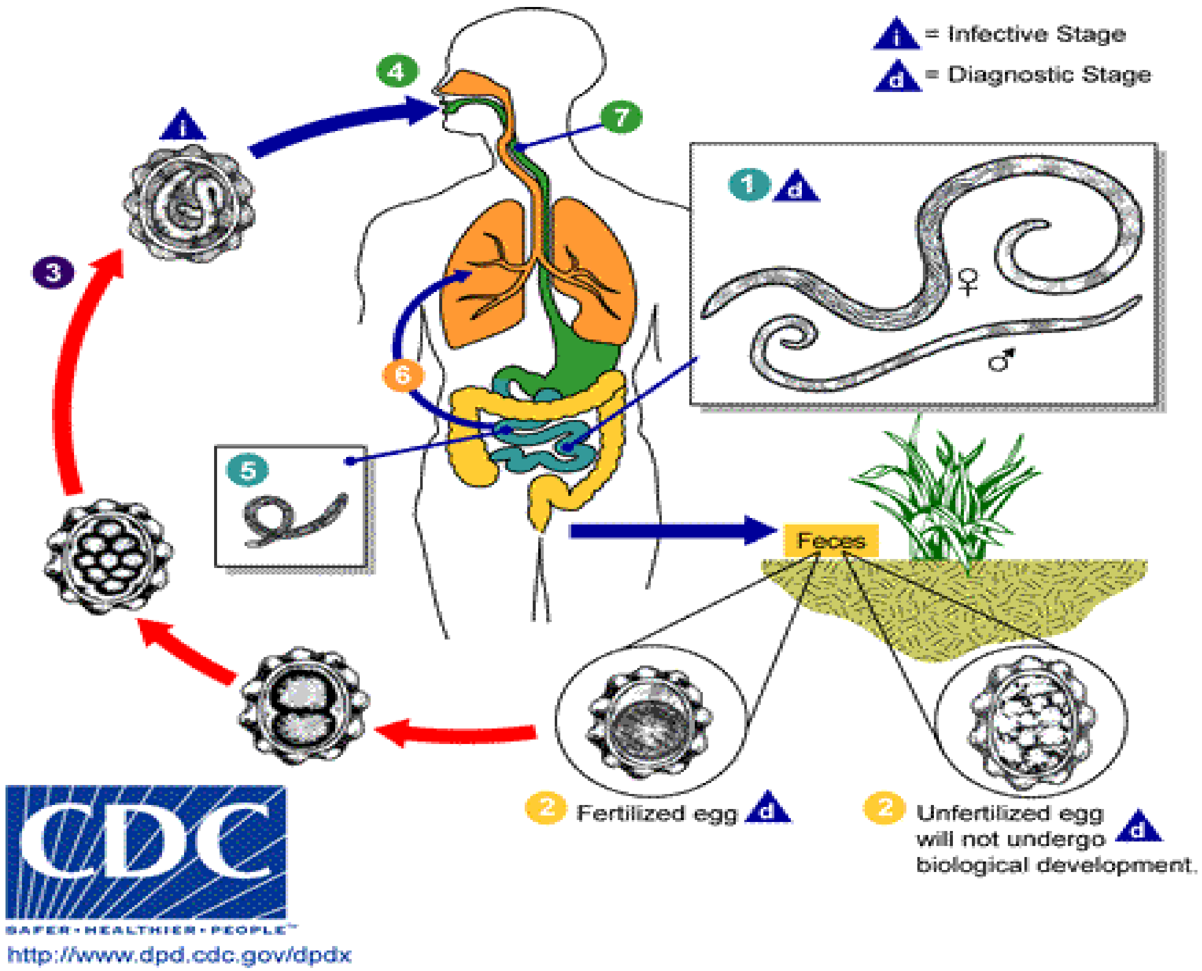


Figure 1: The lifecycles of a) Trichuris trichiura[26] *and b) Ascaris lumbricoides*[27]

Life cycle of *Ascaris lumbricoides*:

1) Adult worms are established in the lumen of the small intestine

2) Eggs are excreted via the faeces of the host

3) Eggs embryonate in the soil for just less than three weeks, after which they are infective

4) The infective eggs are swallowed by an individual

5) The larvae emerge from eggs and breach the intestinal mucosa

6) The larvae migrate to the liver, then the lungs

7) After a short lived maturation period the larvae cross the epiglottis then enter the gastrointestinal tract, with the whole process taking at least 2 months

1.2. Morbidity and burden of disease

Trichuriasis is a potentially serious disease that can cause colitis (inflammation of the colon), impaired growth and, in rare cases, Trichuris dysentery syndrome which features rectal prolapse, chronic iron deficiency anaemia and chronic dysentery[28].

Ascariasis can also cause severe problems in those affected, from lactose intolerance and problems absorbing vitamins[29] to perforation of the intestines and bowel infarction, all of which are caused by conglomeration of adult worms in the ileum or small intestine. The latter two symptoms are most frequently found in young children and can lead to potentially fatal peritonitis[15] (inflammation of the peritoneum, tissue coating the abdomen's inner walls).

Relatively few deaths are attributed directly to the STH infection, with available estimates varying from 12,000[15] to more than 100,000[16] per year. Estimates of the number of deaths caused by *T. trichiura* every year range between 3,000 and 10,000 deaths per year[25] and *A. lumbricoides* is thought to be responsible for 10,500 deaths a year worldwide[30]. Anaemia complications due to hookworm infections (as hookworms attach themselves to villi in the gut then cut into the mucosal surface in order to feed on blood) are commonly indicated to be the largest reason for STH related mortality, with between approximately 3,000 and 65,000 deaths per year[25, 31] attributed to them.

For both ascariasis and trichuriasis, it is generally believed that there is a positive correlation between infection intensity and morbidity – children are of course most likely to suffer from high intensity infections. There are various studies documenting the severe impairment caused to school performance by STH infection and the corresponding impact that this can have on the future earning potential of the individual, and the socio-economic development of the country[32, 33].

A number of papers have researched the accuracy of defining infections as light, moderate, heavy, etc. by imposing threshold values on the faecal concentration of eggs in those infected[34]. For example, for *A. lumbricoides* the WHO put forward a threshold of 50,000 eggs per gram (EPG) of faeces for a heavy infection[35], which has been questioned[34] because of the large variations observed in the fecundity of adult female *A. lumbricoides* worms worldwide.

The effects of chronic infection are the predominant contributors to the high estimates for the global burden of STH infection. Disability Adjusted Life Year (DALY) estimates for trichuriasis vary between 1.6[24] and 6.4 million[36] DALY's lost per year. The large discrepancy between the two estimates is the result of differing focuses on the effects of the helminth on the health of those infected[15, 37] and the lower threshold placed on what constitutes a high intensity infection in children between the ages of 0 and 5 years old.

In total, somewhere in the region of between 4.7 and 39 million DALY's are thought to be lost per year to STH infections[36], meaning that the maximum estimated burden is comparable to that of tuberculosis or malaria for example[38].

1.3. Epidemiology and age profiles of infection

Globally there are currently estimated to be 5.3 billion people living in urban and rural areas of stable *A. lumbricoides* or *T. trichiura* transmission, with 71% of this group living in Asia or Oceania[39]. Prevalence of infection varies greatly from region to region. A further 143 million people[39] are thought to live in areas of unstable transmission. In 2003 it was estimated that there were 140 million cases of infection by *A. lumbricoides* in India alone, along with 73 million cases of *T. trichiura* infection. This pattern of a greater number of infections by *A. lumbricoides* than by *T. trichiura* holds throughout Asia in general, but in Latin America and the Caribbean the opposite is true, whilst in Sub-Saharan Africa hookworms are responsible for the greatest number of infections (198 million compared to 173 and 162 million for *A. lumbricoides* and *T. trichiura* respectively)[40].

The geographical heterogeneities seen are at least partly the result of the different climatic conditions between separate areas: the infective stages of hookworm are much more sensitive to low temperatures of below 10^{O}C than either roundworm or whipworm, whilst at very high temperatures, of greater than 35^{O}C, the situation is less clear and can vary depending on location[39].

Children between the ages of 5 and 15 years seem to suffer from the most intense infections of *A. lumbricoides* and *T. trichiura*[19] with infection intensities declining as individuals enter adulthood. There are a number of plausible hypotheses having being offered to explain this so-called 'convex' age-intensity profile[41, 42]; for example, this age dependency could be the result of acquired immunity to infection, or lifestyle changes that alter the individuals exposure to possible infections[43] (the significantly higher intensity of ascariasis in girls compared to boys in a study in Madagascar was thought to be down to differences in exposure[44], or instances of childhood geophagia which cease as young individuals mature).

The basic reproduction number R_0 for *T. trichiura* has been estimated as ranging between 4 and 6 whilst R_0 for *A. lumbricoides* has been calculated to vary between 1 and 1.8[48]. In macroparasites, the basic reproduction number is the average number of mature female worms produced by a (mated) adult female during her reproductive lifespan in the absence of density-dependent constraints, and therefore the estimates indicate the maximum potential of a parasite population in a specific environment[20]. In order for the infection to be endemic, R_0 has to be equal or greater than 1. The larger the value of R_0, the larger the coverage of an intervention needs to be to bring the infection under control.

These estimations suggest that *A. lumbricoides* would be less resilient to control measures than *T. trichiura,* which has a potential impact on proposed mechanisms of combatting helminth infections. It must be stated however that given the large differences seen worldwide from region to region in terms of fecundity of worms, drug efficacy, etc. that these estimates should not be extrapolated as being suitable global estimates. They may instead be a reflection of the infection ecology in the areas from which they were estimated, as well as a number of other assumptions included into the equations of the basic reproduction number.

It is very likely that there will be substantially more heterogeneity in R_0 values across the distributional range of these parasites. Also, if host populations and transmission intensities/patterns within these host populations are not homogeneous, because of different levels of predisposition or household transmission, etc., the values of the basic reproduction ratio are likely to be impacted by these heterogeneities, and the parasite populations are likely to be more resilient to control interventions (e.g. maintained by ‘core groups’ of highly wormy people, who are more predisposed to infection, or who live in particularly wormy households, etc.). Although these heterogeneities are well recognised in other infectious diseases, they have not been taken into account in available estimates of STH basic reproduction ratios.

1.4. Predisposition and susceptibility to infection

There are, in humans, suspected candidate genes that are linked to susceptibility to STH infection[45] which could possibly explain to an extent why some individuals are predisposed to infection[46, 47], though exposure and other factors are also important. However, the existence of this genetic susceptibility or resistance to infection is not currently of primary importance when considering control because of the overall effectiveness of the anthelmintics currently used, and because of the relative difficulty in ascertaining the genotype of an individual compared to merely treating as many people as possible in a MDA programme. If interventions against STH infections were to shift from morbidity control to elimination, a more targeted approach that focused on the determinants of individual infection would possibly become more important.

Age-mediated host behaviour is often considered important in determining susceptibility to infection[44], as childhood activities which place individual at risk of infection (i.e. geophagia or sanitary habits) change drastically as children reach teenage years and adulthood.

As chemotherapy merely treats an individual's infection and otherwise does nothing to change the infected hosts susceptibility to infection, or the environment in which these infections occur, re-infection rates by the STHs are all extremely high[48]. This is also partly due to the short generation times of the STHs. One year after treatment, *A. lumbricoides* typically reaches 94% of its pre-treatment prevalence and *T. trichiura* reaches 82% of its pre-treatment prevalence level. Given however the strongly nonlinear relationship between infection intensity and infection prevalence[49] it is also important to document the levels of re-infection reached in terms of (usually indirect) measures of infection intensity (commonly EPG of faeces). There is a positive correlation between the status of an individual's infection before treatment and the intensity of re-infection. Further than this, the order of infection by helminth species can also play an important role in interspecific competition[50], so re-infection attempts by a helminth species may be inhibited by the incumbent helminth species.

