HUMAN EXPOSURE TO *ASCARIS* INFECTION THROUGH WASTEWATER REUSE IN IRRIGATION AND ITS PUBLIC HEALTH SIGNIFICANCE

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Abstract

A longitudinal study of *Ascaris lumbricoides* reinfection over a 9-10 month period was carried out in a wastewater irrigated farming region in Central Mexico (September 1989 - May 1991). The study assessed the relationship between different levels of exposure and its components, in the context of wastewater irrigation, and the prevalence and intensity of *Ascaris* infection.

Three farming populations were studied: (1) those who irrigated with untreated or raw wastewater; (2) those who irrigated with wastewater that had sedimented during passage through a storage reservoir (a form of partial treatment); and (3) those who lived in a rain-fed area. Individuals' exposure to *Ascaris* eggs was estimated by in-depth interviews (characterising the frequency and type of contact with wastewater). Wastewater contact through agricultural activities was also estimated by structured observation.

Predisposition to *Ascaris* infection was largely a consequence of behavioural and environmental factors that caused wastewater contact. Overdispersion of *Ascaris* intensity in the study population was not age- or gender-dependent.

Over the 12-month monitoring period, untreated wastewater had a mean concentration of 96 *Ascaris* egg/litre and sedimented wastewater a concentration of <1 *Ascaris* egg/litre.

Contact with untreated wastewater during various activities was associated with differing degrees of excess risk of *Ascaris* infection in the respective groups:

- crop irrigation: 3-fold risk among children
- chilli production: 5-fold risk among men and higher intensity infections
- tending livestock: 4-fold risk among women
- consumption of crops irrigated with wastewater: 2-fold risk in men and children and higher intensity infections in children
- sweeping the yard: 5-fold risk in women

Contact with sedimented wastewater during play was associated with more than a two-fold risk in *Ascaris* infection among children, and during maize production, with higher intensity infections among men.

The nematode egg guideline of ≤1 egg/litre is adequate to protect the health of farmers using wastewater in agriculture, but is not sufficient to protect children. Any future modifications of the guideline must consider this.

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CHAPTER 1. INTRODUCTION

Predisposition to intestinal nematode infection has been discussed at great length. There is much debate over the relative importance of behaviour, environment and genetics in determining the prevalence and intensity of intestinal helminth infection (Bundy, 1988; Keymer & Pagel, 1988). Globally, *Ascaris lumbricoides* is the most prevalent intestinal nematode. Estimates suggest as many as 1472 million people are infected worldwide (Crompton, 1999). Few studies have estimated human exposure and infection in the same population. Reports from the literature indicate that increased faecal contamination of the environment is associated with *Ascaris* infection. A reduction in such contamination results in a corresponding reduction in *Ascaris* infection (Moraes, 1996; Smith, 1993).

This thesis aims to further the understanding of the role of human exposure to faecal contaminated environments in determining the prevalence and intensity of *Ascaris* infection. The study was based in the semi-arid Mezquital Valley in Central Mexico, about 100km north of Mexico City. The risk of *Ascaris* infection associated with exposure to wastewater of differing qualities was measured. Three farming populations were studied: those who irrigated crops with untreated or raw wastewater; those who irrigated with wastewater that had sedimented during passage through a storage reservoir (a form of partial treatment); and those farming families that lived in a rain-fed area. Structured observation studies and interviews provided an in-depth understanding of the complexities of human exposure to wastewater in this population.

Unravelling the complexities of predisposition to *Ascaris* infection will assist in directing efforts to control this intestinal nematode, which is estimated globally to cause morbidity in around 59 million individuals (de Silva *et al.,* 1997) and be responsible for 10.5 million DALYs (Chan, 1997).

Wastewater is a valuable resource. Wastewater reuse in irrigation is essential in many countries, where there is a desperate shortage of fertile agricultural land and where arid conditions mean that productivity is low. However, without adequate microbiological guidelines for wastewater reuse, safe and efficient management of this resource can not be achieved. This thesis aims to comment on the validity of the WHO nematode egg guideline for wastewater reuse in restricted crop irrigation (WHO, 1989).

The objectives of this thesis can be summarised as follows:

Ascaris Epidemiology

1) To assess the relationship between different levels of exposure and the prevalence and intensity of *Ascaris* infection
2) To assess the effect of the components of exposure on determining the prevalence and intensity of *Ascaris* infection
3) To assess the impact of the intensity of *Ascaris* infection on morbidity levels

Wastewater Reuse and *Ascaris* infection

4) To quantify the impact of raw wastewater reuse in irrigation on the prevalence and intensity of *Ascaris* infection and morbidity among farm workers and their families
5) To assess the effect of the lower concentrations of *Ascaris* ova in sedimented wastewater on reducing the excess risk of *Ascaris* infection and high intensity infection,
6) To evaluate on the validity of the nematode egg guideline (WHO, 1989), for the health protection of agricultural workers using wastewater in restricted crop irrigation

Human Behaviour

7) To study the behaviour of farmworkers and their families in the fields, so as to measure the degree of contact with the wastewater and identify specific activities that result in increased contact with wastewater

8) To identify those 'risky' behaviours that are associated with high intensity *Ascaris* infection and that could be amenable to change

CHAPTER 2. LITERATURE REVIEW

2.1 *ASCARIS LUMBRICOIDES* - A GLOBAL ISSUE

Ascaris lumbricoides is one of the most prevalent human parasites today. Over 50 years ago, Stoll estimated that 644 million people world-wide were infected with *Ascaris lumbricoides* (30% prevalence for the human race) (Stoll, 1947), Since then several authors have recalculated the numbers infected with *Ascaris* (Peters, 1978; Walsh & Warren, 1979; Crompton, 1988). Evidence suggested that in many developing countries, as many as one in two are infected with *Ascaris* infection. Current calculations estimate that 1472 million people are now infected with *Ascaris* (28% prevalence) (Crompton, 1999). World prevalence of *Ascaris* infection thus appears to have hardly altered over the last 50 years. However there has been a significant shift in the distribution of infection away from North America and Europe, a reduction in infection in Africa and Asia, and an increase in infection in Latin America and the Caribbean (Chan, 1997). The prevalence of *Ascaris* infection in Latin America, estimated at 32.1% by Peters (1978) is now reported at nearly 40% (Chan, 1997). In Mexico, the prevalence of infection varies in urban and rural areas from 43% in the state of Guerrero to nearly 100% in the state of Yucatan (Gonzalez R. *et al.*, 1985; Forrester *et al.*, 1988; Lara Aguilera, 1984). In Asia, estimates put the prevalence at 40.5%, with prevalence ranging from 21% in some parts of Malaysia (Kan *et al.*, 1989) to 100% in parts of Taiwan (Chen & Hsieh, 1969). Recent control efforts in some countries in Asia have resulted in around a 10% reduction in *Ascaris* infection (Chan, 1997). There seems to have been a reduction in *Ascaris* infection in Africa from 40% to just over 20% (Chan, 1997).

Ascariasis is a chronic infection and in endemic areas, the prevalence can approach 100% in early childhood (Peters, 1978). It is distributed throughout poor and socio-economically deprived communities in the tropics and sub-tropics, where sanitation and hygiene are poor and the climate is favourable to the development of the infective stages of *Ascaris*. *Ascaris* infection is principally a cause of morbidity rather than mortality, but around 20,000 are estimated to die each year as the result of *Ascaris* infection (Walsh & Warren, 1979). The level of morbidity is very difficult to estimate because so few non-life threatening cases are ever reported. Several authors have estimated morbidity. Crompton (1999) estimated that about 335 million people are affected by *Ascaris* related disease. De Silva *et al.,* (1997) estimated 59 million were at some risk of morbidity and that ascariasis was responsible for about 10,000 deaths each year. Chan (1997) estimated that 10.5 million DALYs (disability-adjusted life years) were lost through *Ascaris* infection.

Ascaris infection can affect the host in a variety of ways (de Silva *et al.,* 1997). Physical fitness and growth can be affected, however these effects can often be reversed by reducing worm burdens. The presence of *Ascaris* worms can be associated with damage to the mucosal lining of the intestine and impaired protein absorption. Also, heavy infections of *Ascaris* can lead to obstruction of the intestine which can cause the intestine to cease to function. With the exception of intestinal obstruction, most symptoms relating to *Ascaris* infection are non-specific.

Despite differences in estimates between authors, it can be concluded that *Ascaris* infection still affects at least 25% of the world's population. Effective chemotherapy exists, and is possibly the reason for reductions in *Ascaris* prevalence in Africa and Asia (Bundy, 1997). A greater understanding of the epidemiology of *Ascaris* infection would enable the implementation of other control measures as well as chemotherapy. This is especially important where reinfection with *Ascaris* is rapid.

2.2 LIFE-CYCLE OF *ASCARIS LUMBRICOIDES*

Ascaris lumbricoides has a direct life-cycle with man as the definitive host. Transmission of the parasite occurs by the faecal-oral route. The ova are released by the fertilised female worm and excreted by the infected host. In favourable conditions (moist and 20-26°C) the larva develops inside the ovum in 10 to 15 days. When the infective ovum is ingested, it passes into the small intestine where the larva emerges. The larva then penetrates the intestinal wall and migrates in the bloodstream to the liver at 4 days post infection. The larva migrates in the bloodstream from the liver via the heart to the lungs at 7 days post infection where the larva develops. The immature worm returns to the small intestine 17 days post infection, where the worm matures. The pre-patent period is about 6-8 weeks, after which the female worm produces about 240 000 eggs per day. The adult worms have a life span of 6-12 months and are usually located in the ileum. The male worm is about 20 cm and the female worm 30-40 cm in length. The male is more slender than the female which has a diameter of 8 mm. Both female and male worms are creamy white with a tinge of pink.

Transmission of *Ascaris* infection occurs by ingestion of the infective ova. Transmission can occur through occupational exposure, for example workers that irrigate crops with wastewater or those that collect nightsoil to be put on the fields. If the wastewater or the nightsoil is contaminated with *Ascaris* eggs then there is a risk of *Ascaris* transmission. Transmission can also occur through consumption of food or water contaminated with *Ascaris* eggs, this is called consumer exposure to infection. Consumer exposure can be the result of crop irrigation with wastewater or fertilisation with nightsoil. The other route of transmission is through poor hygiene and sanitation in the home. This often occurs when there is promiscuous defecation in the yard around the house; the soil is then contaminated and subsequent contact with the soil could lead to transmission of *Ascaris* infection (Feachem *et al.*, 1983).

2.3 *ASCARIS* EPIDEMIOLOGY

2.3.1 Introduction

The direct life-cycle of *Ascaris lumbricoides* and other intestinal nematodes, together with their widespread distribution and high prevalence has meant that a wealth of information has been collected on the distribution of these parasites. Since the 1920's there have been many studies that have gathered information from one or two communities on a range of intestinal nematodes. In the early 1920's much of the research concentrated on hookworm infection (Cort, 1922; Stoll, 1923; Sawyer, 1925; Chandler, 1925; Augustine, 1926). The papers published at this time on other intestinal nematodes seem mainly to concern reports of clinical disease caused by intestinal nematode infections. Then, in the late 1920's and 1930's a series of epidemiological studies of *Ascaris* infection were carried out in Panama, the United States and China (Cort, 1931). Interest has gradually increased since this early work and in recent years there has been much research on the epidemiology of *Ascaris* and other intestinal nematodes.

Epidemiological studies of *Ascaris* have provided important information about distribution of the infection in many parts of the world. There have been studies for example in Iran (Arfaa & Ghadiran, 1977), Mexico (Stoopen & Beltran, 1964), Burma (Thein-Hlaing *et al.*, 1984), Ethiopia (Tedla & Ayele, 1986), Tanzania (McCullough, 1974) and Kenya (Bell, 1965; Schaller & Kuis, 1972). The numerous studies carried out have enabled the epidemiology of *Ascaris* infection to be characterised in a variety of settings.

2.3.2 Age dependence

In many of the epidemiological studies carried out, similar age-prevalence and age-intensity profiles for *Ascaris* infection can be observed in endemic areas. The prevalence of infection rises steadily from early childhood with children first becoming infected as young as 4 months. Prevalence of infection then peaks in late childhood, generally between 8 and 14 years of age. In adulthood prevalence often remains at the high levels attained in late childhood. The intensity of infection follows a different pattern, rising steeply from early childhood, peaking in late childhood but unlike the prevalence pattern, intensity of infection then declines during adulthood to a lower steady level (Bundy *et al.*, 1987; Forrester *et al.*, 1988). Elkins *et al.*, (1986) observed this profile of *Ascaris* infection in a study in Southern India. The study was carried out in seven fishing villages on the coast of the Bay of Bengal. The prevalence of *Ascaris* infection increased from 46% in the 0-2 year age class to a peak of 93% in the 5-9 year olds, prevalence then declined slightly to 80% in the adult age classes. The intensity of infection, in terms of egg counts and worm burden, rose to a peak in the 5-9 year age class, but then declined significantly in late childhood and adulthood. Bundy (1988) in describing the age-intensity profile of *Ascaris* infection stated that the intensity of infection exhibits marked convexity, reaching a maximum in the 5-9 year age class and is lower in adulthood. However other studies have found conflicting results to this 'typical' profile of *Ascaris* infection.

Croll *et al.*, (1982) also obtained a profile of intensity of infection that differed from the profile often reported. A study was carried out on 216 people selected at random from two adjoining rural villages in Iran. It was found that the prevalence of *Ascaris* infection rose rapidly in early childhood to reach 93.3% in the 5-9 year olds and a peak of 100% in the 15-19 year age class. The prevalence of infection remained at a high level during adulthood. The intensity of infection however, while rising rapidly in early childhood, did not then decline in adulthood but remained at a consistently high level throughout the adult age classes.

Stoopen and Beltran (1964) carried out an epidemiological study of *Ascaris* infection in Mexico, in the village of Santiago Yancuictlalpan, in the Sierra Madre. The prevalence of *Ascaris* infection rose from 28% in infants aged 4-23 months to 70% in the 2-6 year olds and to 74% in the 7-14 year age group. The prevalence of *Ascaris* infection then fell to 58% in adulthood. This is different to the profile of infection often reported, where the prevalence of infection remains at the high level attained in childhood.

Several epidemiological studies in Burma have found that both the prevalence and intensity of *Ascaris* infection decline in adulthood (Tu *et al.*, 1970, Hpay *et al.*, 1970, Thein-Hlaing *et al.*, 1984). Thein-Hlaing *et al.*, (1984,1987) carried out a study among 783 people from a rural village in Rangoon Division, Burma. Prevalence rose rapidly in early childhood to a peak of 92.5% in the 5-9 year age class and then declined to 65% in the oldest age class. The average worm burden increased rapidly during early childhood to a peak of 17.3 worms in the 5-9 year age class and then decreased to 4 worms in the oldest age class.

Clearly it is not possible to generalise, since variations in the profile of *Ascaris* infection can be seen between communities and between age classes and between individuals within age classes. There are no age-prevalence and age-intensity profiles for *Ascaris* infection that can be applied to all communities in the tropics and subtropics where *Ascaris* infection is endemic. There appear to be factors that affect the age-prevalence and age-intensity profiles of *Ascaris* infection differently at both the group and individual level, depending on the situation.

2.3.3 Sex dependent variations

While much information on the age stratified profiles of *Ascaris* infection has been obtained, only a few of these papers also report the profiles of infection separately for the males and females. A variety of results have been observed however, from the papers that have reported gender differences in the profile of *Ascaris* infection.