1.5. Immunological responses and the peak shift hypothesis

The immunological response of humans to helminth infection is multifaceted and detailed information is limited. Without any doubt the infections prompt a strong response from the host's immune system[51], but which and how exactly immune responses may confer resistance to infection/re-infection are not completely understood. Moreover there is no direct immunological model for *A. lumbricoides* in humans and *Trichuris muris*, which infects mice, is often used to infer possible processes affecting *T. trichiura* when discussing immunologically-mediated mechanisms of resistance.

It has been shown in mice that immunoglobins (with immune responses for all serotypes having been noted[52, 53, 54, 55]) and B-cells can have a role in protecting the rodents against helminths; there is a strong implication however that this plays a greater part in protection against reinfection rather than combatting primary infections[51]. In addition to this, it has been shown that T-cells play a crucial role in determining the balance between infection and disease, with (mutually regulatory) T_H1 and T_H2 phenotypes being predominantly linked with susceptibility and protection respectively[56].

It has been hypothesised that helminth infections are masters of immunoregulation, tending to promote an immunological environment dominated by T_H2 responses, which downregulate the T_H1 arm and facilitate worm survival whilst decreasing host damage. However, the elicitation of an immune response does not necessarily mean that the response is protective and although there are a large number of papers detailing noted correlations between immune responses and helminth infections[56, 57, 58], the interpretation of such correlations in terms of susceptibility, protection, or immunopathology should be treated with caution.

The possible consequences of removing worms from human hosts have also been investigated to an extent. The motivation for these studies is that in regions of endemic helminth infection cases of atopy, the misregulation of T_H2 responses that seem to be elicited by helminths, have remained extremely low[51] with field (ecological) studies showing a negative association between helminth infection prevalence and incidence of atopy[51]. It has been suggested[51, 59] that removing worms, and therefore upregulating T_H1 responses, may result in problems with exacerbation of T_H1-derived inflammatory responses. Given that infections with protozoan parasites seem to primarily elicit T_H1-like responses, the exacerbation of the latter could cause issues in regions endemic with diseases such as malaria, leading to severe immunopathological effects. There are studies linking presence of helminth infection with fewer cases of severe malaria[60], though this topic is hotly debated and, as contrasting evidence also exists, there is no definitive answer regarding the issue of malaria-helminth co-infection and the impact of antimalarial or anthelmintic intervention on either infection or their associated morbidity.

If acquired, protective immunity is important when considering helminth infection, it has been hypothesised that there should be a clear negative correlation between the maximum level of infection in an individual and the age at which this maximum, or peak, is reached when analysing age-profiles of infection in populations exposed to different transmission intensities[61]. The decline in the intensity of infection in an individual would be a result of the protective response that has been acquired by the individual, with transmission rate being the key factor in the development of this immunity. This is known as the peak shift hypothesis, and although there is some evidence in support of this theory, particularly in schistosomiasis[62, 63, 64], the hypothesis has not substantiated by analysis of other helminthiases, especially the filariases[7, 65].

1.6. Treatment and control

The current recommended treatment to 'cure' infections by *T. trichiura* is a single 400mg or 500mg dose of a benzimidazole anthelmintic[66] (mebendazole or albendazole). These drugs can be made in a generic form for low cost and do not require high levels of training to be distributed[19]. The benzimidazoles have pooled cure rates (percentage of those treated that tested negative for helminth eggs after drug treatment) for *T. trichiura* of about 30% for albendazole[67] and 36% for mebendazole[67] and egg reduction counts (percentage reduction in the number of EPG of faeces) of between 0%[68] and 89.7%[69] for albendazole and 81%[70] and 92.8%[71] for mebendazole. Trials involving multiple doses of mebendazole have noted higher cure rates in the range of 56% to 98%[19] and multiple doses of albendazole (either three days of 400mg of albendazole) result in similar improvements in efficacy compared to a single dose[8].

On the other hand the benzimidazoles have a much greater efficacy against *A. lumbricoides*, with cure rates of over 80%[8] and 90%[8] being recorded regularly for albendazole and mebendazole respectively. Egg reduction rates are similarly high, with values of over 90%[7, 8] being commonplace.

Other noted anthelmintics, such as levamisole, are used much less frequently and there are less data with regards to drug efficacy.

The drug used in the studies from which this projects data are sourced was pyrantel pamoate, which has been noted to have a low cure rate against *T. trichiura*, with values of between 11.5% and 38.1% being found in the few clinical trials that have been conducted[72, 73]. By contrast, cure rates of pyrantel pamoate against those infected by *A. lumbricoides* range from 85.1%[73] to 93.8%[72], with an egg reduction rate of 87.9% also being estimated (the egg reduction rate for *T. trichiura* was 52.0%[73]).

Clearly therefore pyrantel pamoate has a much greater effect against *A. lumbricoides* worms than against *T. trichiura* based on the information from these trials, though it must be stressed that relatively few convincing trials have been ran and in every trial only children were treated (meaning that the impact on adult hosts is unknown). The implications of these differential cure rates are that, given the high likelihood of being infected with both of these worm types in areas with a high prevalence of infection for the two species, a significant percentage of those treated will probably continue to be infected with *T. trichiura*, but not with *A. lumbricoides*. Although of course pyrantel pamoate is not actually used in actual modern control programmes, given the different efficacies of the benzimidazoles that are currently used the question is still relevant. The potential ramifications of this failure to treat trichuriasis are the focus of this project, with regards to the effect on the remaining whipworm of the removal of another worm species from the hosts system.

1.7. Helminth frequency distributions

It has been widely shown that helminth frequency distributions (the distributions of the number of parasites or of transmission stages in a population per person) are strongly overdispersed compared to a Poisson or random distribution and are often best approximated empirically by a negative binomial[13], or zero-inflated negative binomial distribution[67]. These distributions are described by the mean worm burden, the prevalence of infection by the worm and the ‘clumping’ parameter *k*. Parameter *k*, also known as the overdispersion parameter, is inversely proportional to the degree of aggregation of the helminths in a host population, with a usual value range of between 0.1 and 1.0[20], indicating strong overdispersion. As *k* values become larger (for example, above 5 or more), the distribution tends to the Poisson or random distribution.

Estimates for values of *k* for the STHs vary significantly depending on the factors being considered i.e. age group or region, or from the relationship between prevalence and intensity in a group of populations[49]. For example, estimates from children in North Bangladesh for *A. lumbricoides* calculate a *k* value of 0.44[16], whilst estimates in Dhaka find values between 0.412 and 1.195 depending on the age and sex class investigated[13]. Often therefore a single value of *k* is not used; instead *k* is often defined as a linear function of the mean worm burden (i.e. *k* is equal to a constant plus a multiple of the mean worm burden)[13] in estimates that are derived from the analysis of the prevalence vs. intensity relationship across a collection of populations. These estimates, however, are derived from aggregate measures instead of from the raw distributional data of single populations. The best practice, if possible, is to estimate the overdispersion parameter from the frequency distribution analysis in as many single populations as available, determine the relationship between these values and the intensities of infection obtained for each group and compare the estimates obtained with those derived from the aggregate measures.

Parameter *k* is very important when researching the impact of a helminth species in a host population as the greater the aggregation of worms in a handful of hosts, the fewer the people in principle that would need to be treated to make a significant contribution to controlling the infection. This is because those with the heaviest infections are not only those who suffer the most but also make the largest net contribution to helminth transmission in a region[13].

Targeted treatment, though extremely attractive in theory, can be difficult to implement practically because of problems with the cost, logistics and accuracy of testing for helminth infections before treating. For example, when attempting to calculate the burden of the STH’s faecal samples are often taken from random individuals in the population and a variety of copromicroscopic techniques (frequently Kato-Katz smears) are used. The Kato Katz technique has been criticised[74] for its relatively poor sensitivity and attempts have been made to find a suitable replacement[74].

As a result of the problems with targeting treatment, in most cases mass treatment of individuals in a population or age group is implemented, especially if the intervention is taking place in a highly endemic area. Whereas this type of drug delivery (MDA) is easier to implement then targeted treatment, MDA does bring a number of problems such as an increased risk of development of drug resistance by the targeted helminth, as it decreases the amount of refugia[75] (parasite population not subject to anthelmintic pressure).

1.8. Density dependence in helminth species

Density dependent mechanisms can impact on many different stages of a helminths life-cycle. For example, rates of female worm fecundity, adult mortality and establishment of new infections may depend on density of established (or incoming) worms[76]. Of particular importance when discussing anthelmintic treatment programmes are the negative density-dependent mechanisms which curb parasite population growth in a limited environment. Such density dependencies will be relaxed after treatment, facilitating the rapid rebounding of parasite populations and their general resilience to perturbation[77]. Positive density dependent effects are, on the other hand, those whose rate increase with parasite density, such as the parasite mating probability[78] whereby the probability of worms of different sexes (males and females in dioecious species) meeting and mating within a host increases, leading to the so-called breakpoint densities. These are (non-zero) densities below which parasite populations cannot persist and would be pushed into terminal decline (due to, for instance, single-sex infections in which female worms remain unfertilised).

The stage of the helminths life-cycle upon which negative density dependent mechanisms act can have a large effect on the rate at which host re-infection occurs after the individual has been treated with an anthelmintic – helminth species whose growth is restricted by density dependent fecundity alone tend to have slower rates of re-infection than those whose establishment rate in a host is controlled by density dependent mechanisms[77]. In practice, it is likely that more than one mechanism alone is responsible for the regulation of parasite infrapopulations.

Density dependence can influence the spread of drug resistance, because the resistant helminths that remain after treatment will be subject to lesser constraints on fecundity (if that is the manner in which the parasite population size is regulated) so there could be an increase in resistant allele frequency in subsequent generations[79].

It has been suggested that for certain nematode populations which have infected individuals to be subject to density dependent effects the host must demonstrate an immunological response to infection i.e. density dependent effects are immunologically mediated[80]. Studies on the nematode *Strongyloides ratti* in mice showed that the immune response of infected individuals played a role in mediating factors such as female worm fecundity, etc., whilst in animals with a compromised immune system no density dependent effects were observed. This finding could have great significance in terms of co-infection between helminths and other pathogens, which may result in different regulation pathways of the immune response.

2. Project objectives, rationale and hypotheses

The general aim of this project is to explore any possible influence of the removal of *A. lumbricoides* from infected hosts via chemotherapy on the intensity of *T. trichiura* infection using data collected in Dhaka, Bangladesh between 1988 and 1990 by Hall and co-workers[13], and data collected from the village of Vairavan Kuppam in Pulicat, South India in 1984 by Elkins and co-workers[14]. This objective is achieved using analyses which are structured as follows. First, as no previous analysis of the *T. trichiura* egg count data that were collected has been done, some descriptive information is presented, such as the host age and sex profiles of infection and the percentage of hosts infected at baseline, for *T. trichiura*. For comparative purposes between the worms, similar statistics are also shown for *A. lumbricoides*. A statistical model is then developed and used to explore the potential interaction between the two helminths following effective chemo-expulsion of the *A. lumbricoides* populations. Finally the implications of the results for on-going STH treatment programmes are discussed.

The hypotheses under consideration are:

Null hypothesis: There is no interaction between whipworms and roundworms. Therefore, the removal of the latter does not affect the former, as these species are effectively independent. There is no statistically significant increase or decrease in T. trichiura egg counts following the removal of *A. lumbricoides*.

Alternative hypothesis: The two species interact biologically, and there is either an increase in the density of whipworm (indicative of an antagonistic interaction), or a decrease in such a density (synergistic interaction) following the removal of *A. lumbricoides*.

3. Study designs and data sets

This project focuses on two data sets that have kindly been made available.

The Bangladesh data set: The first data set was collected during a study of the intensity of infection and reinfection with regards to various intestinal nematodes in an urban slum in Dhaka, Bangladesh[13]. This study ran over the course of 18 months between August 1988 and February 1990 and involved the treatment of 1765 people with pyrantel pamoate, an anthelmintic that paralyses the worms, allowing for their expulsion more or less intact in the faeces. To once again reiterate the point, pyrantel pamoate is very effective against *A. lumbricoides* but it is not particularly efficacious against *T. trichiura*[72, 73].