Arfaa and Ghadiran (1977) in a study in Iran found that the prevalence of infection was consistently higher among females (93%) than males (86%) in all six of the villages studied, though the same proportion of females and males were observed to have high intensity infections. Henry (1988) in a study that focused on pre-school children (under 4 years) found that in the 12-23 and 24-35 month age classes, males had marginally higher prevalence of infection (34% and 36%), than females (32% and 25% respectively), however these differences were not significant. Tedla and Ayele (1986) found that in the younger age classes the prevalence of infection was slightly lower in males (34.1%) than the females (39.0%), and in the older age classes the prevalence of infection was slightly higher in the males (46.4%) than the females (41.5%), however again these differences were not significant. Stoopen and Beltran (1964) in Mexico failed to find a difference in the prevalence of *Ascaris* infection between the females and males. Forrester *et al.*, (1988) also in Mexico, found no difference in prevalence of infection between the females and males. The intensity of infection however appeared to be consistently higher in the females than the males in all the age classes. Elkins *et al.*, (1986) also found that females had higher intensities of *Ascaris* infection than males in most age classes.

Generally the differences between females and males are marginal when the prevalence of infection was measured, although there were small differences found when intensity of infection was measured. However, there are no clear trends prevalence and intensity profiles of *Ascaris* infection for males and females. In some of the papers, differences in the profile of *Ascaris* infection were observed between the females and males in all the age classes, in others only in certain age classes, while in some papers there were no differences observed. It is evident that factors are affecting the prevalence and intensity profiles of *Ascaris* infection of males and females differently in different settings. The reasons for these differences are unclear, however the fact that the differences vary according to the community setting suggests that the factors may relate behavioural or environmental factors, rather than immunological or genetic factors.

2.3.4 Socioeconomic status, hygiene and sanitation dependent variations

Several studies have investigated the effect of socioeconomic status, hygiene and sanitation on the prevalence and intensity of intestinal helminth infection (Arfaa & Ghadiran, 1977; Croll *et al.,* 1982; Henry, 1988; Holland *et al.,* 1988; Thein-Hlaing *et al.,* 1987, Kightlinger *et al.,* 1998, Curtale *et al.,* 1999).

A study in Mexico found a relation between poor housing and low socioeconomic status and the intensity of *Ascaris* infections (Stoopen & Beltran, 1964). Higher intensity infections were found in people living in houses of wood or bamboo with dirt floors. In the centre of the town where housing and sanitation were better, only 6.5 % of the people had higher intensity infections, and the majority (62.5%) of the people were diagnosed by stool examination as uninfected. However, no data were collected on the occupation or income of the members of the households, and these two variables could have acted as confounders. To obtain a clear understanding of the relationship between *Ascaris* infection and housing and sanitation it is important that all the variables that could cause confounding are measured.

Holland *et al.,* (1988) collected data on socioeconomic status, housing, hygiene and sanitation in Panama. Some evidence, though not strong, was found to suggest that poorer housing and lower socioeconomic status are associated with higher intensity of *Ascaris* infection. It was found that infected children (on the basis of stool examination) lived in significantly poorer housing than those uninfected. More lived in houses of bamboo or wood (rather than concrete), with dirt floors and used communal or surface water. More infected children failed to use sanitary facilities (even though they were available in some cases). The level of the mother's education was on average lower in infected children. Crowding was greater in the families of infected children. Also the percentage of families with a regular income was lower than for uninfected children. Holland *et al.,* (1988) found there was a strong association between socioeconomic status and the method of faecal disposal of a child, and infection with *Ascaris.*

The rate of *Ascaris* reinfection following treatment was compared in pre-school children in three villages in St Lucia, West Indies, with different levels of sanitation (Henry, 1988). Results showed that children living in villages with poor sanitation (no household water supply and no latrine) were more likely to be infected with *Ascaris* and were more likely to be reinfected following treatment. Also it was found that the method of faecal disposal and the degree of household crowding were significantly different between children that had never been infected and children that had been always infected during the course of the study.

Clearly socioeconomic status, sanitation and hygiene can play a role in determining whether an individual is infected with *Ascaris* and possibly also affect the intensity of this infection. However, many of the studies mentioned have failed to collect data on possible confounding variables. Therefore it is very difficult to make any clear-cut statements concerning the importance of particular measures of socioeconomic status, sanitation and hygiene in the transmission of *Ascaris* infection and the subsequent intensity of *Ascaris* infection.

2.3.5 Debate over profile of intensity of *Ascaris* infection

The profile of intensity of gastrointestinal helminth infection in a population affects the transmission dynamics and the level of morbidity, unlike viral or protozoan infections where infection is an "all-or-nothing" event (Bundy, 1988b). Consequently, in helminth infection, disease is related to the intensity of infection and not to the mere presence of the infection.

In endemic communities in the tropics and sub-tropics, the intensity of infection in a community or within age classes is often overdispersed, with the majority of worms being harboured by a few individuals. A number of authors have demonstrated that following treatment, individuals with high (or low) infections often reacquire correspondingly high (or low) intensity infections (Anderson, 1986; Bundy *et al.,* 1988; Forrester *et al.,* 1988). Such people are described as being predisposed to a particular intensity of infection. Croll and Ghadiran (1981) called individuals that are predisposed to high intensity infections "wormy people". Despite repeated chemotherapy, predisposition to *Ascaris* infection has been shown to persist (Chan *et al.,* 1992). On the other hand, Hall *et al.*, (1992) found that after three reinfections, 10% of the population consistently reacquired high intensity infections, while 60% of the sample had an intense infection at least once.

This distribution of helminth infections can usually be described by the negative binomial distribution (Anderson, 1982). It is clear however that the reason for a particular distribution can vary between helminth infections and communities. One of the problems is that predisposition and natural variability in infection profile occur together. Many ecological reasons have been put forward to account for the age- intensity profile of *Ascaris* infection, but these can not be used to account for all the variability seen (Anderson & Gorden, 1982). While there is natural variation (heterogeneity) in the distribution of the intensity of *Ascaris* infection within age-classes, the distribution is also highly overdispersed (Anderson & Medley, 1985).

These individuals that are predisposed to high (or low) intensity infections are often found to be aggregated within certain families (Chai *et al.,* 1985; Forrester *et al.,* 1988). A similar pattern is also seen in families in endemic areas, with more families than would be expected by chance having high or low intensity infections (Forrester *et al.*, 1990; Chan *et al.*, 1994a).

Currently there is a debate over the reasons for this skewed or overdispersed distribution of worms, whether within a community or an age class. The debate centres on the role of exposure and susceptibility in the determination of the age-related distribution of intensity of infection (Keymer & Pagel, 1988). It is essential to disentangle and to understand the reasons for the age-intensity profiles of *Ascaris* and other helminth infections. Once the causes of predisposition are identified, control measures can be focused on the appropriate groups of the population whether it involves chemotherapy, improved sanitation or health education, or a combination of these measures (Keymer & Pagel, 1988; Bundy, 1988a).

Several authors have put forward a range of reasons to account for the distribution (Bundy, 1988a; Keymer & Pagel, 1988, Schultz & Kroeger, 1992). It could be due to differences between individuals in contact with the infective stages of *Ascaris* due to diet, hygiene or activity, to differences in genetic susceptibility or to resistance to helminth invasion and establishment acquired by exposure in the younger age classes, or a combination of these mechanisms. It may also relate to the aggregated distribution of *Ascaris* eggs in the environment (Schultz & Kroeger, 1992). A study in Burma suggested that the high intensity infections in a small proportion of the population, may be related to poorer personal hygiene and to more contact with contaminated soil in childhood (Thein-Hlaing *et al.,* 1987). Bundy *et al.,* (1987) in describing the two extreme age-prevalence distributions that are seen, one in which prevalence remains high in all the age classes and the other in which prevalence declines in adulthood, suggested that the different distributions reflect differing environmental and cultural factors.

The profile of intensity of *Trichuris trichiura* infection increases to a peak in the 5-14 year age class and then declines in the adult age classes (Bundy, 1986). This subsequent decline in the intensity of infection in adulthood may be due to an age-related change in establishment of the worms (due perhaps to acquired immunity), or to an age-related change in exposure (due to behavioural changes), or to a combination of these processes.

Epidemiological studies of *Ascaris* and *Trichuris* infections have implicated behaviour, culture, hygiene, sanitation environment and various socioeconomic factors as causal in the age-intensity profile of infection (see section 2.3.4).

A study of 2-8 year old children in a children's home in Jamaica found that the distribution of *Trichuris* worms was overdispersed, although the age range was small and all the children remained within the confines of the children's home and so were assured to have had contact with a similar level of contamination (Bundy *et al.,* 1985). Behavioural differences, such as geophagia, may have been important in determining the distribution of worms in the children. Behavioural factors that affect exposure levels need to be measured to determine the relation between exposure and infection. Bundy and Cooper (1988) compared the results of this study with a study conducted in a community in St. Lucia (Bundy *et al.,* 1987). The children in the community showed predisposition to a particular intensity of infection, but the children in the children's home did not. Differences in the pattern of environmental contamination between the children's home and the village could explain this. As said above however, to show the impact of exposure, exposure must be measured and this study did not do this and the differences here could be also due to other factors. Several studies of *Ascaris* and *Trichuris* have indicated that differences in intensity of infection may be related to changes in sanitation (Feachem *et al.,* 1983; Arfaa & Ghadiran, 1977; Holland *et al.,* 1988; Henry, 1981). More research however is needed to assess the importance of sanitation in determining the intensity of *Ascaris* infection.

The results of a study of sensory impaired children in Guatemala indicated that behavioural differences between deaf and blind children may account for the differences in the profile of infection observed (Scott *et al.,* 1989). The age-intensity profiles of *Trichuris trichiura* were very different in deaf and blind children. In deaf children, intensity of infection peaked in 7-8 year age class, declining to a low level in the teenagers. In the blind children, intensity of infection remained low until the early teens, peaking in the 13-14 year age class. These findings suggest that the differences in the intensity profile of infection may relate to differences in the development of deaf and blind children, with deaf children able to take an earlier interest in their surroundings than blind children. The subsequent decline in the intensity of infection may relate to adults observing more hygienic practices than children, reducing exposure to infection, and so predisposing themselves to lower intensity infections (Bundy, 1986).

Exposure to the infective stages of *Ascaris* and other helminth infections is clearly influential in determining the intensity profile of infection, whether exposure is a result of a behavioural, cultural or socioeconomic factor, or a combination of these factors. To demonstrate the part of exposure in the determination of the prevalence and intensity of *Ascaris* infection, it is necessary to measure exposure and *Ascaris* infection, as well as controlling for possible confounding variables. Infection can only occur via ingestion of the infective eggs, either directly with contaminated water or soil or indirectly with contaminated food. Therefore to measure exposure it is necessary to measure the amount of contact an individual has with the source of infection, whether it is water, soil or food. Although any aggregation of the infective stages of *Ascaris* in the contaminated medium will affect this exposure measure. It is also important to consider any climatic changes that could affect the survival of the infective stage, such as temperature or humidity (Tedla & Ayele, 1986). Clearly there are problems here in accurately quantifying exposure.

Several studies have suggested that greater contact with infected water, soil or contaminated food by a particular sector of the population has resulted in a greater likelihood of *Ascaris* infection and in higher intensity *Ascaris* infections (Stephenson, 1985; Elkins *et al.*, 1986; Thein-Hlaing *et al.*, 1987; Bundy *et al.*, 1987), but few quantitative studies have been carried out. A study in Kenya suggested that children with earth floors in their homes are more likely to have *Ascaris* infection and are more likely to be reinfected (Stephenson, 1985). Peridomestic exposure was suggested as a causal factor in the high intensity *Ascaris* infections seen in children. Since exposure was not actually quantified it is not possible to say whether these observations were due to peridomestic exposure or to another route of transmission. Also the intensity of infection was not measured in the adults in the families studied. Therefore it is not possible to say whether the presence of an earth floor increases the likelihood of infection in the family as a whole or only in the children. This is important in determining whether hygiene and sanitation practices of the family or the behaviour of the child are more involved in deciding the distribution of high intensity *Ascaris* infections in this community.

A study in Jamaica measured geophagia indirectly by measurement of silica levels in stool samples. Results indicated that high intensity *Ascaris* and *Trichuris* infections among children may be associated with increased geophagia (Wong, 1988; Wong *et al.,* 1988). The often seen decline in the intensity of infection in adulthood may be associated with the fact that adults seem to exhibit less geophagia than children (Halstead, 1968). Cooper and Bundy (1988) stated that reportedly geophagia occurred in 30% of 2-8 year olds in a community studied in St. Lucia. If this is the case, then geophagia could be important in determining predisposition to high intensity infection in this community.

While these studies show associations between exposure to infection and the intensity of infection, there has been no attempt to directly quantify exposure and determine the reduction in exposure required to reduce infection to defined levels. This is due to the problems associated with accurately measuring exposure. In the study of geophagia mentioned above, peridomestic exposure was measured in children in Jamaica. However geophagia was measured indirectly by measuring the amount of silica in the faeces (Wong, 1988; Wong *et al.,* 1988). Moreover the relationship between geophagia and the intensity of *Trichuris* infection and to some extent *Ascaris* infection (Cooper & Bundy, 1988) is speculative since the intensity of infection and geophagia were not measured in the same study.

Moraes (1996) found that communities where drains had been introduced, which prevented flooding of sewage, had reduced levels of *Ascaris* infection and intensity compared with communities with no drains. Smith (1993) showed that faecal contamination of streets due to flooding of sewage was associated with an increased risk of *Ascaris* reinfection prevalence among children.

Several studies have indicated that exposure due to farming practices may be important in determining the intensity of *Ascaris* infection, including two studies in Iran. Arfaa and

Ghadiran (1977) in a study in Iran reported that the intensity of infection did not decline in the adult age classes. While exposure was not measured in the study, it was reported that nightsoil was used as the principal fertiliser for vegetables that were later eaten raw. It may be that consumption of *Ascaris* contaminated vegetables resulted in the intensity of infection remaining high in adulthood. It was also found that higher intensity infections were found in females than males. The authors suggested that closer contact with infected dust and with vegetables and nightsoil heavily contaminated with *Ascaris* eggs may explain these high intensity infections. Another study in Iran found that the intensity of infection varied on a seasonal basis, and implicated climatic changes on both human habits and the rate of development plus survival of the transmission stage (Croll *et al.,* 1982). Another possible determinant also related to climatic changes is the seasonal use of nightsoil as a fertiliser for vegetables and the resulting increase in exposure to infection in workers and consumers. A study in Macao City, where many families live in concrete blocks of flats with piped water and sewerage and where the roads have concrete surfaces, showed that schoolchildren were infected with *Ascaris* (Chan Chac-Tai, 1989). Since soil transmission of infection was unlikely, there were two possible routes of transmission; firstly fresh vegetables imported from China and contaminated with *Ascaris* eggs and secondly the highly infected immigrant population from the farming communities in China that lives in Macao City. Studies have also indicated that farm workers and sewage farm workers exposed to wastewater have significantly higher intensity infections of *Ascaris* than controls (see section 2.4.3).

Research has indicated that exposure to infection may be important in determining the profile of intensity of infection, and that a reduction in the level of exposure could reduce reinfection. Theoretical research has indicated that repeated, targeted drug treatment of these heavily infected groups in the population can have a significantly greater impact on morbidity than mass or random application (Anderson & May, 1982; Anderson & May, 1985). Though helminth infections are persistent and reinfection in many communities is rapid, safe and effective chemotherapeutic agents are available today for many of the major helminth infections. (Anderson & May, 1982). However, targeted control measures depend on the ability to repeatedly identify those individuals in the community with high worm burdens (Anderson & Medley, 1985). Chan *et al.,* 1992 found that after repeated chemotherapy, predisposition to *Ascaris* infection persisted, while Hall *et al.*, (1992) found such individuals were not consistently heavily reinfected. Recent advances in thinking regarding effective and cost-efficient helminth control through chemotherapy suggest a strategy of targeting three particularly vulnerable groups; pre-school children, schoolchildren and women of reproductive age (Hall *et al.,* 1997). As well as chemotherapy, a range of other control measures are available including reduced environmental contamination, whether in soil or water, domestic or public, and improved sanitation and hygiene in combination with health education programmes. It is important to understand fully the impact of exposure and the components of exposure e.g. contamination levels, behaviour and hygiene, in determining the both the likelihood of *Ascaris* infection and the profile of intensity of *Ascaris* infection, so that appropriate health protection measures can be identified. The extent by which exposure needs to be reduced to reduce the public health significance to negligible levels also needs to be understood.

2.4 WASTEWATER REUSE AND *ASCARIS* INFECTION

2.4.1 Microbiological quality of wastewater

There is much documentary evidence of the high concentrations of protozoans, helminths, bacteria and viruses found in wastewater (reviewed by Feachem *et al.,* 1983 and Shuval *et al.,* 1986). Some lakes and rivers have been found to hold as much as 50% wastewater by volume (Zapponi, 1989). Faecal coliform levels between 10^7 and 10^9 faecal coliforms per 100ml have been reported for untreated wastewater. Studies have shown that the concentration of *Ascaris* eggs in developing countries can be as high as 558 *Ascaris* eggs per litre of wastewater (Mara & Silva, 1986). The extensive literature on the survival times of pathogens in wastewater and on land and crops irrigated with wastewater has been reviewed by Feachem *et al.,* (1983) and Strauss (1985). Evidence indicates that helminths and protozoans survive long enough in wastewater and on soil to lead to a risk of infection (Shuval *et al.*, 1986). *Ascaris* eggs can remain intact and viable for several months in the soil if conditions are favourable. It is evident that human contact with wastewater could in the right circumstances lead to transmission of helminth infections.

2.4.2 Why the need for wastewater reuse ?

Wastewater reuse began in Europe with the beginnings of sewerage, in the middle of the nineteenth century. It started in Britain as an environmentally friendly solution to the problems of sewage disposal (Shuval *et al.,* 1986). Previously sewage had been dumped in rivers and lakes causing pollution. Water shortages around the globe have now made wastewater a valuable resource in many countries and the use of wastewater for irrigation in many regions is now widespread to maintain and increase arable land, especially in developing countries (Mara & Cairncross, 1989; Rose, 1985). A nineteenth century solution to river pollution has now developed into a lifeline for agricultural stability and development, particularly in developing countries. There have been reports of considerable and sometimes dramatic increases in agricultural production through the introduction of wastewater irrigation. Worldwide, although wastewater irrigated agriculture represents only 13% of all arable land, it represents 34% of total production (Pescod & Arar, 1988). Currently in Latin America more than 500,000 hectares of land are irrigated with wastewater (CEPIS, 1996). Over the past 30 years wastewater reuse in China has expanded considerably and now 1.33 million hectares in China are irrigated by wastewater (Pescod & Arar, 1988). In Israel 67% of wastewater is reused.

With the benefits to the environment and to agricultural production from wastewater reuse, the goal must be to ensure that wastewater reuse for crop irrigation occurs without risk to the health of consumers or workers, or to the environment (WHO, 1989). Epidemiological studies are essential to assess any excess infection due to wastewater reuse above the level of infection in the general population. To ensure that there are no health risks, guidelines based on epidemiological evidence are necessary to steer policy makers when wastewater irrigation projects are proposed and to ensure adequate management of existing wastewater irrigated areas.

2.4.3 Epidemiological evidence of increased *Ascaris* infection associated with wastewater reuse in agriculture

Many epidemiological studies of wastewater reuse in agriculture have investigated the relationship between enteric diseases and contact with wastewater. Unlike drinking water related infections, it is not possible to make a direct correlation between exposure and the pathogen levels in the wastewater (Zapponi, 1989), because the relation differs considerably depending on the purpose of reuse, the degree of contact and hygienic practices employed. Consequently it is difficult to assess the real risk of infection due to occupational exposure to wastewater. For example, for a farm worker, exposure will depend on the length and degree of contact and the frequency of contact.

The picture is different with consumer exposure; the risk of infection from the consumption of wastewater irrigated food could be said to follow to some extent the conclusions from research on the risks related to consumption of contaminated drinking water i.e. the level of exposure being directly related to the level of contamination on vegetables. Special circumstances are generally required for epidemiological studies of consumer risk to be feasible because the origin of the food needs to be known. For example, Shuval *et al.,* (1986) described a study in Tara Prison, Egypt (Khalil, 1931). Incidence of schistosomiasis and ancylostomiasis declined as the length of time in prison increased, but incidence of *Ascaris* and *Trichuris* infection remained at the high levels found in the local population. The low levels of schistosomiasis and ancylostomiasis in the prisoners were attributed to the fact that the prisoners did not go outside the prison during their stay, also a system of bucket latrines was strictly enforced and these were emptied outside the prison, so there was no possibility of direct transmission of infection from contact with excreta. The high levels of *Ascaris* and *Trichuris* infection in the prisoners and the wardens were attributed to the vegetables eaten by prisoners and wardens alike that were grown at a small sewage farm, which used the sewage from the prison to irrigate and fertilise the soil (Shuval *et al.,* 1986). This study provided the first evidence to indicate that *Ascaris* and *Trichuris* infections can be transmitted by vegetables irrigated with wastewater.

Another study in Egypt, of the prevalence of *Ascaris* infection in Port Said and Kom Ombo in Aswan province, found the prevalence of *Ascaris* infection to be significantly higher in Port Said than in Kom Ombo. However, sanitation in Kom Ombo was very poor in comparison with Port Said, with few houses having latrines or a water supply. Khalil was unable to explain these results until he found that vegetables eaten raw were irrigated with sewage in Port Said, whereas in Kom Ombo no wastewater or human excreta was used in agriculture (Shuval *et al.,* 1986). This study though in itself weak, added further weight to the evidence of consumer risk through wastewater irrigation of vegetables found in the Tara Prison study.

Data from Jerusalem clearly demonstrate consumer risk of increased helminth infection through consumption of vegetables irrigated with wastewater (Shuval *et al.,* 1986). Before 1952 and the foundation of the State of Israel, wastewater irrigated vegetables were available throughout Jerusalem. After 1952, the western section of Jerusalem was separated from the rest of Jerusalem, and the supply of wastewater irrigated vegetables suddenly stopped to western Jerusalem. The results of examination of stool specimens dating from 1934 to 1960 by Ben-Ari (1962) revealed that before 1952 the prevalence of *Ascaris* infection was 35%. After the partitioning of the city and the cessation of the supply of wastewater irrigated vegetables, the prevalence of *Ascaris* infection in the western section declined to 1%. In 1967 the city of Jerusalem was reunified and the supply of wastewater irrigated vegetables to the western section of Jerusalem resumed. A study of the population of the western section revealed a very low prevalence of *Ascaris* infection prior to reunification, which rose steeply to 12% following reunification. Wastewater irrigation of vegetables was prohibited in Jerusalem and the surrounding area in 1970 and within a few years the prevalence of *Ascaris* infection fell again to less than 1%. The evidence from Jerusalem together with the study at Tara Prison in Egypt provides strong evidence of increased helminth infections due to the reuse of wastewater in the irrigation of vegetables.

Research also indicates that direct occupational contact with wastewater is associated with increases in *Ascaris* infection above the level found in the rest of the population. A study in India found that the prevalence of *Ascaris* infection in sewage farm workers was 47% compared with only 13% in the controls (Krishnamoorthi *et al.,* 1973). The intensity of infection was also found to be higher in the sewage farm workers than the controls, with 80% of sewage farm workers having medium and heavy infection, compared with only 33% of the controls. Despite there being considerable variations in the prevalence of infection between workers at different sewage farms in India, a similar pattern of infection is found throughout, with a higher incidence of parasitic infection among the sewage farm workers than local control populations. A study of children living in a sewage farming area in Marrakech (Bouhoum & Schwartzbrod, 1998) also found *Ascaris* prevalence greater among those exposed to raw sewage than among controls.

Two small studies from Mexico looked at *Ascaris* infection related to contact with wastewater. Families from two communities were compared, one using untreated wastewater for restricted crop irrigation and the other using uncontaminated water for crop irrigation (Rivera Ramirez, 1980). Amoebiasis was the only outcome studied and incidence was greatest in the community using untreated wastewater irrigation. However the incidence data came from health centre records, which have often been found to be incomplete and unreliable (Shuval *et al.,* 1986). The second study compared school children from two communities, one using untreated wastewater and the other using "clean" water for crop irrigation (Sanchez Leyva, 1976). It was concluded that the direct or indirect use of wastewater did not increase the prevalence of gastrointestinal infection in a community. Shuval *et al.* (1986), in reviewing this study, suggested that the "untreated" wastewater may have passed through a local reservoir before being used for irrigation. This could have resulted in the sedimentation of protozoan cysts and helminth eggs, which would have reduced any excess risk of infection associated with the reuse of raw wastewater. Also no attempt was made to determine the level of contact the children or their families had with wastewater, so there is no reason to suppose that all the children in the community using untreated wastewater had contact with the wastewater. Another study in Mexico, to the south of Guadalajara, in the state of Jalisco (Castaneda *et al.,* 1983) found a significantly higher prevalence of *Ascaris* infection among the workers using wastewater (50% prevalence) compared with those using spring water for irrigation (16% prevalence). The study concluded that a high prevalence of protozoan and helminth infections in both groups was due to poor food hygiene practices and a general lack of knowledge about health and hygiene among workers using wastewater and those using spring water. The use of raw wastewater however did represent an increased risk of infection through contamination of soil, food and drinking water. The authors felt that there was a need for improvement in the quality of wastewater used in agriculture. It was accepted that wastewater was a valuable resource, but emphasised that authorities must protect the health of workers and consumers.

Work from East Germany found that in Darmstadt, there was an association between epidemics of ascariasis and economic depression and food shortages (Baumhogger, 1949). The vegetables and salad crops grown on the Darmstadt sewage farms made up an important part of the diet during times of economic hardship. Prevalences of *Ascaris* infection between 45% and 89% were reported during these times (Krey, 1949; Schieper & Kalles, 1949). The highest prevalence (89%) was recorded in a section of Darmstadt called Griesheim, where local farmers irrigated vegetables with wastewater. In Berlin, during the same period of economic hardship, the prevalence of *Ascaris* infection was only 2.2% (Baumhogger, 1949). Baumhogger pointed out that though wastewater irrigation was practised in Berlin, all wastewater was treated before reuse, the treatment including sedimentation and biological oxidation. The prevalence of *Ascaris* infection in other cities in Germany where there was no reuse of wastewater for irrigation, treated or not, ranged from 2.7% to 9.8%. Therefore reuse of untreated wastewater was associated with an increase in the prevalence of *Ascaris* infection but reuse of treated wastewater was not. The highest prevalence of *Ascaris* infection was recorded in an area where farmers were using wastewater. This suggests that occupational exposure of workers and contact infections of their families, together with consumer risk, may have contributed to the prevalence of *Ascaris* infection in this area. Though little information was provided on the methodology of these studies, Shuval *et al.*, (1986) concluded that they provided strong circumstantial evidence that irrigation of vegetables and salad crops with raw wastewater can lead to increased transmission of *Ascaris* infections to the general public and also to those occupationally exposed to wastewater irrigation. This work from Germany also provides circumstantial evidence that there is an association between treatment of wastewater before reuse and a reduction in the risk of *Ascaris* infection.

Despite the fact that there have been only a limited number of epidemiological studies of the risk of transmission of *Ascaris* infection associated with wastewater reuse, evidence seems to indicate that contact with untreated wastewater may increase the risk of intestinal nematode infection but that treatment of wastewater before reuse can reduce this risk. There is also evidence that reuse of untreated wastewater may be associated with an increase in the risk of high intensity infections for both consumers and workers.

2.4.4 Guidelines and treatment practices for wastewater reuse in agriculture

Early guidelines for wastewater reuse in agriculture were concerned only with the pathogen content of the wastewater. The aim was to achieve full removal of pathogenic micro-organisms. To this end the California State Health Department introduced strict standards of microbiological quality for wastewater used in irrigation, particularly for vegetable crops. In the case of crops that were processed, there were to be no more than 23 faecal coliforms per 100ml in wastewater used in spray irrigation and, for surface irrigation, primary effluent was to be used. For crops likely to be eaten raw, standards required no more than 2.2 faecal coliforms per 100ml in wastewater used in surface irrigation.

A WHO meeting of experts on the reuse of effluents in 1971 reviewed the treatment methods currently available (WHO, 1973). Under field conditions it was feasible to produce an effluent containing not more than 100 coliforms per 100ml. Despite the fact that information concerning health risks was sparse, the meeting felt that only a limited health risk would result from unrestricted crop irrigation with wastewater containing no more than 100 coliforms per 100ml. A wastewater quality guideline of 100 coliforms per 100ml was proposed, which was technically feasible for countries to achieve. The meeting also felt there was a need to give the wastewater quality guidelines an epidemiological basis. Until the proposed guideline, guidelines and standards for wastewater reuse were designed to remove pathogens completely; however this was not possible with the treatment technology in many countries. There was an urgent need for epidemiological assessment of the risk of infection associated with wastewater reuse, so that appropriate microbiological guidelines could be proposed.

Following the recommendations of the WHO meeting in 1971, new epidemiological evidence was accumulated and earlier studies re-evaluated. There was epidemiological evidence that in many developing countries the main risks to human health through wastewater reuse were due to helminth infections (Feachem *et al.,* 1983). A meeting in Engelberg in 1985 of public health experts, environmental scientists and epidemiologists reviewed the work that had been done (Engelberg Report, 1985). The risk of infection was lower than previously thought, particularly in the case of bacterial and viral pathogens. The meeting felt that the guidelines for wastewater reuse were inappropriate and overly conservative in the light of current understanding. While some countries such as the United States of America were able to reach the "no-risk" California standards for wastewater, even the standards proposed by WHO in 1973 had proved a stumbling block to wastewater irrigation projects in many developing countries, where the level of treatment needed to reach these standards was both economically and technically unfeasible (Shuval, 1987). It was felt that maintenance of such strict guidelines for the microbiological quality of wastewater would only lead to further unregulated use of wastewater, often with no treatment at all (Engelberg Report, 1985).

The Engelberg meeting proposed the introduction of new guidelines for the microbiological quality of wastewater. In proposing these new guidelines, the meeting considered not only the results of epidemiological studies but also the level of microbiological quality that was achievable in developing countries where resources are often limited. A nematode guideline was introduced and the bacterial guideline relaxed. The nematode guideline was introduced in the light of a growing body of evidence that in many countries the main health risks from wastewater reuse are due to helminth infections. It required that, in both restricted and unrestricted crop irrigation, there should be no more than a geometric mean of 1 nematode egg per litre of wastewater. The figure of 1 nematode egg per litre of wastewater was chosen because it was achievable with existing low cost treatment technologies. There was no epidemiological basis behind the nematode egg guideline. The faecal coliform guideline was relaxed to less than or equal to a geometric mean of 1000 faecal coliforms per 100ml for unrestricted crop irrigation, which was in line with the river quality used for unrestricted irrigation in Europe and the United States with no known health risks (WHO/UNEP, 1987). No bacterial standard was recommended for restricted crop irrigation, although a minimum degree of treatment equivalent to a 1-day anaerobic pond followed by a 5-day facultative pond was recommended. The guidelines proposed by the Engelberg Report were among several papers reviewed in 1987 at a meeting of a WHO Scientific Group on Health Aspects of Use of Treated Wastewater for Agriculture and Aquaculture in Geneva (WHO, 1989). The guidelines were broadly endorsed and slightly extended (Table 2-1).