All individuals who took part in the study donated a faecal sample which underwent microscopic analysis via a quantitative ether sedimentation technique[81]. Egg counts for *A. lumbricoides, T. trichiura* and hookworm (the eggs of *N. americanus* and *An. duodenale* tend to be counted together because of high levels of co-infection) were recorded for each participant included in the study, and the number, weight and sex of *A. lumbricoides* worms that were passed out in the stool of those treated was also noted (a count of roundworm larvae was also completed). Unlike roundworms, adult whipworms are not expelled from those infected after treatment with pyrantel and so were not counted.

Individuals were treated three times overall, with a 6 month gap between each treatment round. Stool samples were taken before treatment for egg counting purposes, and over the 48 hours immediately after treatment in order to collect and count expelled *A. lumbricoides* worms. As a result of practical considerations about collection and time needed to perform the ether sedimentation technique, the data for each round were collected over the course of the 6 months between treatments, ensuring that for each individual or group of individuals, the time between collections was effectively 6 months.

In terms of those who took part in the study, more women were able to participate than men and the numbers were significantly different ($P<0.002$) in the 6 oldest age groups[13], although there do not seem to be any significant differences between months in terms of the mean age of individuals sampled in each month.

The South India data set: The second study was conducted in a village north of Madras, in the Pulicat region of South India over the course of 1984[14]. Once again the treatment used was pyrantel pamoate, but rather than being treated once every six months individuals were first treated in January and then again 11 months later in November. Stool samples were collected in January and November before chemotherapy, and also in March and July, in order to measure the efficacy of the anthelmintic and to monitor the amount of re-infection that had occurred. Not everybody who was treated in January was followed up in each month i.e. some donated faecal samples once and then dropped out, or donated samples in July but not in March.

Faecal samples were processed by an ether sedimentation technique and the number of *A. lumbricoides* (fertilized and unfertilized), *T. trichiura* and hookworm eggs were counted. In addition to this, worm counts were made for *A. lumbricoides*, and the counted roundworms were also sexed.

4. The epidemiology of roundworms and whipworms in Bangladesh and South India

4.1. Methods

Worm count data provides a more accurate measure of helminth burden than data on EPG of faeces. This is partly due to the aforementioned problems associated with calculating egg output via ether sedimentation techniques (though the technique used in these studies is one of the better techniques available, any egg counts made from relatively small faecal samples will be inherently highly variable) but also because the large amount of variability inherent in collecting egg counts. Consequently *A. lumbricoides* worm counts are used for descriptive statistics when analysing the data from Bangladesh, though results reached using egg count data for roundworms are also included so that the contrasts between *T. trichiura* and *A. lumbricoides* in terms of egg output can be displayed and compared.

As there are no worm counts for whipworms, egg counts are utilised as an indirect measure of infection intensity instead for this species. Unfortunately, only egg counts from the South India data set are used in the analysis from this location because of the incompleteness of the worm count data from this location.

The analysis of Hall et al.[13] used two sex classes and 11 age classes: 1-2, 3-4, 5-6, 7-8, 9-10, 11-12, 13-16, 17-26, 27-36, 37-46 and >46 years old. The data analysis of Elkins et al.[14] again used two sex classes but instead had 9 age classes: <2, 2-4, 5-9, 10-14, 15-24, 25-34, 35-49 and 50+ years old. To be consistent with these previous evaluations these classifications are maintained for the relevant data sets.

For each sex and age group, the prevalence of infection was calculated as the proportion of those infected (with adult worms and/or eggs) out of the total examined, displayed as a percentage. The intensity of infection was calculated as the arithmetic mean number of worms or of EPG of faeces. The results were plotted by age and sex for the total population examined in the two locations. Prevalence and intensity data were also plotted by month/year to elucidate any seasonal effects, understand the effects of treatment, and visualise any possible interactions between these variables. By analysing the patterns of infection according to host age and sex, locality, and seasonality, an insight was obtained into the variables that would be important to take into account in any subsequent analysis of the relationship between the densities of roundworms and whipworms before and after treatment.

4.2. Results and Discussion

4.2.1. Age-prevalence profiles

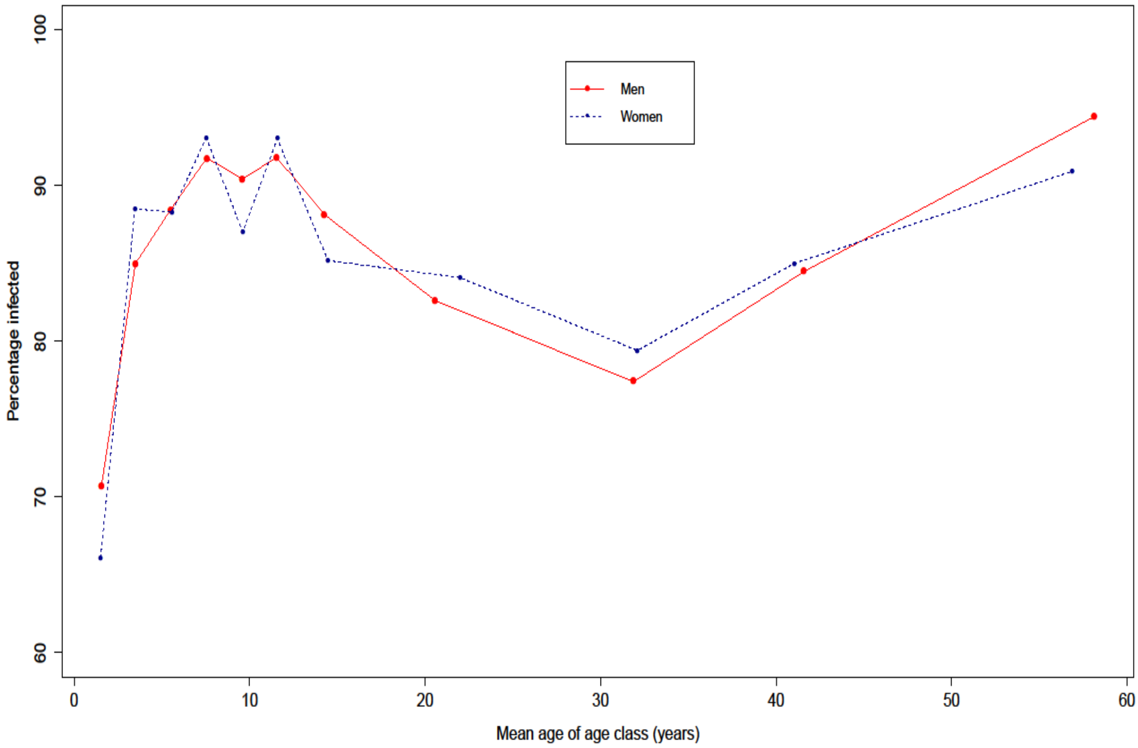
Bangladesh

Figure 2 presents age-prevalence data at baseline (after the 1st treatment) from Bangladesh. The figure illustrates that there is no significant difference in the prevalence of infection for *T. trichiura* in men and women in older classes despite this being the case for *A. lumbricoides*[13]. This result seems at odds with the assumption that the two worm species have very similar modes of transmission and implies that there could be a distinction between them regarding determinants of infection.

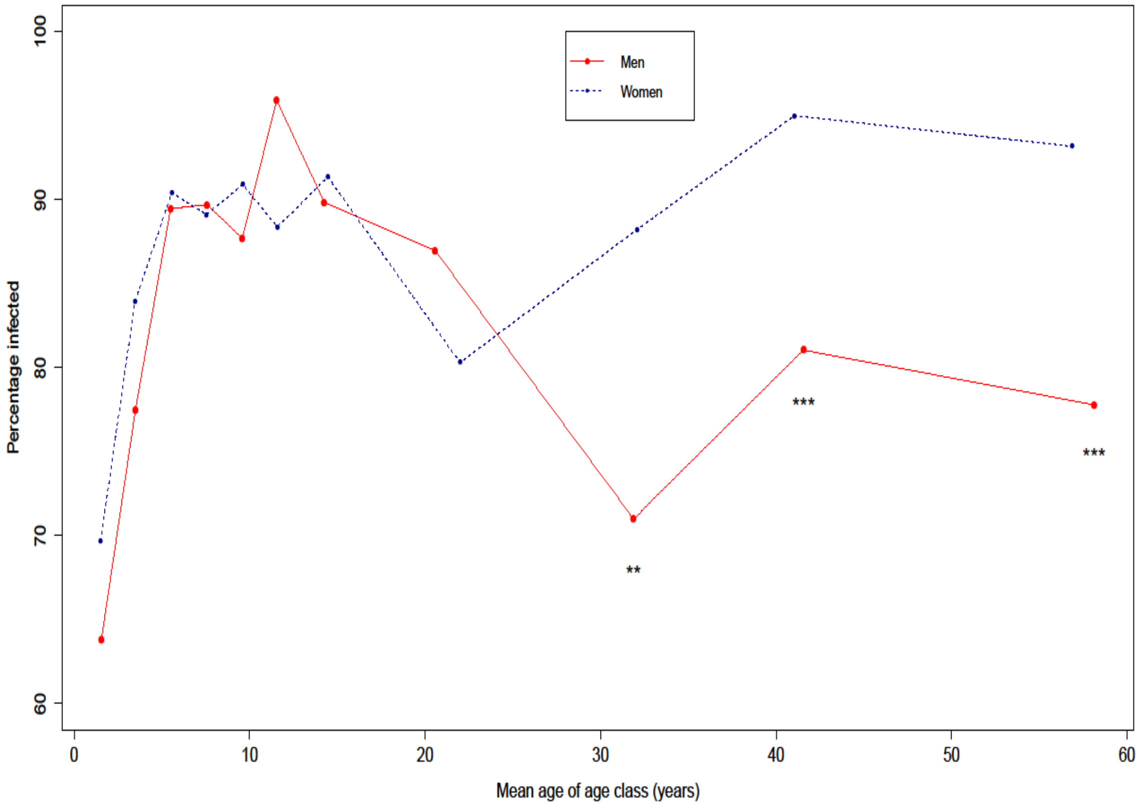
Apart from this, the prevalence of infection in younger age groups appears very similar for both worm species. Approximately 70% of children in the youngest age group (1 – 2 years old) are infected, with this percentage rising to about 90% in school age children before falling as individuals enter adulthood. The much lower prevalence estimate for the youngest age group seen when using egg count data (over 20% lower than when using worm count data for roundworms) is possibly because those individuals are infected predominantly with immature female worms. The increase in prevalence in the older age classes may be as a result of changes in exposure or immunological condition, though for *A. lumbricoides* it is men, unlike women, who experience a significant decrease in prevalence after reaching adulthood. Women maintain a prevalence of over 90% in the three oldest age classes. The different age-prevalence profiles for men and women indicate an exposure component, as if these differences were due solely to the development of a protective immune response, one would expect to see a peak shift between the sexes with the most heavily infected group reaching the peak of infection earlier (rather than later, as observed). This result is shown in both the egg counts and the worm counts, though the significance is weakened slightly when considering egg counts due to the greater level of variability expected.