The guidelines for wastewater reuse proposed by the Engelberg meeting in 1985 and reaffirmed in 1989 were said to provide a high degree of health safety to workers and consumers, but at the same time to be realistic in the light of resource constraints and limited technology in many countries.

Waste stabilisation ponds are capable of eliminating helminth eggs and protozoal cysts, and reducing bacteria and viruses to very low levels (Shuval *et al.,* 1986). Waste stabilisation ponds are economically feasible for developing countries where resources are often limited but land is available and cheap. Studies have found that under tropical conditions a system of 2 ponds with a total retention time of 12.3 days can achieve the WHO guideline for restricted crop irrigation (Mara & Silva, 1986). A study in Mexico State, Mexico reported 100% removal of helminth eggs from sewage with 200-400 *Ascaris* eggs per litre when passed through a single facultative pond (Rivera A. *et al.,* 1985). Conventional treatment plants with primary and secondary treatment are unable to achieve these guidelines for wastewater reuse. Other technologies are however available; high technology tertiary treatment can virtually remove all pathogens and produce a quality approaching that of drinking water (Pescod & Arar, 1988). The problem with tertiary treatment plants is that they are highly sophisticated and expensive to construct. Tertiary treatment plants require highly trained personnel for maintenance and spare parts are often difficult to obtain.

While wastewater treatment can provide a high degree of health safety to workers and consumers, health protection does not automatically have to signify wastewater treatment; there are other low cost measures that can be employed. Preventing or reducing contact with the wastewater can be as effective as treatment of the wastewater. Four types of control measure should be considered;

(i) treat the wastewater to remove or reduce the potential risk of infection;

(ii) restrict the crops that can be grown;

(iii) select appropriate methods of wastewater application and

(iv) modify human behaviour and reduce exposure to infection.

In many circumstances a combination of several control measures is most appropriate (Blumenthal, 1988; Blumenthal *et al.,* 1989; WHO, 1989; Mara & Cairncross, 1989). In order to implement appropriate control measures the relationship between exposure and intensity of *Ascaris* infection and the public health significance of the intensity of *Ascaris* infection needs to be understood.

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Table 2-1 Health guidelines for the use of wastewater in agriculture (WHO, 1989)[1]

Category	Reuse conditions	Exposed group	Intestinal nematode[2] (arithmetic mean no. eggs per litre)	Faecal coliforms (geometric mean no. per 100ml)[3]	Wastewater treatment expected to achieve the required microbiology
A	Irrigation of crops likely to be eaten uncooked, sports fields, public parks	Workers, consumers, public	≤ 1	≤ 1000[4]	A series of stabilisation ponds, designed to achieve the microbiological quality indicated, or equivalent treatment
B	Irrigation of cereal crops, industrial crops, fodder crops, pasture and trees[5]	Workers	≤1	No standard recommended	Retention in stabilisation ponds for 8-10 days or equivalent helminth and faecal coliform removal
C	Localised irrigation of crops in category B if exposure to workers and the public does not occur	None	Not applicable	Not applicable	Pre-treatment as required by irrigation technology, but not less than primary sedimentation

[1] In specific cases, local epidemiological, sociocultural and environmental factors should be taken into account, and the guidelines modified accordingly.

[2] *Ascaris* and *Trichuris* species and hookworms.

[3] During the irrigation period.

[4] A more stringent guideline (≤200 faecal coliforms per 100ml) is appropriate for public lawns, such as hotel lawns, with which the public may come into direct contact.

[5] In the case of fruit trees, irrigation should cease two weeks before fruit is picked, and no fruit should be picked off the ground. Sprinkler irrigation should not be used.

2.5 MEASURING EXPOSURE

It is important to measure exposure to *Ascaris* infection for several reasons, (i) to establish the relationship between differing levels of exposure to *Ascaris* eggs and the prevalence and intensity of *Ascaris* infection, (ii) to understand the importance of the different components of exposure to *Ascaris* eggs in determining the intensity of *Ascaris* infection and (iii) having established the importance of the components of exposure, to determine the importance of different activities and behaviours with respect to the intensity of *Ascaris* infection. The relation between a reduction in exposure to *Ascaris* eggs and a reduction in high intensity *Ascaris* infections can then be assessed.

Many studies have measured levels of environmental contamination with *Ascaris* ova (reviewed by Kagei, 1982) but few have looked at the degree of contact with the environment and the relationship of contact and environmental contamination to levels of infection. It is relatively easy to measure the pathogen level in an environment, whether it is water or soil, but environmental contamination does not necessarily imply transmission. Transmission of *Ascaris* infection will depend on several factors, including the survival time of the ova in the environment and the frequency and extent of contact with the contaminated environment. If human behaviour is such that there is little or no contact with the contaminated environment, then no matter how heavy the environmental contamination is, it will not affect the intensity of *Ascaris* infection.

There has been very little research into the impact of exposure on the intensity of *Ascaris* infection. The only research comes from studies of geophagia carried out in Jamaica (Wong *et al.*, 1988) (see page 29). While this study provided useful information on the possible association between geophagia and infection, geophagia is only one factor that affects peridomestic exposure. Helminth infections are cumulative infections and, to assess the impact of exposure on the intensity of *Ascaris* infection, it is important to have a direct measure of exposure over a defined period of time that can then be related to the *Ascaris* infection acquired over the same period.

There have been many studies that have measured exposure to schistosome infection through contact with infested water (Dalton, 1976; Dalton & Pole, 1978; Kloos & Lemma, 1980; Tiglao & Camacho, 1983; Kloos *et al.*, 1983; Butterworth *et al.*, 1984; Wilkins *et al.*, 1984; Blumenthal, 1985; Etard, J, Audibert, M & Dabo, A., 1995, Kloos *et al.*, 1997). Unlike schistosome infections, the transmission sites for *Ascaris* and other geohelminth infections are not clearly defined (Bundy & Blumenthal, 1990). Consequently most of the research examining the effect of exposure on helminth infection comes from studies of schistosome infection and water-contact activities.

Early work on exposure to schistosome infections examined the relationship between the frequency and duration of contact with water and the prevalence of schistosome infection. Dalton (1976) carried out a study of water contact with *Schistosoma mansoni* in an endemic valley in St. Lucia, where a domestic water supply was to be introduced. Water contact was quantitatively measured. Cycles of human behaviour relating to age and sex were established. Activities were found to be linked together. Laundry and bathing were linked activities; many women when they had finished washing the clothes then bathed. The level of body contact with the water and the duration of contact varied according to the activity. For example, contact was high in bathing, but duration of contact was shorter than in washing. The observations indicated that water contact could be broken into different types that related to distinct domestic and economic activities. The activities were complex and water contact relating to each activity varied according to age and sex. It was felt that a decrease in the number of water contact activities would lower transmission and so reduce infection. It was hoped that the provision of a domestic water supply would achieve this, by stopping washing and the related activities from occurring in the river, thus leading to an overall reduction in domestic river water-contact activities. However the extent to which contact needs to be reduced, in order to lower transmission and so reduce infection to negligible levels, was uncertain. The rivers had social value as a meeting place for the community. Also fording of the river and other economic activities would continue and this would need to be considered (Dalton, 1976). The study by Dalton (1976), while providing one of the first quantitative studies of water contact, only measured the frequency and duration of water contact when considering exposure to infection.

Dalton and Pole (1978) were the first to examine the relation between water contact and infection by age class. The study on Lake Volta in Ghana found that, although there was a similar pattern of egg output by males and females, the age-related distribution of egg output rose to a peak in the 10-19 year age class and declined to low levels age over the age of 20 years.

Kloos and Lemma (1980) and Tiglao and Camacho (1983) added to the work by Dalton (1976) and Dalton and Pole (1978) in studies of schistosomiasis, measuring the amount of body surface contact as well as the frequency and duration of contact with water when examining the relation between water contact and infection. Tiglao and Camacho (1983) observed that higher intensity schistosome infections, in males than in females, correlated with a higher duration of water contact in males than in females. The study by Kloos and Lemma (1980) found that the site where contact took place was an important factor in the relation between exposure and infection. It also emphasised the importance of measuring exposure and infection in the same population.

Following these recommendations Kloos *et al.,* (1983) carried out a study of *S.haematobium* in children in Upper Egypt. Many contacts recorded were found to have occurred at non-infective sites and this may explain why there was no correlation found between water contact and infection. The level of water infectivity (ie concentration of infectious cercariae) at the transmission sites together with the frequency and duration of water contact and the extent of body surface contact were all important in assessing the relation between exposure and infection. Unfortunately, an exposure index considering all these factors was not calculated, as had been proposed by Kloos and Lemma (1980).

Butterworth *et al.,* (1984) carried out a study of the relationship between water contact and the intensity of *S.mansoni* infection. There was no correlation between the duration of water contact and the intensity of *S.mansoni* infection; however when water infectivity was also considered the pattern of infection and exposure were similar. Blumenthal (1985) developed an index of exposure that considered the frequency and duration of water contact, the amount of body surface contact with the water and the level of water infectivity. A correlation was found between exposure and reinfection levels in a group of 8-13 year olds, with a higher level of exposure among children reinfected than those who were not reinfected. It was found that in children aged 2-9 years, the mean intensity of reinfection increased with increasing duration of water contact. In adult males, low levels of reinfection were associated with low levels of water contact. It was also found that there was a stronger relation between exposure and infection when the exposure index was considered than when duration of water contact alone was considered (Wilkins *et al.,* 1987).

These studies demonstrate the importance of considering all the factors that affect transmission of infection when attempting to examine the relationship between exposure and infection. The enormous complexities of measuring exposure were demonstrated by a study of water contact in Kenya in schistosome endemic communities (Fulford *et al.,* 1996). Sites of water contact were identified for seven communities and exposure was described in terms of the activities performed, time of day, month, type of activity and the degree of immersion. Kloos *et al.,* (1997) examined the relationship between human water contact and intensity of infection using stepwise linear regression. Several factors indicative of infection intensity were identified, but variations in the level of exposure and patterns of transmission between study groups meant that these factors also varied. The transmission sites for *Ascaris* infection are not clearly defined as for schistosomiasis, because exposure is often peridomestic. Several studies have measured peridomestic exposure related to *Ascaris* infection, but have failed to identify specific activities related to *Ascaris* infection (Peng *et al.,* 1996; Kightlinger *et al.,* 1998).

The relationship between occupational contact with wastewater through crop irrigation and *Ascaris* infection can be considered in a similar manner to the water contact studies of schistosome infection, since there is a more clearly defined site of transmission where occupational exposure is concerned. In the particular scenario of wastewater reuse, measurement of exposure to wastewater of differing qualities would provide an opportunity to assess the role of exposure in the determination of *Ascaris* prevalence and intensity of infection, and any associated morbidity. It would also enable a clearer picture of the impact of raw and treated wastewater reuse on the health of those in contact with the wastewater.

Studies have provided circumstantial evidence that raw wastewater reuse is associated with increased prevalence and intensity of *Ascaris* infection among consumers and workers (Khalil, 1931; Krishnamoorthi *et al.*, 1973; Shuval *et al.*, 1986); this excess infection may disappear when wastewater is treated before use (Shuval *et al.*, 1986) or other preventive measures are taken.

A recent paper compared the prevalence of *Ascaris* infection in infants, school-aged children and adults. It suggested that the prevalence of *Ascaris* infection in school-aged children could be used to assess community prevalence (Guyatt *et al.*, 1999). This would eliminate the need for high-cost community wide studies to identify communities with high intensity infections and target them for chemotherapy. Such a comparison assumes that the sources of exposure to *Ascaris* infection will be similar for all age groups, but when transmission is not limited to the peridomestic environment this may not be the case. An increasing number of studies have demonstrated an association between prevalence of infection and exposure for particular groups of the population. Under such circumstances, the prevalence of *Ascaris* infection for school-aged children could either over-estimate or under-estimate the prevalence of infection in adults.

Further research is needed to assess the extent by which exposure influences *Ascaris* infection levels and the amount by which exposure needs to be reduced to lower the prevalence and intensity of *Ascaris* infection. In the wastewater reuse scenario, the public health importance of reuse, as a route of transmission of *Ascaris* infection can then be assessed. Once the importance of different activities and behaviours in a wastewater reuse setting are assessed, appropriate control measures including partial treatment of the wastewater and health promotion could then be identified.

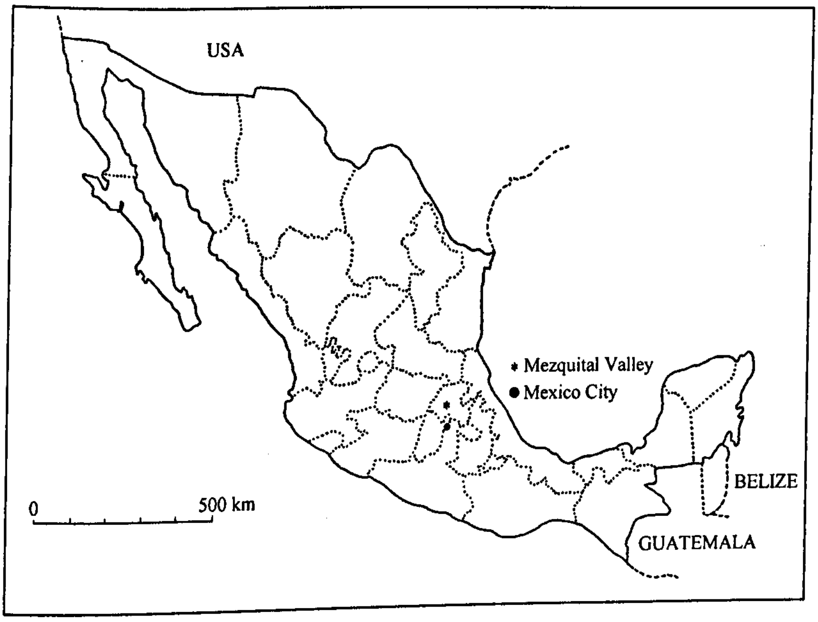
CHAPTER 3. THE STUDY AREA

3.1 PHYSICAL CHARACTERISTICS

Geography

The study was carried out in Mexico, in the Mezquital Valley, about 100 km north of Mexico City (Figure 3-1). The Mezquital Valley was formed by a series of neovolcanic chain reactions and includes the valleys of Tula, Mixquiahuala, Actopan, Ixmiquilpan, and Ajacuba. The area is 1985 metres above sea level and is between latitudes 20'00" and 20'20" north and longitudes 98'55" and 99'30" east. There are several large towns in the region, together with a patchwork of villages ranging in size from several hundred to a few thousand inhabitants. There are also isolated houses usually occupied by families farming the adjacent field. Details of the actual communities studied in this thesis may be found in Chapter 4, Materials and Methods.