Comparing prevalence of roundworm using the two different sets of count data, it seems that prevalence values based on egg count continuously underestimate the values calculated based on worm counts (which is almost certainly the result of the lower sensitivity of egg-detecting methods compared to counting worms). Given that worm count data are generally considered to be a more reliable source of infection intensity, this may mean that egg count based prevalence estimates of *T. trichiura* are also underestimated as analysis of egg count data for the worm was the only available option. Despite this the overall trends in sex and age profiles of infection for *A. lumbricoides* are similar regardless of the type of count data used.

a)


Men
Women
Percentage infected
60
70
80
90
100
0
10
20
30
40
50
60
Mean age of age class (years)

b)


Men
Women
Percentage infected
60
70
80
90
100
0
10
20
30
40
50
60
Mean age of age class (years)
**
***
***

c)

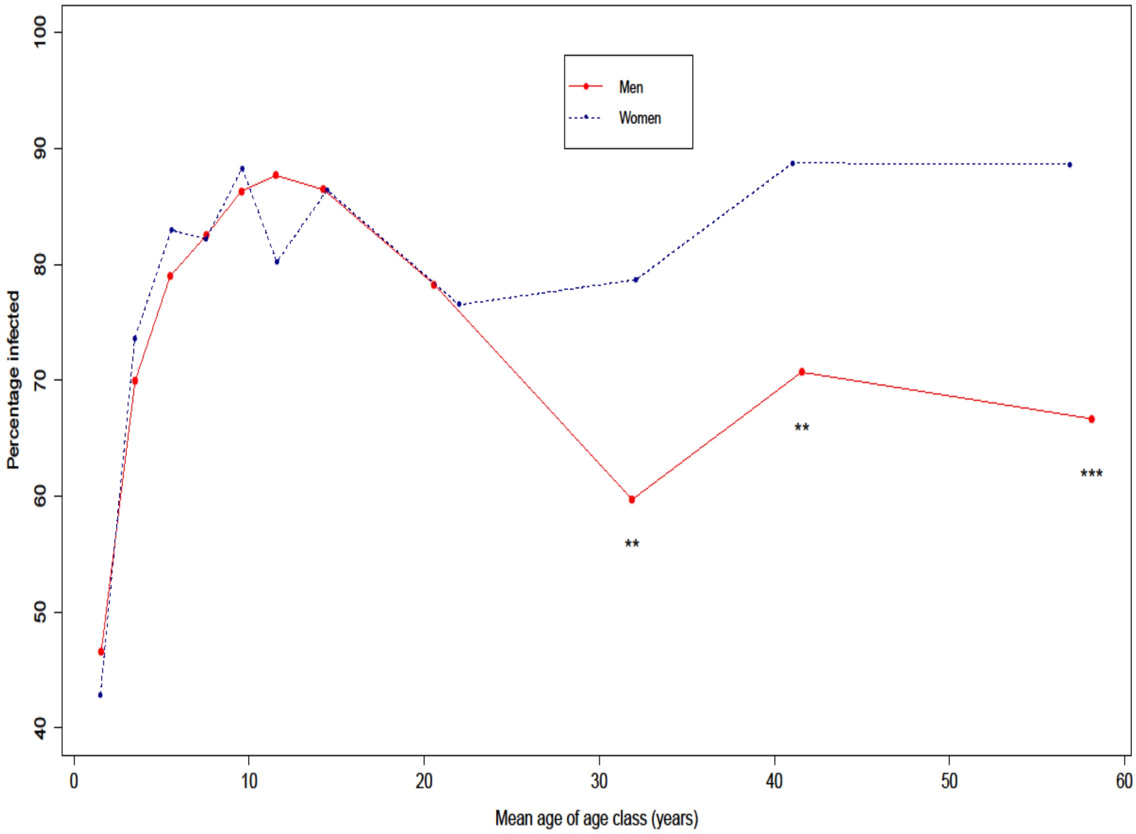


Figure 2: Prevalence of infection at baseline from the Bangladesh data for a) Trichuris trichiura (based on egg count), b) Ascaris lumbricoides (based on worm count) and c) Ascaris lumbricoides (based on egg count)

$*P < 0.05$, $**P < 0.01$ and $***P < 0.001$

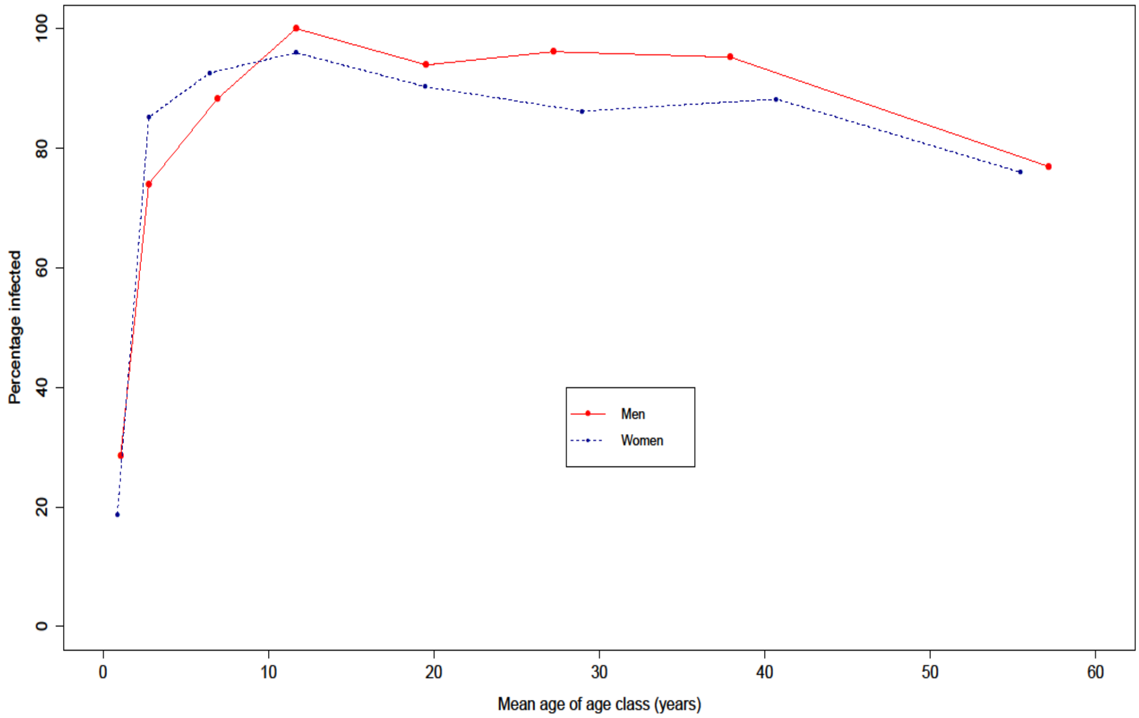
South India

Figure 3 illustrates that both species seem to exhibit the same trends and that there are no significant differences between men and women in any age class. This supports the general assumption that roundworms and whipworms share a similar mode of transmission. For both of the worm species, prevalence in the youngest age class is approximately 30%, which rises swiftly to over 90% in school age children. There is then a general decrease as age increases, though prevalence of *A. lumbricoides* and *T. trichiura* is still over 60% and 70% respectively in the oldest age categories. Since both of these prevalence graphs were constructed using egg count data, it is possible that, as seen in the above example of Bangladesh, there is an underestimation of the actual prevalence of infection for both species.

Comparing the two locales, it is clear that, as expected, children between the ages of 5 and 16 years old suffer from the highest prevalence rates. However, it seems that there are some differences in prevalence rates between the two locations; in South India there does not seem to be any difference in *A. lumbricoides* infection between the sexes and in addition to this there is an overall decline in prevalence in the older age groups, which is not the case in Bangladesh (this could be the result of smaller sample sizes in those age categories).

Following on from this, it is important to note that whilst *A. lumbricoides* and *T. trichiura* have similar prevalences in many age categories, whipworms can have a prevalence of infection of over 90% in children. Given the anthelmintic efficacies discussed in the Introduction, most current treatment programmes will not do anywhere near enough to combat trichuriasis.

a)



b)

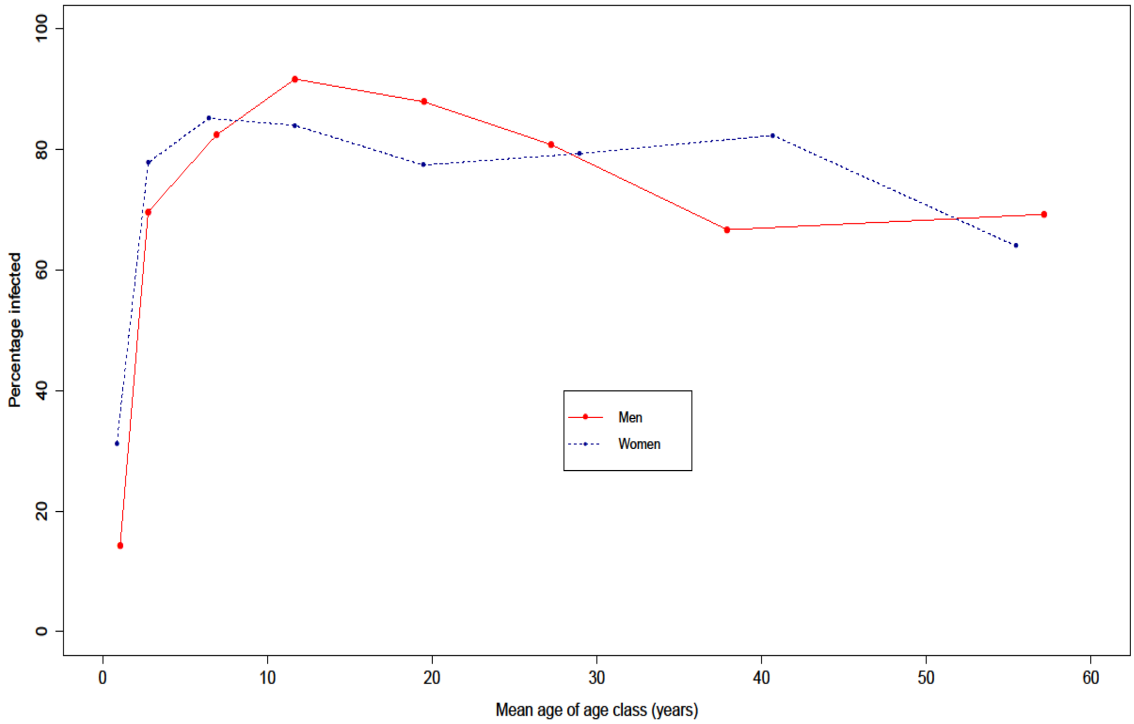


Figure 3: Prevalence of infection at baseline from the South India data for a) Trichuris trichiura and b) Ascaris lumbricoides (both based on egg counts)

4.2.2. Age-intensity profiles

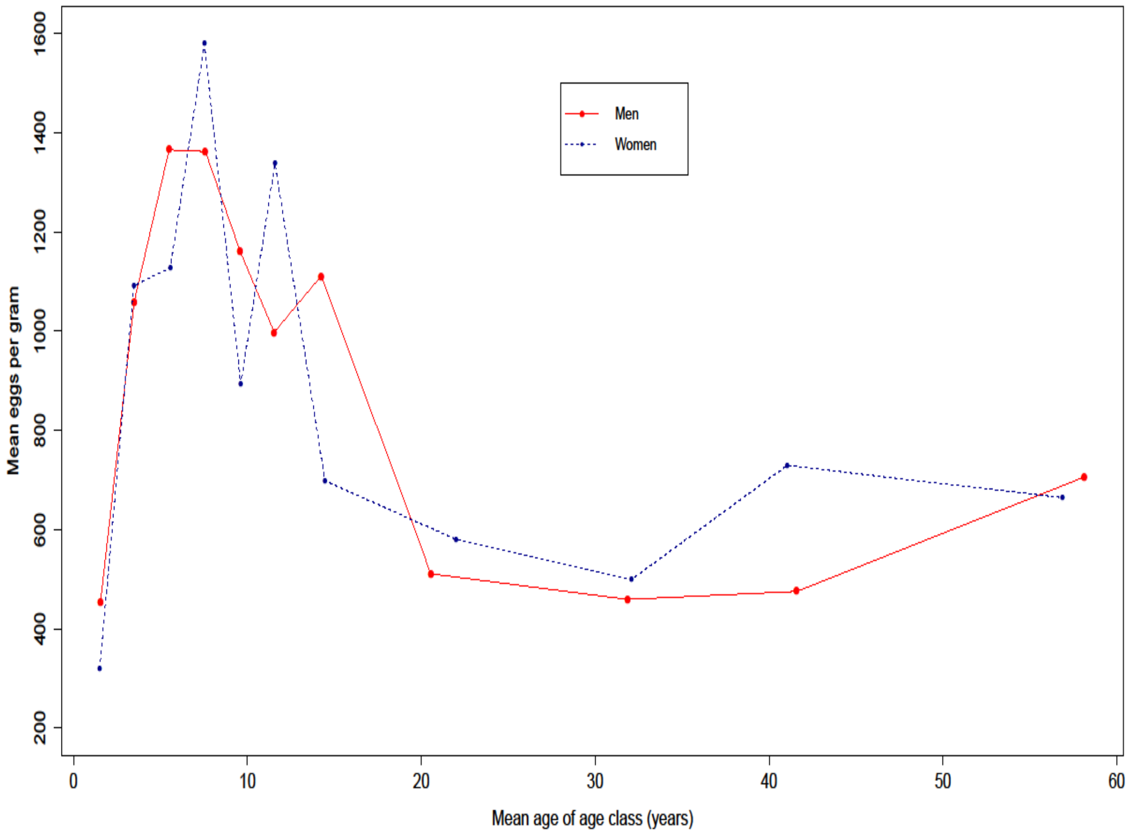
Bangladesh

Figure 4 shows the subtle differences between the age-intensity profiles of *A. lumbricoides* and *T. trichiura*. In the older age groups there are significant differences in the mean intensity of infection between men and women in *A. lumbricoides* but not in *T. trichiura*. These differences are more apparent when comparing the mean worm burden, but are also present, in a diluted form, in the egg count data. Apart from this, the overall trends are similar; school age children suffer the most intense helminth infections, with decreases in intensity as age increases.

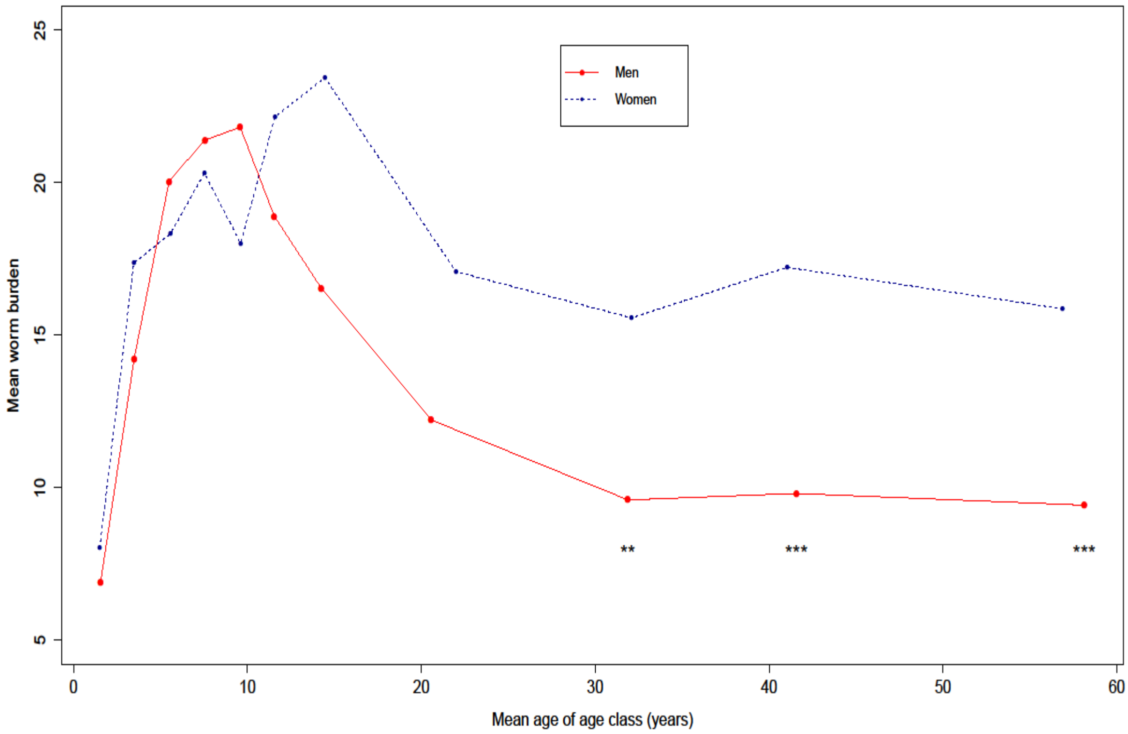
There are a number of differences between the sexes apart from those seen in the oldest age classes. The peak *A. lumbricoides* worm burden in men occurs in the 9 – 10 year age class, whilst in women the highest intensity is observed in the 13 -16 year age category. On the other hand men aged between 5 and 6 years old have the highest EPG values for *T. trichiura* whilst for women it is those between 7 and 8 years old that have the greatest intensity infections.

In terms of absolute numbers, the egg counts for *A. lumbricoides* are greater than those of *T. trichiura*, which may be expected given the much greater eggs per day output of the roundworm compared to the whipworm.

a)


Men
Women
Mean eggs per gram
Mean age of age class (years)
200
400
600
800
1000
1200
1400
1600
0
10
20
30
40
50
60

b)


Men
Women
Mean worm burden
Mean age of age class (years)
**
***
***
5
10
15
20
25
0
10
20
30
40
50
60

c)

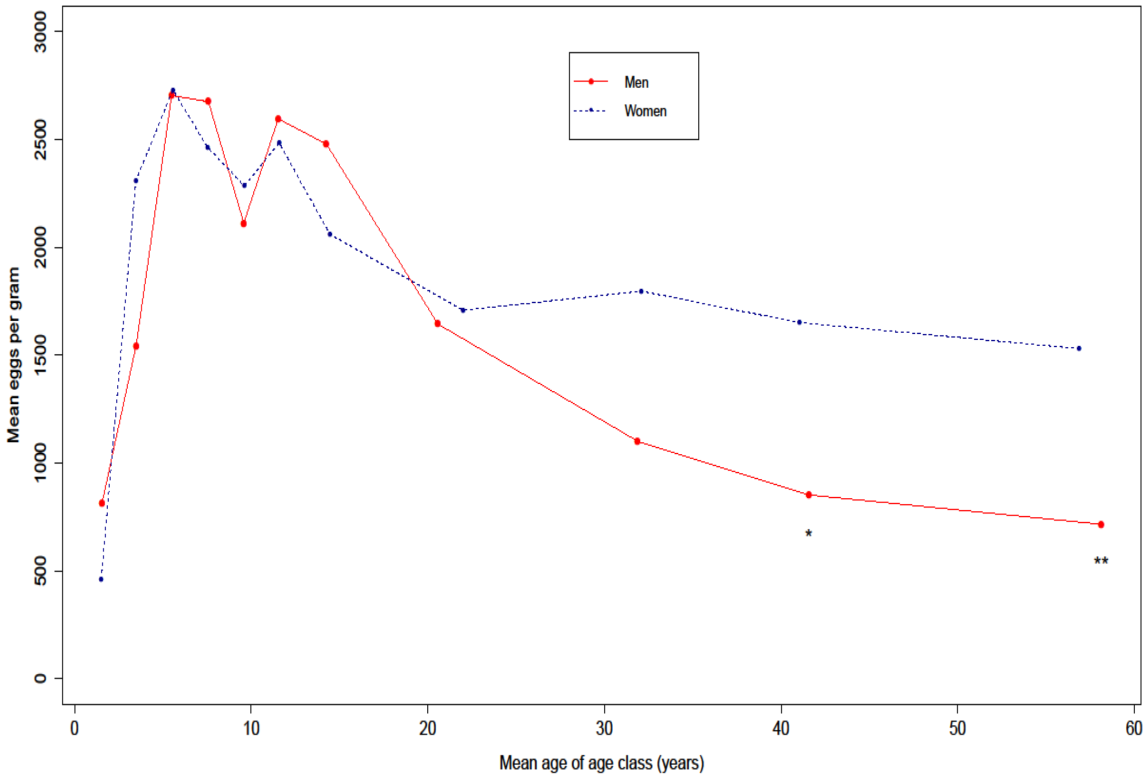


Figure 4: Age-intensity profiles at baseline from the Bangladesh data for a) Trichuris trichiura (based on egg count), b) Ascaris lumbricoides (based on worm count) and c) Ascaris lumbricoides (based on egg count)

****P < 0.05, **P < 0.01 and ***P < 0.001***

South India

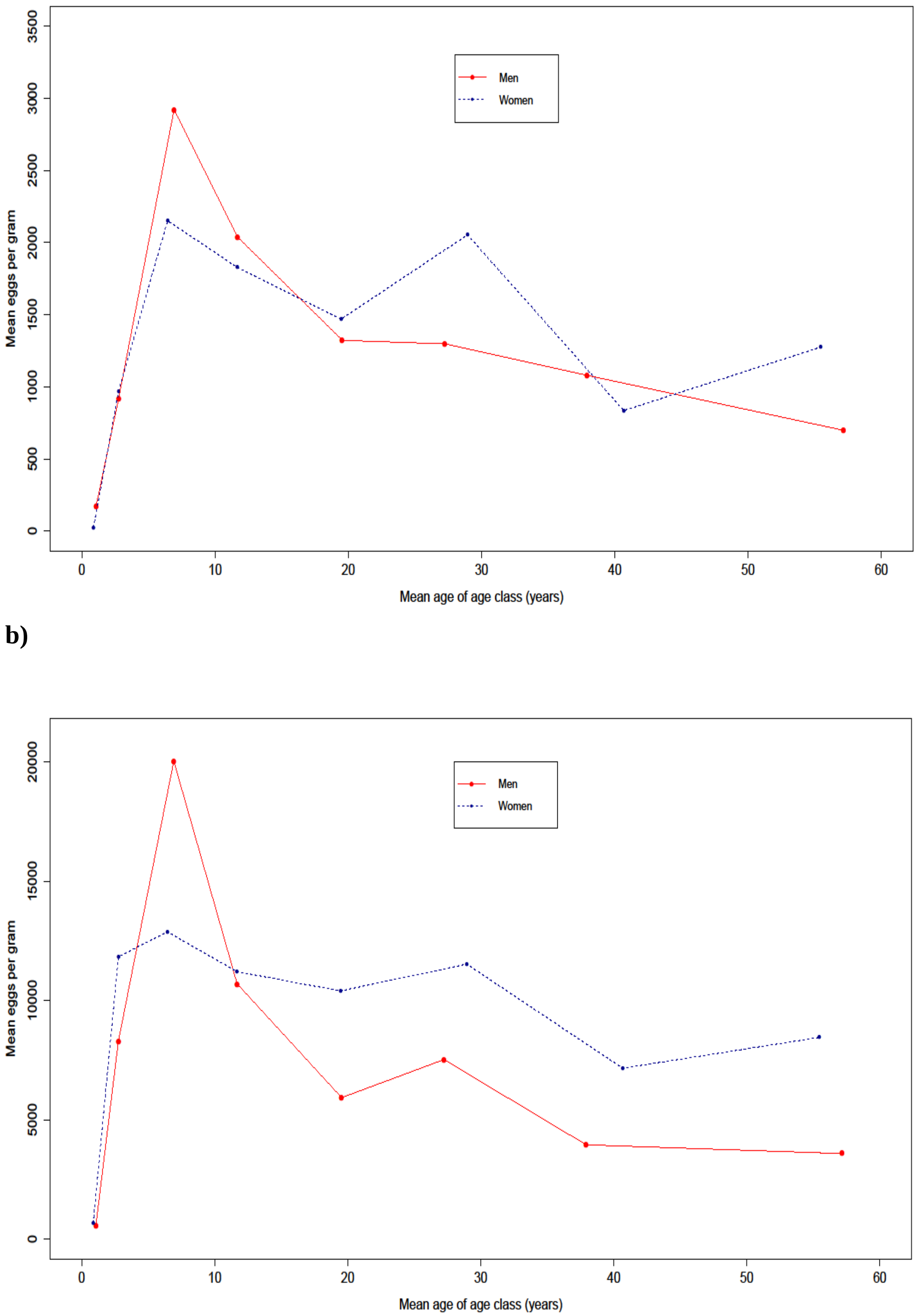
The graphs in Figure 5 show the same general trend for both worms and for both sexes, in that there is a rapid increase in EPG of faeces as age increases, with a peak shown between 5 and 9 years old for both sexes, before a steady decline as the population ages. Again, there is large difference in terms of absolute values of egg output, with men in the 5 to 9 years old age category having mean EPG of faeces of just over 20000 for *A. lumbricoides* compared to approximately 2920 for *T. trichiura*. Whilst egg counts are inherently variable, a large sample size means that mean values can be fairly reliable.