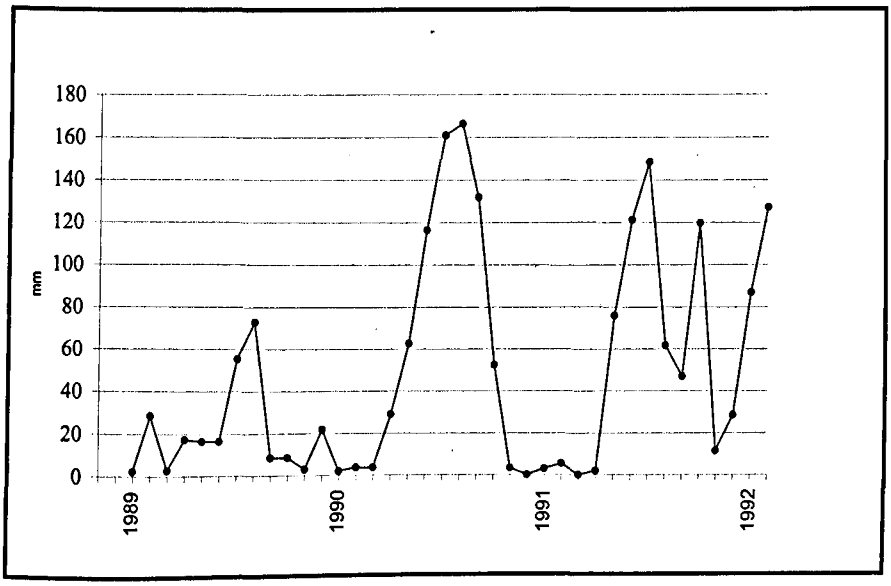
Figure 3-1 Map of Mexico showing Mezquital Valley



Climate

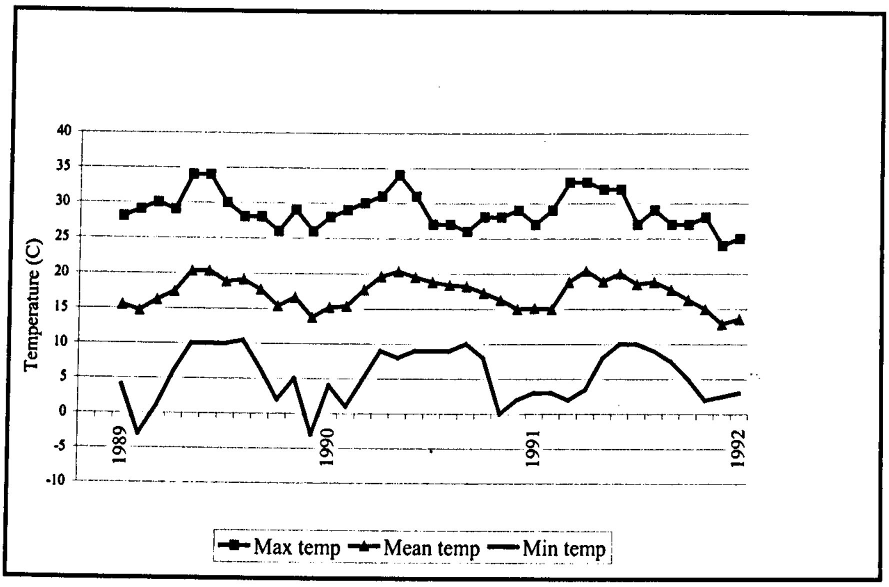
A wide variety of climates can be found in Mexico, from desert to lush rainforest. The study area is classed as semi-arid and has a mean annual rainfall of 495mm, considerably lower than the national average of 760mm (INEGI, 1990). The rainy season occurs between June and September, although rainfall is not sufficient to fully suspend wastewater irrigation (Figure 3-2). The mean annual temperature is 17°C (national mean is 17.4°C), with a minimum temperature of -3°C and a maximum of 34°C (Figure 3-3). Most frosts occur between September and March.

Figure 3-2 Mean monthly rainfall (mm) in Mixquiahuala, Hidalgo (1989-1991)[6]



[6] Raw data obtained from Comision Nacional de Agua, Mixquiahuala (1999)

Figure 3-3 Mean monthly temperature (°C) in Mixquiahuala, Hidalgo (1989-1991) [7]

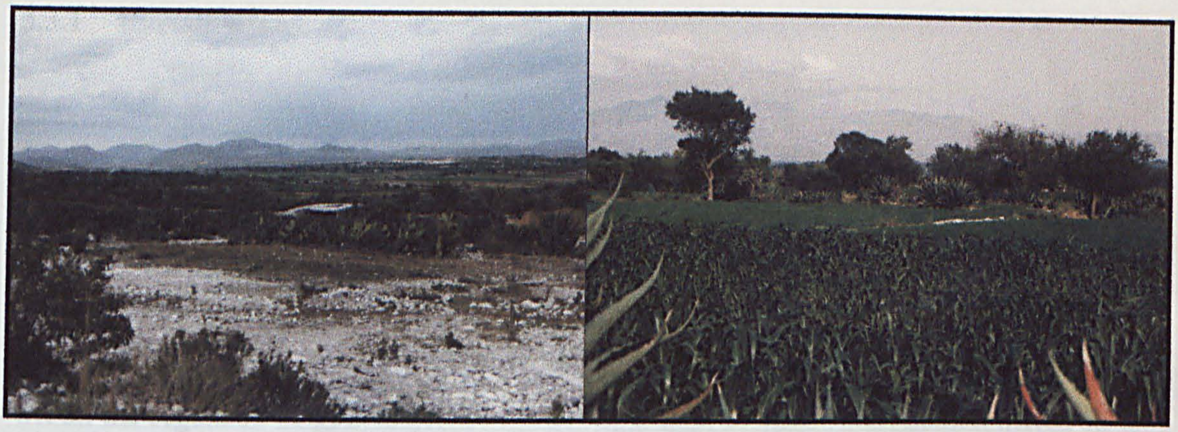


Local Vegetation and Soil Characteristics

Native vegetation in the study area is characteristic of semi-arid areas. The main plants are either thorny scrubs or cacti such as mesquites, maguey, thistles, garambuyo and prickly pear. Due to the climate, 67% of Mexico is arid or semi-arid, however such barren areas, as in much of the study area, have been transformed by the introduction of irrigation (Photo 3-1). The concentrations of most salts in the area are normal with the exception of calcium, which is high. The soil is generally shallow, has low productivity and is unsuitable for many crops without irrigation and the addition of fertilisers. Less than 11 % of the soil in the area is classed as alluvial, deep and fertile. Irrigation has permitted growth of fodder, grain and salad crops, considerably increasing agricultural production in the area (see section 3.3.6 for details). Irrigation has also allowed the growth of wild plants and trees not normally associated with such arid terrain.

[7] Raw data obtained from Comision Nacional de Agua, Mixquiahuala (1999)

Photo 3-1 Comparison of rain-fed and wastewater irrigated fields



3.2 LOCAL POPULATION

In the study area, about half of the agricultural land is privately owned and half is farmed through right of tenure. A plot of land farmed through right of tenure is known as an "ejido" and the farmer that works it as an "ejidatario". Farmers can be classed as small holders, ejidatarios or labourers. Some individuals fit exclusively into one of these categories, whilst others could be assigned to more than one category. For example, an individual may own one field, may have inherited an ejido and may also work for other farmers (small holders or ejidatarios) as a farm labourer. In this situation the individual could be described as a small holder, an ejidatario and a labourer. About 20% of families in the study area are involved directly in farming, with agriculture and related activities accounting for 50-70% of employment. Due, however, to continually mounting economic pressures, many farmers supplement their income by working, for example, as taxi drivers or labourers. At some times of the year, when agricultural work is slack, many farmers are forced to go to Mexico City and to other large towns to work, returning home only at the weekend. The majority of the study population speaks only Spanish, however a few people in the study area speak Spanish and Otomi, the local indigenous language. A detailed description of the study population characteristics can be found in Chapter 5.

3.3 WASTEWATER IRRIGATION

3.3.1 Introduction

In Mexico, it is estimated that over 350,000 hectares of arable land are currently irrigated with wastewater direct from irrigation channels (CNA, 1998), although the figure for indirect wastewater use is much higher (CEPIS, 1995). The quality of the water used for irrigation in Mexico varies considerably from fresh water to untreated wastewater, both within the regulated irrigation system and through indirect reuse from contaminated surface waters. Only about 11% of wastewater within the regulated crop irrigation system has been treated. In areas where wastewater is not officially used, it is thought that there are wastewater discharges into irrigation channels and drains, though the full extent has yet to be quantified (CNA, 1998).

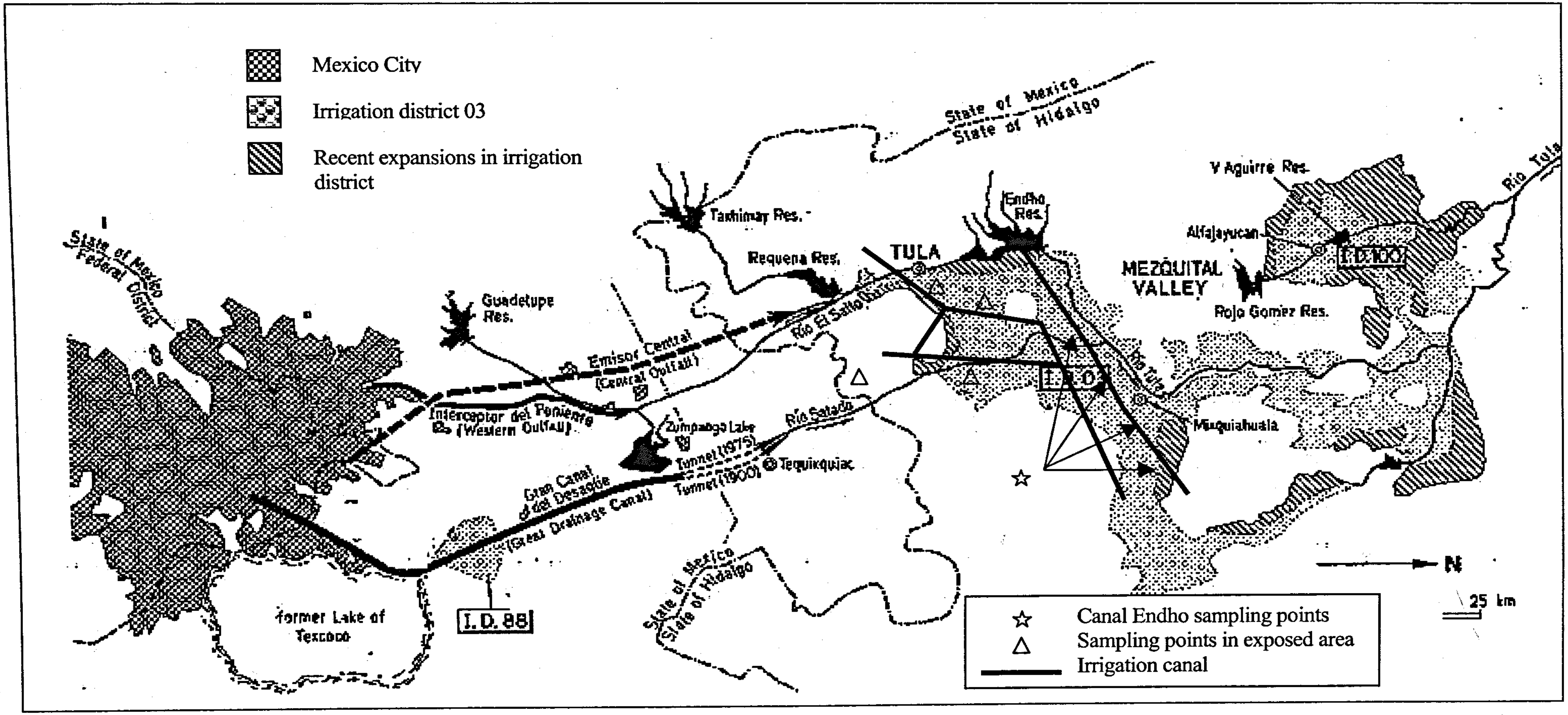
The National Water Commission (CNA) administers the irrigation system through irrigation districts (ID), which manage the distribution of water, irrigation and crop restriction, as well as providing an information service to farmers on such matters as pests, pesticides, fertilisers, and crop varieties. There are over forty irrigation districts in Mexico. The population studied within this thesis lies within ID03, the largest irrigation district (52,270 hectares). The microbiological quality of the wastewater used for irrigation within ID03 varies, some farmers receiving wastewater that has had no form of treatment prior to reuse, whilst others receive wastewater that has first passed through a storage reservoir.

3.3.2 History of Irrigation District 03

There has been wastewater irrigation in the study area, known today as Irrigation District 03 (ID03), since the end of the last century. It was not an anticipated nor planned reuse, but came about as a result of the need to dispose of large volumes of domestic sewage and drain runoff (collectively termed wastewater) produced in the Mexico Valley, and to prevent flooding in this immense urban area. The original plan was to transport the wastewater out of the Mexico Valley to the River Tula and from there to the sea. The first wastewater reuse system was for the generation of electricity in the early 1900's. However, due to the dry climate and the lack of an alternative source of water for crop irrigation, the farmers with fields near the channels built by the electricity company began to use the wastewater to irrigate their crops. Then gradually, with no overall plan or design, the farmers themselves began to build channels to transport the wastewater to the fields, as needed (Personal communication, Ing Romero A). In 1917 the Department of Irrigation was created. Its aim was to promote and organise irrigation projects, establish quotas for the private use of national waters and obtain funds to finance further irrigation programmes. The Department of Irrigation encouraged land reforms and in 1926, with the creation of the irrigation law and the National Irrigation Commission, crop irrigation in Mexico began in earnest.

The first drain built to carry wastewater out of Mexico City was the Great Drainage Canal built around 1900 (Figure 3-4). The Emisor Central is the most recent move by CNA to control the flow of wastewater from the Mexico Valley (Figure 3-4) and was constructed between 1967 and 1975. It collects the wastewater from the Mexico Valley, and works by gravity with the level of the channel varying from 50 to 270 metres below that of the city.

Figure 3-4 Map of wastewater irrigation system in the Mezquital Valley, Mexico



Reservoirs were constructed to retain both fresh water and wastewater. The first to be constructed was the Taxhimay dam in 1912 (expanded 1933-34), then the Requena dam in 1919-22 (expanded 1926) and finally the Endho Reservoir (1947-1951). The characteristics of these three reservoirs are displayed in Table 3-1.

Table 3-1 Characteristics of Reservoirs in Irrigation District 03

Characteristic	Taxhimay	Requena	Endho
Completed	1912	1922	1951
Expanded	1933-1934	1926	-
Main inflow	Sn Luis de las Presas	River Tepeji	River Tula
Dam width (m)	23.0	214.0	1400.0
Dam height (m)	39.0	27.0	55.0
Total capacity ($m^3 \times 10^6$)	50.0	70.7	182.9
Maximum volume ($m^3 \times 10^6$)	42.7	52.3	138.5
Discharge to:	River Tepeji	River Tula	Central & Endho Canal
Max Discharge Rate (m^3/s)	750	600	Central:1100. Endho:30
Type of outlet	Gravity	Gravity & controlled by 4 gates	Gravity & controlled by 2 gates

3.3.3 Current Infrastructure in Irrigation District 03

Today, Mexico City and the surrounding urban areas (commonly known as the Mexico Valley) produce wastewater at an average rate of 40 m^3/s. It is estimated that something in the order of 1,600 million cubic metres of wastewater are produced each year, of which 80%

is sewage and 20% is clean water, run-off from rainfall. This wastewater is utilised in four of the irrigation districts :- 03 (Hidalgo), 28 (Hidalgo), 88 (Mexico), and 100 (Hidalgo).

There is a complex system of reservoirs and irrigation channels in ID03 to manage the flow of wastewater, freshwater and rainfall. Wastewater enters from the east of ID03 via the River Salado and from the west via the River Tula. On the eastern side, the wastewater passes directly into the system of irrigation channels. In contrast, a proportion of the wastewater entering from the west is stored in the Endho Reservoir, where it undergoes partial sedimentation, prior to reuse. This storage reservoir can be seen as a form of treatment, and the effluent from the dam as partially treated wastewater.

River Salado

The River Salado receives wastewater from the Great Drainage Canal. In places, the drain is above the level of Mexico City and as a consequence, wastewater must be pumped to avoid flooding the city. From the River Salado, wastewater passes into the irrigation channels, Dendho and Tlamaco-Juandho.

River Tula and associated reservoirs

The wastewater from the Mexico Valley enters the River Tula by two routes, directly from the Emisor Central and indirectly from the Western Outfall via the El Salto River. The irrigation system on the western side of ID03 is a little more complex than that on the eastern side. There are three reservoirs within the system, Taxhimay, Requena and Endho. The Taxhimay dam feeds the Requena dam, which flows into the River Tula. The River Tula also receives untreated wastewater before the flow of the river divides. A proportion of the flow from the River Tula passes into the Requena Canal and the remainder continues along the River Tula and enters the Endho Reservoir. The proportions of untreated wastewater and freshwater in the River Tula, and so also in the Endho Reservoir and the Requena Canal, are dependent on the season.

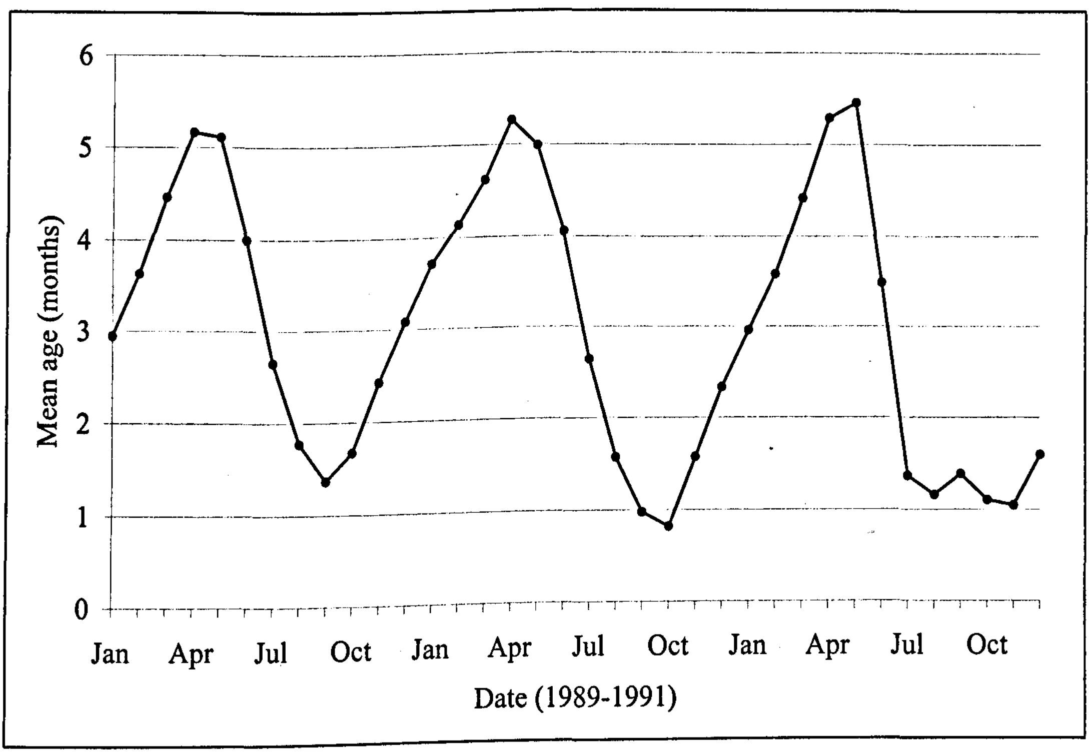
Endho Reservoir and the subsequent irrigation system

The water entering the Endho Reservoir is a mixture of freshwater and untreated wastewater, the proportions of which, as mentioned above, depend on the season. The average retention time for water leaving the reservoir has been calculated (Figure 3-5). It was calculated on the basis of total mixing from known exit flow rates and storage volumes[8]. The mean age of the water leaving the reservoir in month (n),

$$a_n = \frac{(a_{n-1}+1)(V_{n-1}-O_n)+(0.5*I_n)}{(V_{n-1}-O_n+I_n)}$$

where V_n=volume at end of month n (m^3), I_n= inflow in month n (m^3), O_n= outflow in month n (m^3)

Figure 3-5 Mean age of water leaving Endho Reservoir



[8] Raw data from CNA (1999)

There are two channels receiving wastewater from the Endho Reservoir, the Central Canal and the Endho Canal. The Endho Canal flows east and receives a small flow from the irrigation channel, Rapida, carrying untreated wastewater (from Requena Canal) before being used for crop irrigation. There are seasonal variations in the amount of untreated wastewater entering the Endho Canal from the Rapida Canal. When the level of water in the Endho Reservoir is low, towards the end of the dry season, the untreated wastewater component of the Endho Canal increases slightly.

The remainder of the wastewater from the Endho Reservoir flows into the Central Canal, which carries the wastewater to ID100 and the Rojo Gomez reservoir. The wastewater is stored here before being using for crop irrigation, storage time in the Rojo Gomez reservoir depending on the demand.

3.3.4 Irrigation Management within the study area

Irrigation district 03 is composed of a vast network of channels, dams and gates that carry the wastewater to the fields. There is a series of main irrigation channels that expand across the entire district. Most of the main channels are concrete lined and the flow along these channels is controlled by electronically operated irrigation gates (with the option of manual override) that are spaced along the length of the channel. The flow of wastewater in these channels is closely monitored by hourly readings. This serves several purposes. Firstly, during the rainy season, there is always the danger that a sudden increase in rainfall could lead to the wastewater breaking its banks and flooding fields and neighbouring communities. In the event that the level in a channel should reach maximum capacity, the irrigation gates can be fully opened and the excess wastewater diverted to the network of drainage ditches, to relieve the pressure on the irrigation system. This unfortunately means that potential irrigation water is lost. The second reason for detailed monitoring of the flow of wastewater is to optimise the capacity of the irrigation system and allow planning for expansion of the system. Thirdly, there must be an accounting for the flow through the system by the CNA to the farmers.

The wastewater flows from the main channels to lateral channels (sometimes concrete lined) and from there to sub-lateral channels through irrigation gates (Figure 3-6). The gates on the lateral and sub-lateral channels are smaller than those found on the main channels and are manually operated by canaleros[9], employed by the CNA. It is their job to regulate the flow of wastewater by operating these gates, thus ensuring an adequate flow of wastewater to each field. Wastewater irrigation is practised throughout most of the year. Despite an increase in rainfall between June and September, the rain alone is not sufficient for many crops and, if not supplemented by wastewater, low productivity would result, and in the case of some crops, the harvest would be lost.

3.3.5 Health protection measures adopted for wastewater irrigation in Mexico

Wastewater reuse regulations in force at the start of this study stated that wastewater for irrigation of vegetables eaten uncooked and fruit should contain ≤1000 total coliforms per 100ml. Where wastewater exceeded this imposed limit, crop restriction was enforced by the CNA, though it was not mentioned in the prevailing legislation. It was not until 1991, partly due to the WHO guidelines (1989), that the Mexican Government reviewed the regulations governing reuse of municipal and urban wastewater in agriculture. The revised guidelines (NTE, 1991) (Table 3-2) considered the wastewater quality, the form of irrigation and the interval between the last irrigation and the harvest. The conditions for wastewater reuse in crop irrigation were set using four microbiological qualities of wastewater (Table 3-3), which took into account the concentration of total and faecal coliforms, and the concentration of helminth ova. The crops covered by these regulations are listed in (Table

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[9] A "canalero" is a man who is employed either by the CNA or the farmers. He is responsible for managing the distribution of wastewater to fields within a particular area.

3-4). All other crops could be grown without restrictions, for example maize and alfalfa. It should be noted, however, that while cucumber, red tomato, courgette and green tomato are restricted when grown as free-standing plants, they are not restricted if grown on climbing frames. The climbing frame lifts the plant above the soil and prevents the plant, or more importantly the crop or fruit, from coming into contact with either the soil or the wastewater during or after irrigation.

Figure 3-6 Stylised diagram of irrigation channels

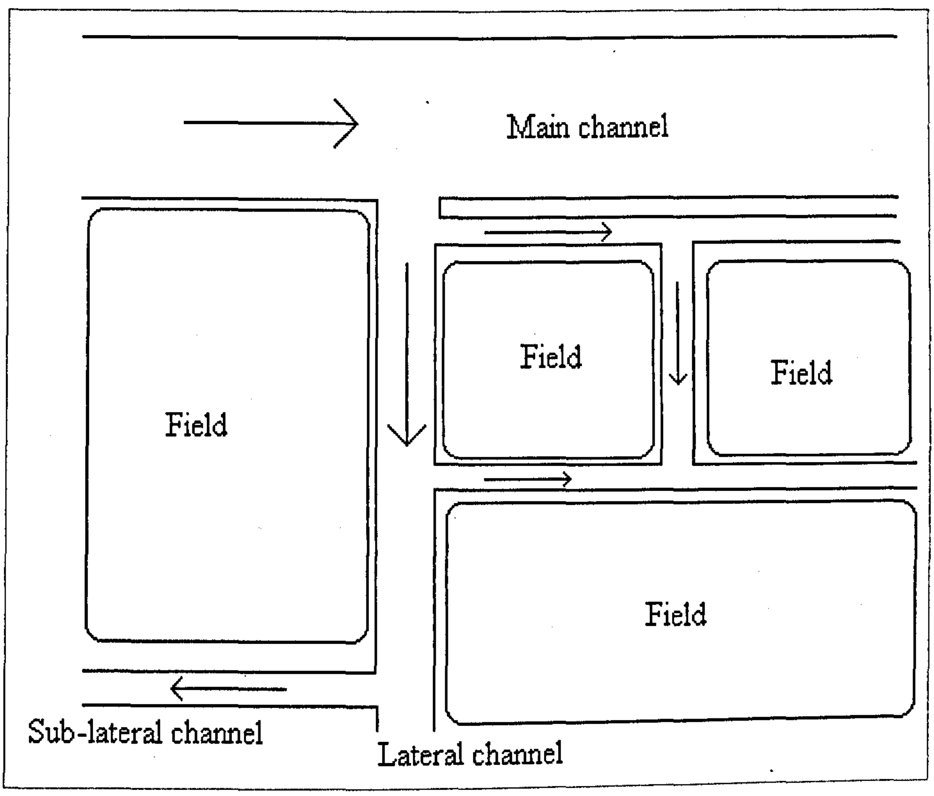


Table 3-2 Conditions for Irrigation of Restricted Crops for 1991 Mexican guidelines

Type of Irrigation	Type of Wastewater	Minimum time between the last irrigation and the harvest (days)	Banned Crops
Flood	1	20	All crops listed in Table 3-4 except garlic, cucumber, sweet turnip, melon and water melon
	2	20	All crops listed in Table 3-4 except melon and water melon
	3	20	All crops listed in Table 3-4
	4	20	All salad crops, vegetables and fruits in general
Furrow	1	15 20	All crops listed in Table 3-4 except garlic, cucumber, sweet turnip, melon, water melon and green tomato No restrictions
	2	20	All crops listed in Table 3-4 except garlic, cucumber, sweet turnip, melon, water melon and green tomato
	3	20	All crops listed in Table 3-4 except melon and water melon
	4	20	All salad crops, vegetables and fruits in general
Spray	1	20	All crops listed in Table 3-4 except garlic, cucumber, sweet turnip, melon and water melon
	2,3,4	20	All salad crops, vegetables and fruits in general

Table 3-3 Microbiological qualities of wastewater for 1991 Mexican reuse regulations

Wastewater Type	Total Coliforms (no./100ml)	Faecal Coliforms (no./100ml)	*Ascaris* Eggs (no./litre)
1	< 1000	-	0
2	-	1-1000	≤ 1
3	-	1001-100,000	Not required
4	-	> 100,000	Not required

Table 3-4 Crops regulated by 1991 Mexican reuse guidelines

Salad Vegetables	Vegetables	Herbs	Fruits
Beetroot	Beet	Coriander	Blackberries
Celery	Broccoli	Epazote	Melon
Cucumber	Cabbage	Mint	Strawberries
Lettuce	Carrot	Parsley	Sweet turnip
Radish	Cauliflower		Water Melon
Red tomato	Courgette		
Water Cress	Garlic		
	Green tomato		
	Mushrooms		
	Onion		
	Papalo		
	Spinach		
	Wild greens		

3.3.6 Benefits and drawbacks of wastewater irrigation

The wastewater in the study area has high levels of suspended organic matter, so that wastewater irrigation together with the action of ploughing can lead to considerable increases in productivity. It is estimated that 50% of crop production in Mexico originates from the 30% of the agricultural land that is irrigated. It was estimated in 1986-1987 that whilst production from the irrigation districts accounted for 86% of the total production from wastewater irrigated and rainfed agriculture in ID03, it represented only 26% of the area.

The wastewater from Mexico City is used to irrigate 52,270 hectares in Irrigation District 03. In the order of 48,000 families are thought to benefit directly from the increase in agricultural production resulting from wastewater irrigation. As well as increasing productivity of crops such as maize, wastewater irrigation also means that many crops generally not feasible in semi-arid areas can be grown. This is the case with alfalfa, which cannot be grown in the rainfed areas. As well as for fodder and cereal crops, wastewater irrigation opens up the way for vegetable production. Results from ID03 demonstrate the economic benefits of cultivating such crops (Table 3-5). Though there are more hectares of maize and alfalfa than vegetable crops, the economic gains from vegetables far outweigh those from maize and alfalfa. However the cost of treating the wastewater, so as to enable vegetables to be grown more widely, would need to be considered.

Table 3-5 Agricultural productivity in Irrigation District 03 (1986-87)

Crop	Hectares	Work days	Total Value (£×10^6)	Value/Hectare (£)
Maize & Alfalfa	59,500	642,765	25.06	421
Vegetables	5,720	723,840	14.43	2522
Total	65,220	1,366,605	39.49	-

(personal communication, CNA 1990)

Despite the enormous benefits that come from wastewater reuse in crop irrigation, there are problems associated with reuse that are sometimes forgotten:-

1) The inherent health risks for workers, consumers and the general population associated with reuse of untreated or partially treated wastewater for crop irrigation. Faecal coliform concentrations in untreated or partially treated wastewater can be high (10^3 - 10^8 / 100ml), when considered in relation to both WHO (1989) and Mexican (NTE1991) guidelines. The helminthological concentrations of the wastewater are also high, in spite of partial treatment within the irrigation system, especially in the storage reservoirs. The health risks for farm workers and their families associated with reuse of wastewater is one of the areas addressed in this thesis. More information regarding current knowledge of the health risks associated with wastewater reuse can be found in Chapter 2.

2) Effects of high salinity on the environment and crops. Wastewater has high saline levels, and low or medium levels of sodium, meaning that drainage is important and crops that are sensitive to salts can not be grown.