Compared to the results from Bangladesh, there is once again no difference between men and women in terms of intensity of infection for either worm species, though both sets of results show a comparable pattern of high intensity infections in the children between 5 and 16 years old, with a decrease in intensity thereafter. There was a significantly higher value for EPG of faeces seen for *A. lumbricoides* in South India, which is perhaps an example of the large variability seen worldwide in the fecundity of roundworms[34].

Overall it appears that whilst there are some distinctions to be made between the two separate locations, the general picture is one of extremely high prevalence of both worms, with children bearing the brunt of the intensity. This is probably not due to issues with the ether sedimentation process used to calculate the values given for mean EPG of faeces because not only is this pattern is still evident when using worm count data but as the sample sizes are reasonably large the mean values should be reasonably accurate. In Bangladesh there seems to be an increase in prevalence of infection of both worm species in the oldest age categories to approximately a similar level to that seen in school aged children. However, the infections suffered by children are significantly more intense than those endured by adults.

To summarize, the biggest difference between the two regions is that there are no sex related differences seen in the data from South India, whilst in Bangladesh there were significant differences were visible for *A. lumbricoides* when analysing both egg and worm count data. *T. trichiura* demonstrated no signs of host sex related differences in either location. Further studies in the regions are required to investigate this, ideally involving whipworm worm counts rather than egg counts in order to obtain a more accurate picture of helminth burden (this would be much more difficult than obtaining roundworm counts, given the problem of counting whipworms with any degree of accuracy). In addition to this, the decrease in trichuriasis intensity seen in both locations for both worm species is perhaps indicative of the peak shift hypothesis being true in this case, with acquired immunity helping to combat re-infection by helminths and leading to a reduction in intensity of infection in the older age classes. Testing this hypothesis would require analysis of many data sets deriving from areas with different intensities of transmission, infection, and therefore exposure to helminth antigens that would elicit a protective immune response. Alternatively, behaviourally mediated age-dependent host exposure could help explain the patterns observed. The convex pattern seen could also be the result of two mutually antagonistic species interacting[34], though this explanation should be considered carefully as the degree of convexity of the age-intensity curve may not be significant.

a)



b)

Figure 5: Age-intensity profiles at baseline from the South India data for a) Trichuris trichiura and b) Ascaris lumbricoides (both based on egg counts)

4.3 Seasonality and re-infection

Bangladesh

Figure 6 displays a pattern of decreasing worm or egg counts over the period of treatment for each worm species. This trend is illustrated most clearly by the *A. lumbricoides* worm count data (*Figure 6b*), but is also shown by the egg count data for both *T. trichiura* (*Figure 6a*) and *A. lumbricoides* (*Figure 6c*) despite the greater variability in these data (the last collection of data points has greater variability because of the much diminished sample size as participants in the study dropped out).

This pattern is suggestive of a seasonal component of transmission. Bangladesh has a monsoon season between June and October, is dry and warm between November and February and between March and May it is muggy and hot. There is the potential for greater transmission in the monsoon season as the vastly increased rainfall could possibly affect sewage and sanitation systems, which is important because transmission of the STHs relies on individuals coming into contact with faeces contaminated with helminth eggs.

It has been widely noted that the monsoons of August and September 1988 were particularly severe[82, 83], and as such it is possible that transmission of whipworms and roundworms (and other STH species) increased during these months.

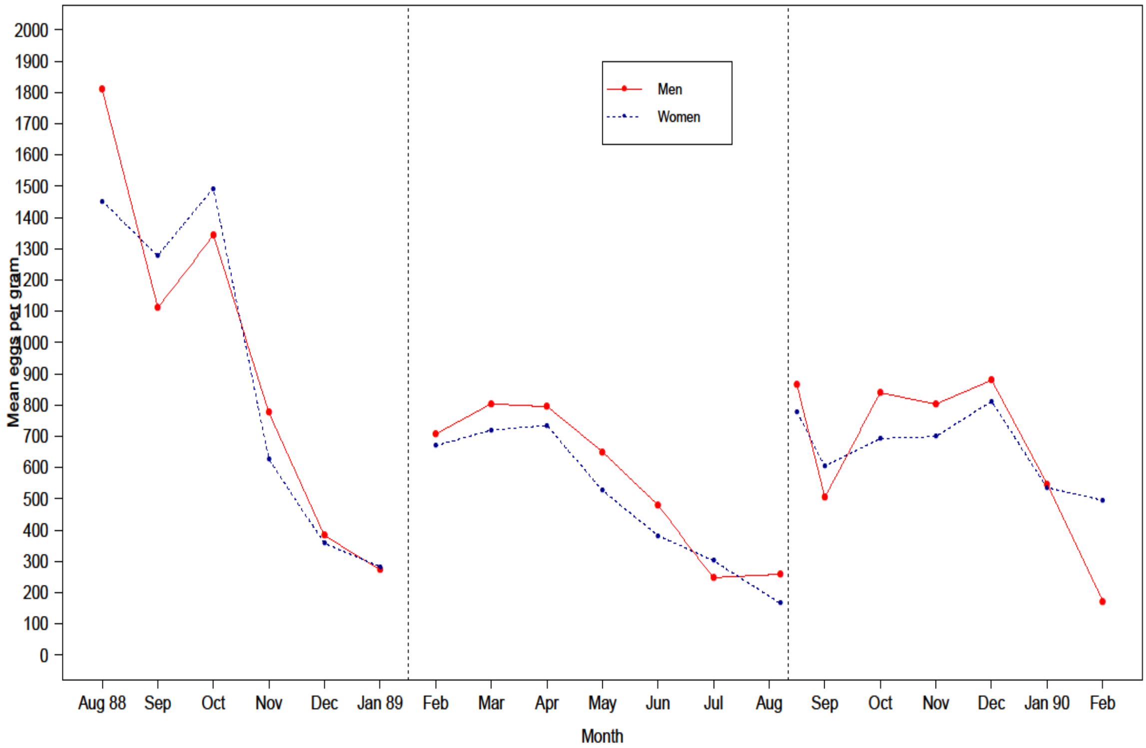
On the other hand, sanitary conditions in the slum area of Dhaka where the data were collected were already appalling and it is entirely possible that transmission intensity was not greatly affected by the severe monsoon. Moreover, existing problems with infrastructure and a large amount of precipitation over the previous two years[84] may have adversely affected health and sanitation systems, compounding the deleterious impact of the monsoon [85].

In either case, by far the highest egg counts occurred in the months when the monsoon was raging, with significant decreases seen as soon as the monsoon season ended. The pattern that follows after successive treatment rounds could be the result of those that had been treated by chemotherapy being re-infected in the period with the greatest transmission intensity, whilst those treated later were re-infected when transmission intensity had decreased.

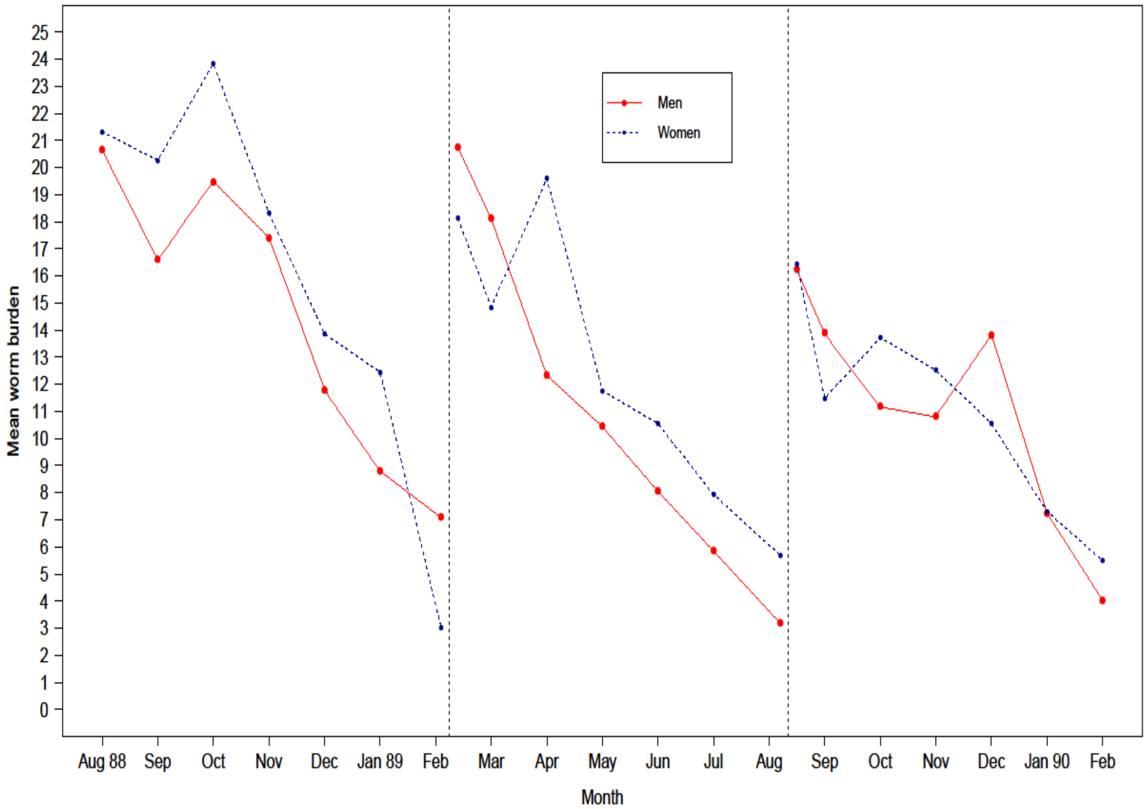
Once again, much higher maximum values of EPG of faeces were recorded for

A. lumbricoides than for *T. trichiura*. Re-infection occurred extremely rapidly, with the total worm burden almost back to pre-treatment levels in every case after only 6 months. To put this in context, the current recommend schedule for chemotherapy is a once yearly treatment by an anthelmintic. These findings suggest that this would be insufficient to properly combat morbidity in this region considering that it would take only half of the time period between treatments for re-infection to become a significant problem.

a)


Men
Women
Mean eggs per gram
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
Aug 88
Sep
Oct
Nov
Dec
Jan 89
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan 90
Feb
Month

b)


Men
Women
Mean worm burden
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Aug 88
Sep
Oct
Nov
Dec
Jan 89
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan 90
Feb
Month

c)