3) Groundwater contamination. There is continual infiltration of wastewater into the subsoil. There is a risk that groundwater supplies can become contaminated by pathogens and chemicals contained in the wastewater, especially in regions where there is fissured rock or a high water table.

4) Health effects associated with the presence of industrial waste products. Investigations of chemical contamination suggest that heavy metal levels in the soil and plants are within limits of tolerance, except for copper.

3.4 CROPS GROWN

The main crops grown in wastewater irrigated areas of IR03 are maize, alfalfa, oats, fodder barley, courgettes, chilli peppers, green tomatoes and red tomatoes (Table 3-6). Other vegetable crops are restricted and can only be grown under certain conditions, due to the use of raw wastewater for irrigation (see section 3.3.5 for details).

In the rainfed areas, the main crops cultivated are maize and beans. Both these are sturdy crops and can be grown without irrigation, although productivity is severely reduced when compared to the irrigated areas.

Table 3-6 General agricultural cycle of crops in ID03

Crop type	Crop	Sow or plant	Irrigate	No. of irrigation sessions per year	No. days between irrigation sessions	Harvest
Fodder or Grain	Alfalfa	Oct-Jan	All year	8-12	30-45	All year
	Maize	Mar-May	Mar-Nov	6	30	Aug-Dec
	Barley	Jun-Jan	Jun-Apr	5	30	Jan-May
	Wheat	Nov-Jan	Nov-Jun	4-5	30	Feb-Jun
	Oats	Oct-Dec	Oct-Mar	4-5	30	Jan-Apr
	Bean	Feb-Jul	Feb-Oct	5	30	Jun-Nov
Vegetable	Tomato	Mar-Apr	Mar-Sep	5	14,21,21,30	May-Oct
	Courgette	Feb-Aug	Feb-Oct	5	nd	Mar-Nov
	Chilli	Mar-May	Mar-Sep	5	14,21,21,30	Jul-Oct
	Green tomato	Mar-Apr	Mar-Sep	5	14,21,21,30	All year

Note : nd=no data

3.5 IRRIGATION PRACTICES IN THE STUDY AREA

Each field is bordered along at least one side by a small irrigation ditch, which connects via one or more channels to the main irrigation channel. In order to control the entry of the wastewater onto the field, each field has a raised border around its periphery. Two forms of irrigation are practised in the study area, flood irrigation and furrow irrigation. Flood irrigation is generally used with alfalfa, maize and beans and furrow irrigation with chillies and courgettes.

3.5.1 Flood Irrigation

Crops such as maize are planted in rows, but the crop is sturdy and the furrows are very shallow. During irrigation, this allows the wastewater to flow past the stem of the crop. The farmer starts irrigating on one side of the field by cutting a hole in the raised turf border and allowing the wastewater to flood across the field. When one section of the field has received sufficient water, the farmer closes the hole in the border and cuts another further along, so that the wastewater passes to the next section of the field. So the farmer continues until the field has been irrigated.

3.5.2 Furrow Irrigation

Furrow irrigation requires more care than flood irrigation, because the wastewater must be directed across the field along deep furrows. The speed of entry of the wastewater must be carefully controlled to ensure that the wastewater does not cut through the furrows, as this will damage the crop. The farmer starts irrigating on one side of the field by cutting a gap in the border. He allows the wastewater to pass along several furrows across the breadth of the field and when the soil has received sufficient water, the farmer closes these furrows and directs the wastewater to the next ones. So the procedure continues until the farmer has irrigated the entire field. The aim of furrow irrigation is to ensure only the roots of the crop come into contact with the wastewater.

3.5.3 Impact of the Agricultural Cycle on Irrigation Practices

The practices described above were general patterns for flood and furrow irrigation, but there are some differences depending on both the crop and the point in the agricultural cycle when the irrigation takes place. There is often a preparatory stage, prior to sowing the seed or transplanting the young plants, when for example, in the week prior to sowing maize seed, the field is irrigated. This irrigation is different to later irrigation sessions because of the need to saturate the soil with water. This initial irrigation is long and very labour intensive, because the farmer must constantly turn over the soil to allow the wastewater to penetrate, rather than just looking on while the wastewater moves across a section of the field.

When the plants are young, the farmer must take care not to let the wastewater enter the field too rapidly and wash the plants away. Also, if the wastewater enters the field quickly and the soil is very hard, the wastewater will not soak into the soil, but will run over the surface, the roots will not receive the water and the irrigation will be ineffective. Crops such as alfalfa need more water than others, so the farmer must direct the wastewater to each section of the field and allow the soil to become saturated before directing the wastewater to the next section of the field. Since the alfalfa plants are sturdy and furrows are low, it is not necessary to watch the movement of the wastewater and the individual can rest at the side of the field while the wastewater soaks into the soil. Maize is similar to alfalfa, the plants are sturdy and require large volumes of water for growth. Chillies, however, are delicate and the farmer must constantly check that the wastewater does not move too quickly and cut through the furrows, washing out the plants.

CHAPTER 4. MATERIALS AND METHODS

4.1 DESIGN, TIMETABLE OF STUDY AND INSTITUTIONAL SUPPORT

4.1.1 Design of the study and timetable

A prospective cohort study design was adopted to investigate *Ascaris* reinfection in farm workers and their families following chemotherapy (see Figure 4-1). Three groups of farming families were defined geographically according to the quality of wastewater used for crop irrigation in each area (see section 3.7 for a full description of the wastewater irrigation system): - (i) raw wastewater, (ii) partially sedimented wastewater and (iii) no wastewater, all crops being rain-fed.

The baseline intensity of *Ascaris* infection was measured in the three study groups (section 4.4.1). All individuals received chemotherapy and 9-10 months post-treatment the intensity of *Ascaris* reinfection was measured. Individual contact with wastewater during the period of reinfection was assessed by structured interviews (section 4.3.1) and direct observational studies (section 4.3.2). *Ascaris* infection related morbidity was assessed in the 5-16 year olds by questionnaire when anthelmintic treatment was given and 9-10 months post-treatment (section 4.4.2). Anthropometric measurements were taken (section 4.5.2). A family socioeconomic questionnaire was administered at the end of the study (section 4.5.1). The wastewater quality was regularly monitored in the raw and partially sedimented wastewater irrigation areas (section 4.3.3).

The methodology adopted and described below, enabled assessment of the relationship between exposure (through wastewater contact) and *Ascaris* reinfection to be investigated.

Figure 4-1 Schedule for study

Activity	89				90												91						
	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J
Recruit & train field staff																							
Recruit & train lab staff																							
Select groups																							
Sensitise groups																							
Stool collection					Pre-treatment										Post reinfection								
Anthropometry																							
Morbidity survey																							
Treatment																							
Worm burden substudy																							
Contact questionnaire																							
Observation studies																							
Socioeconomic quest.																							
Laboratory analysis					Stool analysis - eggs per gram.											Wastewater analysis - ova/litre							
Data entry & data cleaning																							

4.1.2 Institutional and field support in UK and Mexico

The fieldwork for this thesis was facilitated by the collaboration of Dr Ursula Blumenthal (London School of Hygiene and Tropical Medicine) with Dr Guillermo Ruiz-Palacios (Head of Department of Infectious Diseases, National Institute of Nutrition, Mexico City) and Dr Enrique Cifuentes (Phd student at LSHTM at the time of the study). They carried out a parallel study in the region to investigate the health impact of wastewater reuse in agriculture on a range of intestinal infections (Cifuentes, 1996). Initial contacts in the study area were assisted by that study. Dr Ruiz-Palacios provided general field and laboratory support in Mexico.

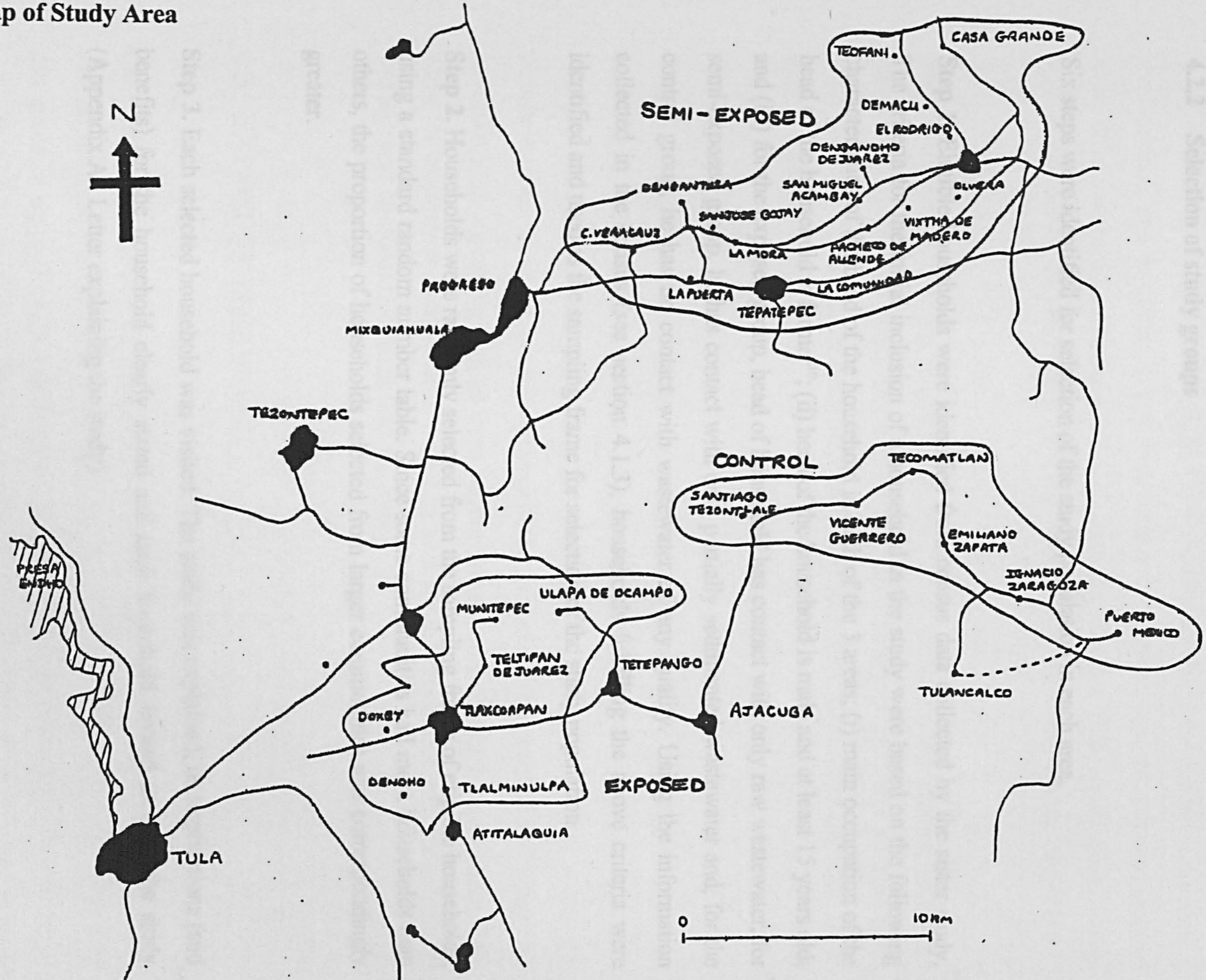
As part of the parallel study described above, a census of communities in Irrigation District 03 and a neighbouring rainfed area was carried out, to identify farmers irrigating with wastewater. Households in each area were visited. If the head of the household was a farmer with wastewater contact, then the source of the wastewater used for irrigation, the address of the household, and the name, age, sex, occupation and education of every individual in the household was registered. It was also recorded whether the farm worker owned land (small holder), rented land, had right of tenure (ejidatario) or was a labourer. This census provided the sampling frame for selection of the study population for that study and for selection of the study population used in the study on which this thesis is based. After this initial census phase of the parallel study, each study continued separately with its individual aims and objectives. The parallel study carried out a large cross-sectional survey of intestinal infections in the area and examined the relationship between each individual's infection and the quality of the wastewater used by the head of the household. While the study described in this thesis, in comparison, used structured obsevation studies to improve understanding of wastewater contact and carried out a detailed assessment of each individual's wastewater contact over a defined time period and its relationship with an individual's *Ascaris* reinfection levels over the same period.

4.2 DEFINITION OF STUDY AREAS AND SELECTION OF THE STUDY GROUPS

4.2.1 Definition of study areas

Three study areas were identified (Figure 4-2), two were wastewater irrigated areas in ID03, one received raw wastewater (exposed area) and the other received wastewater that had been partially sedimented in a reservoir (semi-exposed area), and the third was a rainfed area (control area). It was predicted that *Ascaris* eggs would sediment out of the wastewater during storage in the Endho reservoir, resulting in an improved wastewater quality. It was thought the quality could possibly approach international guideline levels for wastewater reuse in agriculture (WHO, 1989). It was later found that Endho channel actually joins with a minor raw wastewater channel prior to entering the semi-exposed area. However early analysis of the wastewater in Endho channel showed concentrations less than 5 *Ascaris* eggs per litre despite the small raw wastewater inflow, compared with raw wastewater, which had 35-60 *Ascaris* eggs/litre.

Figure 4-2 Map of Study Area



4.2.2 Selection of study groups

Six steps were identified for selection of the study population in each area,

Step 1. Eligible households were identified from census data collected by the sister study. The criteria for the initial inclusion of a household in the study were based on the following characteristics of the head of the household in each of the 3 areas; (i) main occupation of the head of the household is farmer[10]; (ii) head of the household is male and at least 15 years old; and (iii) for the exposed group, head of household has contact with only raw wastewater, for semi-exposed group, he has contact with only partially sedimented wastewater and, for the control group, he has no contact with wastewater of any quality. Using the information collected in the census (see section 4.1.3), households fulfilling the above criteria were identified and used as the sampling frame for selection of the study population.

Step 2. Households were randomly selected from the sampling frame of eligible households, using a standard random number table. Since some communities had more households than others, the proportion of households selected from larger communities was correspondingly greater.

Step 3. Each selected household was visited. The study was explained, its implications (and benefits) for the household clearly stated and each household invited to enter the study (Appendix A : Letter explaining the study).

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[10]A farmer was defined as an individual fitting into one or more of the following categories: owns land, has the right of tenure to land, rents land to grow crops, works on another person's land or helps in the fields.

Step 4. When consent was obtained, the composition of the household[11] was checked and the details collected in the census (Cifuentes, 1996) were confirmed: - (i) name, age, sex and occupation of the head of the household, (ii) occupational wastewater contact of the head of the household and the quality of that wastewater and (iii) name, age, sex and occupation of every other member of the household. Details were also obtained of the occupational contact with wastewater for the other members of the household and the source of that wastewater.

Step 5. Household eligibility to enter the study was reassessed on the basis of the revised and new information collected. The same criteria used initially to select eligible households were employed (Step 1). A household not complying with all these requirements was excluded from the study.

Step 7. Once the eligibility of the household was confirmed the eligibility of the other individual members of the household was reviewed. Inclusion criteria were (i) ≥ 2 years of age; (ii) resident in the household for at least 5 days of the week; and (iii) any wastewater contact is with the same quality of wastewater as that of the head of the household. Individuals not complying with all these requirements were excluded from the study. Only when the majority of individuals in a household failed to comply with these requirements was an entire household excluded from the study.