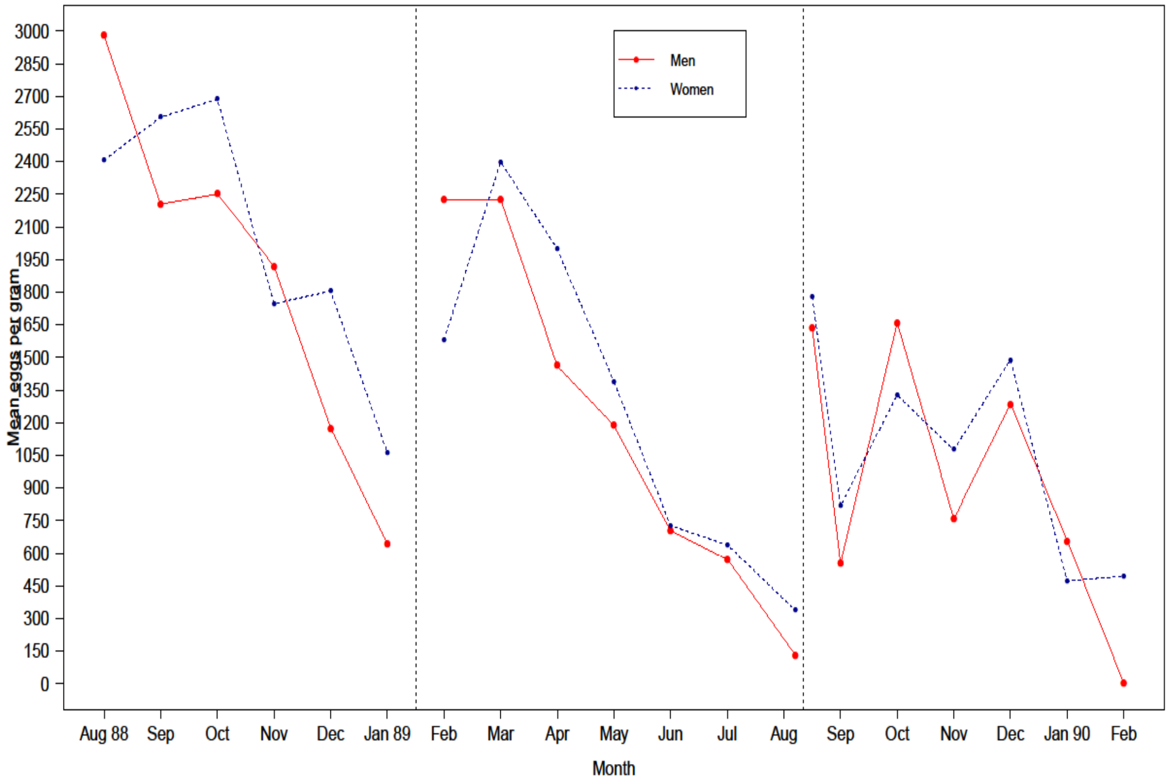


Figure 6: Mean egg or worm count per month from the Bangladesh data for a) Trichuris trichiura (based on egg count), b) Ascaris lumbricoides (based on worm count) and c) Ascaris lumbricoides (based on egg count). Dashed black lines represent the point at which a new round of treatment was started.

South India

The data from South India were collected over a shorter time period than the data from Bangladesh. Figure 7 shows that mean egg counts of *A. lumbricoides* fall sharply after treatment in January (as one would expect given the high efficacy of the drug against roundworm), followed by a general increase over the year as individuals become re-infected. *T. trichiura* egg counts do not follow this pattern however and stay roughly constant throughout the year as although November seems to have a lower egg count than July this difference is not significant. Pyrantel pamoate is of course much less effective against whipworm than against roundworm, and density dependent processes may play a part here. There are no significant differences between the sexes for either worm species.

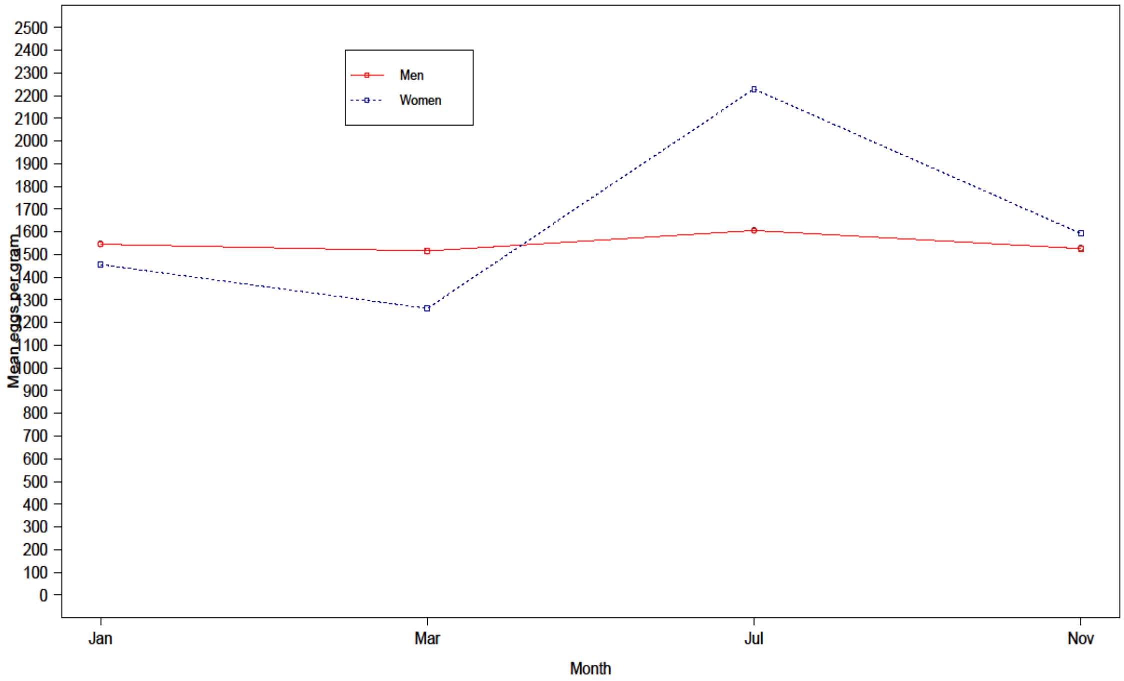
Much as the climate in Bangladesh seemed to have an influence on the results, the climate in South India may have a similarly considerable effect on these data. Tamil Nadu (the state in India containing the village where the investigation took place) has a tropical climate, with constantly high temperatures throughout the year – daily temperatures in the area vary between 24^{O}C and 33^{O}C[14]. The three years before the start of data collection had seen a particularly terrible drought in the area. The monsoon season tends to occur between October and November, with large amounts of rain in August as well[14]. Vairavan Kuppam is a fishing village, unlike Dhaka of course, and this difference in occupations between the two locations could also have an effect on intensity of infection.

It is also possible, given the climate and region in which Vairavan Kuppam is located, that hookworm prevalence may be higher here than in Bangladesh. This could have an effect on the prevalences of whipworm and roundworm because of possible interactions between the species in co-infected individuals.

Unfortunately because the data were collected over the space of less than a year it was not possible to see what possible changes there are over the course of a number of seasons, as it was possible in the Bangladesh data set. Also because no data were collected after treatment in November, it is only possible to compare a single treatment round, as compared to the two rounds in Bangladesh. This problem is further exacerbated by the fact that there are only 4 months in which data were collected, so trends between collection points are impossible to observe, though there are shorter gaps between times when the same individuals donate multiple samples.

Overall, whilst there may be a climatic effect on egg counts in Bangladesh, it is not possible to corroborate this with the data from South India. What is noticeable is that *A. lumbricoides* and *T. trichiura* seem to display similar trends in Bangladesh and that there is no difference between sexes in either location for either worm species according to these results.

a)



b)

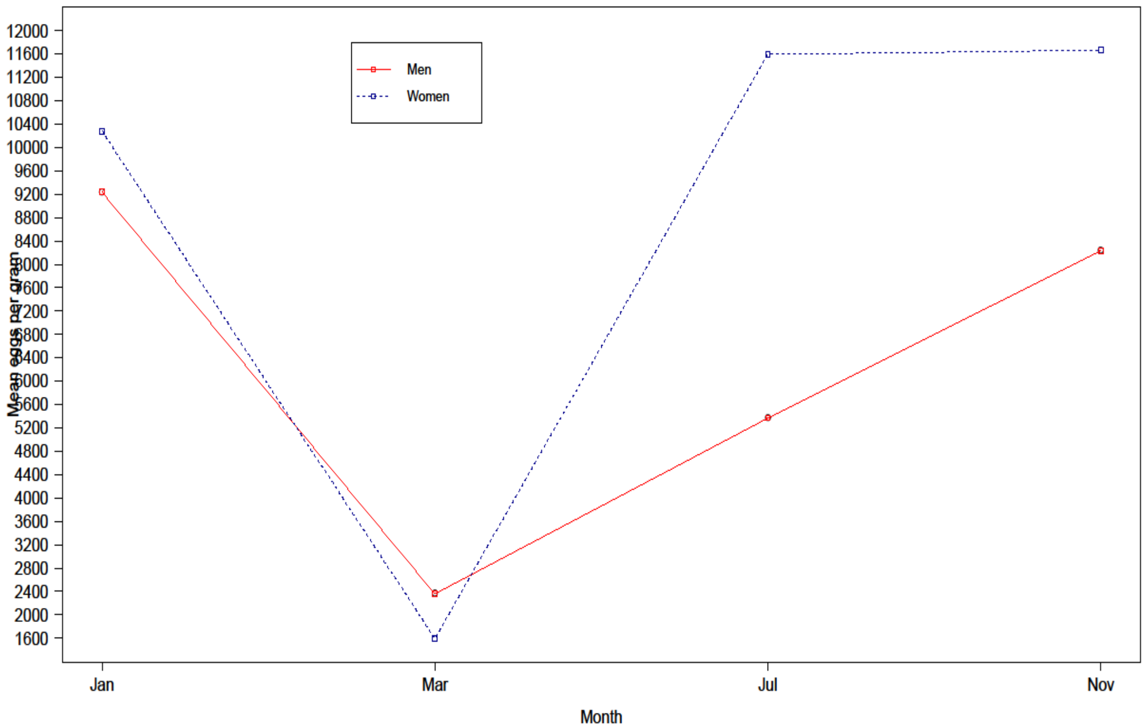


Figure 7: Mean egg or worm count per month from the South India data for a) Trichuris trichiura and b) Ascaris lumbricoides (both based on egg counts)

5. The interaction between roundworms and whipworms in Bangladesh and South India

5.1. Hypothesis

A statistical model was formulated with the overall aim of testing the hypothesis that the removal of *A. lumbricoides* worms via treatment by pyrantel pamoate could give the remaining *T. trichiura* a competitive advantage. The null hypothesis was that the number of *A. lumbricoides* in the host does not have an effect on the proportional change in egg output of *T. trichiura* post-treatment. The alternative hypothesis is that after treatment the surviving whipworms gain a competitive advantage from the removal of roundworms so that their egg output decreases by relatively less - or even increases – if there were many rather than few co-infecting *A. lumbricoides* worms before treatment.

5.2. Models and Methods

Two models (a and b) were fitted to the data from Bangladesh to accommodate the separate rounds of treatment. In this way it was possible to compare *T. trichiura* egg counts in the first re-infection population with *A. lumbricoides* worm counts at baseline (model a), and to compare *T. trichiura* egg counts in the second re-infection population with *A. lumbricoides* worm counts in the first re-infection population (model b).

The number of *A. lumbricoides* worms was treated as a categorical variable, rather than a continuous variable, having formulated both versions of the model and compared the Akaike Information Criterion (AIC)[86] values. The category sizes were chosen by looking at previously published analyses of the data[13].

Sex and age groups were included as factors because of previous indications of the importance of age category and sex in terms of infection intensity (see section 4.2.2. Age-intensity profiles). Month and year were included to allow for the variations expected according to seasonal effects on transmission. –This was especially important for the Bangladesh data (see section 4.2.3. Seasonality and re-infection).

As the response data are longitudinal (since an individual's *T. trichiura* egg counts were measured at multiple time points), it was assumed that egg counts from the same individual were Poisson distributed. A random intercept term was used to account for the overdispersion (extra-Poisson variation) in egg counts among hosts which was evident at each round of treatment.

Therefore the relationship in Bangladesh between the eggs per gram of faeces of *T. trichiura* (EPGTT) and the worm count of *A. lumbricoides* is defined as:

$$EPGTT = \lambda * T + A + S + M + Y$$

where T is the round of treatment, λ is the relevant category of roundworm worm count, A is the age category of the individual, S is the sex of the individual, M is the month in which stool collection took place and Y is the year in which stool collection occurred.

In South India only one year was under consideration, and egg count data for roundworms had to be used instead of worm counts. Thus the model had the structure:

$$EPGTT = \varepsilon * M + A + S$$

where ε is the number of EPG of faeces of roundworm.

The models were fitted using standard Generalized Linear Mixed Model (GLMM) techniques implemented with the lme4[87] package in R[88].

5.3. Results and Discussion

Bangladesh

The results of these two models (*Tables 2a* and *2b*) show that the number of roundworms removed via chemotherapy from an individual who is infected by both *A. lumbricoides* and *T. trichiura* has no significant effect on the egg count of whipworm after a 6 month period of re-infection. This indicates that removal of *A. lumbricoides* from a host who is co-infected with *T. trichiura* doesn't give the remaining *T. trichiura* worms a competitive advantage (or disadvantage).

There are some noteworthy results however. As one may expect given that the heaviest burden of infection for both species is experienced by children between the ages of 5 and 16 years, those age categories exhibited significant relationships in both models with the number of EPG of faeces of *T. trichiura*. Sex does not seem to be significantly associated with egg counts of whipworms in either case, but the month and year of collection are. There were significant differences in egg count between 1988 and 1989, and between 1989 and 1990. The latter result may be explained because the only results taken in 1990 were in the first two months, when transmission rates should be at their lowest compared to the monsoon season in 1989. The former result however may indicate a strong climatic effect on transmission, particularly considering the extreme weather conditions experienced by Dhaka residents during the monsoons of 1988, although there are few significant differences in egg counts between months.

Table 2: Results of the models fitted to the data from Bangladesh with model a) including data from August 1988 to August 1989, and model b) including data from February 1989 to February 1990

a)

Variable	**Category**	**Estimate**	**Standard Error**	**t value**
Intercept		1082.43	302.088	**3.583***
Treatment round	1	121.932	224.042	0.544
Ascaris lumbricoides worm burden (number of worms)	(0,2]	-61.088	144.777	-0.422
	(2,6]	6.645	110.299	0.06
	(6,10]	49.766	119.704	0.416
	(10,20]	174.561	103.731	1.683
	(20,40]	389.815	108.579	**3.59***
	(40,200]	518.321	130.696	**3.966***
Sex	Female	-39.812	49.93	-0.797
Age category (years)	(2,4]	519.935	128.298	**4.053***
	(4,6]	733.138	128.301	**5.714***
	(6,8]	731.076	126.72	**5.769***
	(8,10]	465.346	130.668	**3.561***
	(10,12]	548.716	131.254	**4.181***
	(12,16]	329.761	132.5	**2.489***
	(16,26]	196.122	127.965	1.533
	(26,36]	107.898	128.22	0.842
	(36,46]	181.82	136.717	1.33
	(46,150]	104.532	148.505	0.704
Month	September	208.366	180.491	1.154
	October	388.6	235.623	1.649
	November	395.793	231.986	1.706
	December	306.735	233.796	1.312
	January	244.204	235.808	1.036
	February	-10.344	237.661	-0.044

	March	35.371	259.049	0.137
	April	-514.889	288.978	-1.782
	May	-172.12	284.403	-0.605
	June	-799.309	284.407	**-2.81***
	July	-1143.497	286.106	**-3.997***
Year	1989	-1287.503	269.523	**-4.777***
Treatment Round 1* *Ascaris lumbricoides* worm burden	(0,2]	6.737	201.975	0.033
	(2,6]	-54.766	162.222	-0.338
	(6,10]	-97.686	176.939	-0.552
	(10,20]	-146.349	154.572	-0.947
	(20,40]	-209.478	167.833	-1.248
	(40,200]	-120.585	205.774	-0.586

b)

Variable	**Category**	**Estimate**	**Standard Error**	**t value**
Intercept		521.507	183.484	**2.842***
Treatment round	2	322.016	147.569	**2.182***
Ascaris lumbricoides worm burden (number of worms)	(0,2]	-56	97.626	-0.574
	(2,6]	-56.833	82.742	-0.687
	(6,10]	-51.997	90.799	-0.573
	(10,20]	29.99	80.979	0.37
	(20,40]	173.787	90.82	1.914
	(40,200]	431.899	113.495	**3.805***
Sex	Female	-24.13	42.605	-0.566
Age category (years)	(2,4]	447.221	125.537	**3.562***
	(4,6]	505.904	122.803	**4.12***
	(6,8]	422.486	120.265	**3.513***
	(8,10]	440.278	121.69	**3.618***
	(10,12]	263.989	124.152	**2.126***
	(12,16]	193.245	122.951	1.572
	(16,26]	79.934	124.691	0.641
	(26,36]	36.262	124.159	0.292
	(36,46]	84.082	129.812	0.648
	(46,150]	19.058	140.704	0.135
Month	March	-242.493	123.442	-1.964
	April	-86.072	159.749	-0.539
	May	-68.857	158.437	-0.435
	June	-156.184	159.873	-0.977
	July	-232.176	159.468	-1.456
	August	-478.688	162.46	**-2.946***
	September	-474.83	183.032	**-2.594***
	October	-390.481	224.817	-1.737
	November	-520.472	217.214	**-2.396***

	December	-442.213	218.618	**-2.023***
	January	-385.411	220.91	-1.745
Year	1990	-539.921	198.977	**-2.713***
Treatment round 2**Ascaris lumbricoides* worm burden	(0,2]	4.686	145.116	0.032
	(2,6]	74.63	124.771	0.598
	(6,10]	253.453	135.01	1.877
	(10,20]	76.149	120.872	0.63
	(20,40]	113.442	135.682	0.836
	(40,200]	128.759	181.199	-0.711

Significant results (P<0.05) are indicated in bold and starred

South India

The model fitted to the South India data indicates that the baseline intensity of *A. lumbricoides* infection has a significant effect on the intensity *T. trichiura* infection 6 months later in July. Given the strength of the association (P<0.001) it is highly unlikely that is the result of chance and needs further investigation. However there was no significant effect of the intensity of *A. lumbricoides* infection at any other time point. As for the Bangladesh data, there is no significant relationship between sex and EPG of faeces of whipworm, whilst it seems that age classes between 4 and 34 years do have a significant relationship – of course earlier it was shown that in South India the heaviest burden of infection was experienced by individuals in this age range (see section 4.2.2. Age-intensity profiles), particularly those in school age groups.

Table 3: Results from the model using data from South India

Variable	Category	Estimate	Standard Error	t value
Intercept		1.32E+02	3.23E+02	0.408
Month	March	1.28E+02	1.55E+02	0.829
	July	-8.85E+01	1.63E+02	-0.543
	November	-4.74E+01	1.88E+02	-0.252
Eggs of *Ascaris Lumbricoides* per gram of faeces		4.34E-02	7.25E-03	**5.988***
Sex	Female	4.69E+01	1.68E+02	0.279
Age category	(2,4]	4.40E+02	4.05E+02	1.087
	(4,9]	1.77E+03	3.63E+02	**4.88***
	(9,14]	1.11E+03	3.70E+02	**2.989***
	(14,24]	7.14E+02	3.57E+02	**2.003***
	(24,34]	1.05E+03	3.64E+02	**2.893***
	(34,49]	4.65E+02	3.91E+02	1.19
	(49,150]	6.11E+02	3.90E+02	1.567
March*Eggs of *Ascaris Lumbricoides* per gram of faeces		2.04E-03	1.53E-02	0.133
July*Eggs of *Ascaris Lumbricoides* per gram of faeces		5.94E-02	1.04E-02	**5.687***
November*Eggs of *Ascaris Lumbricoides* per gram of faeces		1.48E-02	1.17E-02	1.269

Significant results (P<0.05) are indicated in bold and starred

6. General Discussion

This project aimed not only to look at co-infection, interactions and the possible consequences of current, imperfect, anthelmintic treatment recommendations but also to discuss the epidemiology of *T. trichiura*, which seems to be the most neglected of the STH's. Funding in 2009 for research and development (R&D) of methods to combat and control the STHs totalled approximately $12.9 million[89] with over half of this sum going into the development of a vaccine to prevent hookworm infection (funding for *T. trichiura* was a little over $1 million in the same time period). Malaria on the other hand received almost $600 million for R&D, illustrating the relative unwarranted neglect of STH infections, and indeed helminth infections overall.

This neglect may be the result of an assumed similarity, in terms of transmission at least, with *A. lumbricoides*, which has been researched in much more detail comparatively. The epidemiological infection patterns presented in this project suggest that there are differences in the patterns of transmission of the two worm species in the locations studied that warrant further attention to trichuriasis.

The absence of an association between *A. lumbricoides* and *T. trichiura* in the data obtained from Bangladesh is in many ways gratifying, as it means that treatment that is ineffective against *T. trichiura* but very effective against *A. lumbricoides* does not impart a competitive advantage to whipworms and potentially facilitate their transmission. However, the generally poor efficacies of anthelmintics against *T. trichiura* is worrying and indicates that anthelmintic treatment programmes should be modified to place a stronger focus on effectively treating whipworm infections.

The widely taken approach of focussing mass chemotherapy on school age children is vindicated by the epidemiological patterns of infection in the two studied communities. However, in addition to school aged children, a comparatively high prevalence of infection was found in the elderly, though infection intensity tends to be relatively low in this older age group. Furthermore, climatic conditions may also have a significant effect on transmission.

The practicality of treatment regimens that include multiple doses of a benzimidazole anthelmintic is unfortunately limited because of difficulties with logistics and in maintaining high rates of compliance. Consequently the currently recommended single dose treatments will almost certainly continue to be used despite their obvious therapeutic weaknesses with regards to *T. trichiura*.

There is also a concern in some quarters over the development on anthelmintic resistance. Such resistance to the benzimidazoles has been found in animals[90, 91] and whilst there are no confirmed reports of resistance for humans (though discussion of ‘sub-optimal response’ has been seen with regards to ivermectin treatment of River Blindness[92]) care should be taken when designing relief programmes that every effort is taken to reduce unnecessary drug distribution (by using targeted treatment if possible for example) and that compliance rates are kept as high as possible. There are no new cheap anthelmintics on the horizon[19], so every effort should be made to maintain the efficiency in combatting helminth infection of the current generation of drugs that are available[93].

Given the apparent similarities in the transmission of roundworm and whipworm, and the fact that the major risk factors for all of the STHs are poor sanitation and hygiene, it is not surprising that concomitant infections are common[5]. These infections can of course not just be infections with more than one helminth, but also co-infections with malaria, HIV[94], etc. depending on the region in question. This project focused on co-infection with roundworms and whipworms because of the similarities in their transmission routes.

Within-host competition[9, 95] between different species within a single host can be over a limited resource such as carbohydrates[96] or can also involve direct interference with the other species life-cycle through toxin excretion[10], etc.. This competition can result in changes to the mortality rate of worms, altered fecundity of female worms or a different rate of worm establishment[10]. Thus although *A. lumbricoides* and *T. trichiura* may inhabit distinct spatial niches within a human host (*A. lumbricoides* predominantly reside in the jejunum, while *T. trichiura* tend to inhabit the cecum), there may still be significant levels of competition between the two worm species.

In terms of the overall design of the model, ideally some measure of weather events would have been included to account for the extreme conditions experienced in Bangladesh during the monsoon season. This was not plausible because of the relatively complicated nature of the 1988 flooding in Bangladesh. Overall the models seemed to be fairly robust and provide some reasonable results, though the use of egg counts rather than worm counts in the South India model was unfortunate but unavoidable. A better, more accurate model would only include worm data for each helminth and would also account for the prevalence of hookworm in the community, especially in the South India model.

In conclusion, focussing on anthelmintic treatments that only remove *A. lumbricoides* from co-infected individuals may not bestow a competitive advantage on the *T. trichiura* worms that remain, but it certainly does little to help effectively control whipworm infections worldwide. This can only be achieved by reviewing and changing current recommended treatment practices.

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