4.3 EXPOSURE MEASURES

Quantitative measurement of exposure to any contaminant, whether biological or chemical, and the subsequent assessment of the exposure pathway requires an understanding of how that exposure occurs and the concentration of the contaminant in the environment that is being studied. In this study, the hypothesis was that exposure to wastewater through

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[11] A household is defined as individuals that eat together and who, when able, contribute to the wealth of the household. Relations living in neighbouring or adjoining houses, but usually eating separately are considered as separate households.

wastewater reuse in agriculture results in an excess risk of *Ascaris* infection, compared to populations where peri-domestic exposure is the main route of transmission and there is no exposure to wastewater. The wastewater exposure can be due to direct contact with the wastewater either during agricultural activities, while performing other activities on wastewater irrigated fields or through accidental contact with the wastewater. Therefore in this study the "environment" considered was wastewater, the "contaminant", *Ascaris*, and the "exposure" of interest, agricultural work. To assess this **particular** exposure pathway to *Ascaris* infection, it was necessary to know, over a defined time period, the number of instances of wastewater contact, how contact occurred, the length of contact and the concentration of *Ascaris* eggs in the wastewater concerned. Three methods were adopted to achieve this and quantitatively measure exposure through wastewater contact,

(i) structured interviews with each person in the study, to gather information concerning the frequency of agricultural and non-agricultural activities involving wastewater contact, during the period of *Ascaris* reinfection (see section 4.3.1),

(ii) direct observation studies of individuals engaged in agricultural activities involving wastewater contact, to determine the extent of wastewater contact related to different activities (see section 4.3.2),

(iii) monitoring the concentration of *Ascaris* eggs in the wastewater in exposed and semi-exposed areas during the period of reinfection (see section 4.3.3).

4.3.1 Wastewater contact structured interview

The overall aim of this interview was to estimate the extent of wastewater contact for each individual in the study during the period of *Ascaris* reinfection and the source of that wastewater.

4.3.1.1 Objectives

The objectives of the interview were, for each individual enrolled in the study,

(i) to assess the frequency of agricultural wastewater contact and establish which agricultural activities resulted in wastewater contact

(ii) to assess the frequency of non-agricultural related wastewater contact and identify the activities which resulted in wastewater contact

(iii) to obtain general information concerning the degree of contact with wastewater during a particular activity

(iv) to obtain general information concerning personal hygiene during activities involving contact with wastewater

(v) to assess the variation between crops in the frequency and occurrence of activities involving wastewater contact

The information obtained through this questionnaire was used in combination with information gathered from the observation studies (section 4.3.2) and the wastewater quality survey (section 4.3.3) to obtain a fuller picture of exposure to wastewater.

4.3.1.2 Interview study design

The wastewater contact interview (Appendix B) was conducted with every individual in the study, 4-5 months after treatment (covering the period from treatment to the date of the interview) and then after the reinfection stool sample (covering the period from the last wastewater contact interview to time of the interview).

To achieve objectives (i)-(iv), the following information was requested for all instances of wastewater contact (agricultural or otherwise) over the recall period (as described above),

- source of the wastewater used to irrigate the field (used to determine whether untreated or sedimented wastewater was used)
- activity being performed (e.g. irrigation, sowing or planting)
- crop grown (if applicable)

Individuals were also asked to describe the details of the most recent instances of wastewater contact, the following information being recorded,

- number of hours performing activity
- extent of body contact with wastewater
- any precautions taken to avoid contact with wastewater
- whether they ate and/or drank during activity
- whether they washed their hands before eating and with what water

Such details were only requested for recent wastewater contact activities. While individuals could remember with surprising precision the frequency of wastewater contact, other details were not remembered so accurately. For example, a farmer would have receipts from the National Water Commission for each irrigation performed, and some wastewater contact activities were performed weekly without fail, while for others, wastewater contact had been a memorable and unforgettable event (e.g. if someone had fallen in a channel).

At the first wastewater contact interview, to achieve the fifth and final objective (and assess crop variations in wastewater contact), individuals having "ejidos" or small holdings were asked for the following information about the agricultural cycle of the crops grown for each plot of land,

General Details

- crop grown or planned to be grown
- location of field
- source of wastewater
- date of sowing or planting
- date and length of the harvest

Irrigation Details

- number of days before sowing or planting that the field is irrigated
- number of days between sowing or planting and the first irrigation
- number of days between subsequent irrigation sessions until the harvest
- how the individual knows when the crop needs irrigating
- influence of growth, rainfall and temperature have on the timing of irrigation sessions

Agricultural Cycle

- month by month, the activities carried out, with or without wastewater contact, the length of the activity and the number of days involving contact with wastewater

4.3.2 Observation studies of wastewater contact

The aim of the observation studies was to measure quantitatively the amount of wastewater contact occurring during agricultural work, and to assess differences in the degree of contact according to the type of job, the crop and individual patterns of work. The intial design identified and observed individuals in the study population who reported wastewater contact during agricultural activities, so that as well as being able to describe wastewater contact, an individual measure of exposure could be assigned. As is explained in section 4.3.2.6, observations were finally performed with farmers in the study areas, although not necessarily in the study population.

4.3.2.1 Terminology

Three terms used in discussing the observation studies in this and subsequent sections of the thesis are defined,

Agricultural activity: an agricultural task, such as irrigation, planting or weeding.

Action: a movement that is a direct result of the activity being done and that could cause contact with wastewater. Actions include "use spade", "hands in wastewater" and "up to knees in wastewater".

Behaviour: a movement that is not essential to the activity being performed and could directly or indirectly cause contact with wastewater. Behaviours include "hand to mouth", "wash hands in wastewater", "eat", "drink" and "smoke".

4.3.2.2 Objectives

The observation studies had the following objectives,

(i) to measure the degree of the variation in wastewater contact according to different activities, crops, and changes in the agricultural cycle

(ii) to identify actions that increase wastewater contact, may enhance *Ascaris* transmission, and that could be modified through changes in irrigation practices

(iii) to assess the variation in the frequency of behaviours (personal hygiene) among individuals engaged in agricultural work (e.g. food consumption patterns and sanitation), that may increase wastewater contact and enhance *Ascaris* transmission

The data gathered through the observation studies enabled statistics such as the mean degree and frequency of contact with the wastewater related to each activity to be calculated. These data were used in conjunction with information gathered from the structured wastewater contact interviews (section 4.3.1) and the wastewater quality monitoring (section 4.3.3), to obtain a fuller understanding of exposure.

4.3.2.3 Development of the methodology

Initially, individuals working in the fields and in contact with wastewater were observed. During a three hour observation period every movement whether considered an action, a behaviour or otherwise, was recorded. However it was decided that many of the movements recorded were not of importance in the context of this study. Also, during long periods of intensive work, it was not logistically possible to record all movements. When attempted, the observer, whether the author of this thesis or a fieldworker, did not have time to write down everything that was happening and so tended to write down those movements that they thought were important. The result was a biased record of the individual's movements. It was decided to try and succinctly describe movements that directly or indirectly involved wastewater contact and that could enhance transmission of *Ascaris* infection e.g. "hand to wastewater" or "hand to mouth". Several additional movements were also included to assist understanding of work patterns and for quality control purposes; for example, the movement "wait". In total, twenty-eight movements were identified and carefully defined to avoid ambiguities between observers (Table 4-1, Table 4-2 and Table 4-3).

Time intervals of 1 to 5 minutes between spot observation points were investigated and a one-minute time interval was adopted. It was decided that a one-minute time interval enabled a representative picture of all actions and behaviours to be achieved, except for two behaviours, 'hand to mouth' (movement 1) and 'washing hands' (movement 2), that occur both rapidly and sporadically. It was decided to record all occurrences of these 2 behaviours, regardless of whether they occurred at the one-minute time point.

A form (Appendix C) was designed to record the selected movements. Each row corresponded to 10 minutes i.e. 10 time points, and each column to a movement (action or behaviour). Columns 1 and 2 corresponded to the two behaviours recorded continuously and columns 3-27 to the movements recorded only at the one-minute time point. All movements could not be noted down at the same time point, since several are mutually exclusive; usually not more than 4 were taking place at any time point. For each time point a cross is placed on the far right of the form to indicate that the time point has passed. If for any reason it is not possible to record the movements that occur at a particular time point, the box is left blank and at the next time point filled with a zero.

Table 4-1 Terms used in defining movements

Hand(s)	:	any part from finger tips to wrist
Arm(s)	:	any part of upper limb above wrist
Foot/feet	:	any part from toes to ankle
Leg(s)	:	any part of lower limb above the ankle
Dry or humid	:	dry or maybe damp but with no wastewater visible
Wet	:	wastewater visible, or saturated with wastewater (wetter than humid)

Table 4-2 Movements recorded continuously

Hand to mouth: put hand to/in mouth, touch lips with hand

Wash hands/arms in wastewater: put hands/arms in wastewater to remove soil and/or vegetation

Table 4-3 Movements recorded at the timepoint

Hand to foot or footwear: touch, scratch or grasp foot with hand

Hand to face: touch or scratch face with hand

Hand to wastewater: put hand in wastewater, but **not** to wash off soil or vegetation (this was recorded as wash hands, see Table 4-2)

Hand to wet soil: touch or pick up wet soil with hand

Hand to dry or humid soil: touch or pick up dry or humid soil with hand

Hand to wet plant: touch or grasp wet vegetation with hand

Hand to dry or humid plant: touch or grasp dry or humid vegetation with hand

Lift up dry or humid clod with hands: lift up dry or humid clod of earth with hands

Lift up wet clod of earth with hands

Lift up dry or humid clod of earth with hands

Lift up dry or humid stones or rocks with hands (if this involves contact with wastewater, movement 5 not movement 12 is recorded)

Arm in wastewater: put arm in wastewater. This movement by definition includes "hand to wastewater". If this is recorded, "hand to wastewater" (movement 5) is not also recorded.

Leg in wastewater: put leg in wastewater or stand in wastewater with wastewater above ankle. If this is recorded, "foot in wastewater" (movement 14) is not also recorded

Foot in wastewater: put foot in wastewater or stand up to or below the ankle in wastewater

Foot in wet soil: put foot in the wet soil or stand in mud up to or below the ankle

Use spade: the spade is actively used for work. For example to move soil, or lift a clod of soil

Hand to spade: the spade is held but not used for work; for example, the person may be waiting with one or two hands on the spade which is resting on the field

Open gate: open irrigation gate

Use sickle: cut away vegetation, shrubs or trees with sickle

Plant in mouth: put plant in mouth or chew plant. For example, chew a blade of grass

Clean mouth/nose with handkerchief: clean or wipe mouth and/or nose with handkerchief

Eat: person eats or lifts food to mouth

Drink: person drinks or lifts the drink container to the mouth

Smoke: person smokes or lifts cigarette to mouth

Walk: person moves across the field

Wait: person is inactive and is not engaged in any work

4.3.2.4 Finalised methodology

One observer followed a single individual for a maximum period of 3 hours or the length of the activity, should this be less. Occurrences of 'hand to mouth' and 'wash hands/arms' were recorded continuously. Every 60 seconds, the movements performed by the individual over a 5-second period were recorded. The time was monitored using a digital watch with a repetitive alarm that sounded every minute for 5 seconds. The observer produced a tally for each movement by row. At the end of the period of observation, the observer totalled the tallies in each column and noted the total at the bottom. This gave the point frequency of each movement during the observation period. The hourly rate for each particular movement was then calculated and used in all subsequent analyses. This methodology is also described in Peasey & Blumenthal (1992).

4.3.2.5 Observation study design

The observation studies began in August 1990, following a pilot study in which the methodology described above was developed. Observations were performed on 529 individuals engaged in agricultural work within the exposed or semi-exposed area (not necessarily those included in the study population).

Following initial visits to the households, discussions with the local "canaleros"[12] and with the local staff of the Ministry of Agriculture and Water Resources (SARH), a strategy for locating individuals was developed. During the initial household visit, it was asked (i) if the individuals, reported in the first wastewater contact interview to work in the fields, were still working or helping in the fields, even if the work was not regular or was not their main occupation and (ii) if anyone-else was now working or helping in the fields. The individuals were then questioned about the work that they would be performing over the next few weeks. A structured series of questions were asked,

(1) the details of probable date, activity and location;

(2) whether the individual or the landowner had paid for the wastewater that was to be used;

(3) if the wastewater had been paid for at the SARH office and the wastewater had been requested from the canalero, then the wastewater could be expected to arrive during the next 7 days;

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[12] A "canalero" is a man who is employed either by the CNA or the farmers. He is responsible for managing the distribution of wastewater to fields within a particular area.

(4) if the wastewater has not yet been paid for, or if the canalero had not been visited, then it would be more than a week before the wastewater arrived;

(5) then, depending on the point reached in the chain of events that led up to the supply of wastewater to the field, the individual was either asked,

- when he would pay for the wastewater, or
- when he would ask the canalero to open the irrigation gate, or
- when the wastewater would arrive.

The next household visit was planned for slightly before the day proposed by the farmer. Often, the farmer could decide to bring forward the planting or irrigation or the canalero could open the irrigation gate earlier than thought. If the activity was missed, the whole procedure would have to be repeated over the next 30-40 days to observe the next contact with wastewater.

4.3.2.6 Problems encountered

There were several problems encountered in locating individuals, due both to the distances between communities and the unpredictability of agricultural work:

- Heavy rainfall the night before a planned irrigation, meant that, depending on the crop and the point reached in the agricultural cycle, the irrigation might be suspended until a later date.

- The supply of wastewater to the field could not be guaranteed and often a farmer was unable to give a precise date for the work. This meant that the fieldworker would need to visit the household on 3-4 consecutive days before the date was fixed

- if the farmer was allocated wastewater at night, it was not possible to observe the individual, both for logistical and safety reasons

Difficulties were also encountered observing children in the fields, whose activities involving wastewater contact were not generally planned. Such activities depended on whether the wastewater was allocated at a weekend or holiday during the day, and whether the child wished to accompany the individual. Because of these problems encountered in locating the individuals, the project was extended to 7 days per week with a weekend rota system set up for the fieldworkers.

4.3.3 Laboratory monitoring of parasitological quality of wastewater

The aim of the monitoring of the wastewater was to measure the quality of the wastewater in the exposed and semi-exposed areas and detect any possible seasonal variations occurring during the period of reinfection.

4.3.3.1 Selection of sampling sites

Due to the complexity of the irrigation system (see Chapter 3), there are many combinations of freshwater and wastewater utilised in the different irrigation canals in the study area. Eight wastewater sampling sites were selected, to measure the quality of the wastewater in all the main irrigation canals supplying wastewater to fields worked by the study population.

Four sampling sites were located in the exposed area and four along the length of Endho canal in the semi-exposed area (Chapter 3,

Figure 3-4).

4.3.3.2 *Collection and storage of wastewater samples*

Wastewater samples were collected from each of the 8 sampling sites every 4-5 weeks during the period of reinfection. A bucket was lowered into the wastewater channel, filled, emptied and then filled again. A five-litre polyethylene container with screw top was then filled to the four-litre mark with wastewater from the bucket. 500ml of 10% Formalin were added to each sample, to prevent fungal growth in the sample. The samples were stored in a cool, shaded area prior to analysis within one day of collection.

4.3.3.3 *Analysis of wastewater samples*

The quality of the wastewater was measured in terms of the number of *Ascaris* eggs per litre of wastewater. Two techniques of wastewater analysis, (i) the Leeds and (ii) the Doncaster, both developed and validated by Ayres (1989) were adopted.

The Leeds technique is designed for use with untreated wastewater containing high levels of suspended solids. This technique was chosen to monitor the quality of the wastewater in the exposed area, which is untreated and has high levels of suspended solids. Other techniques exist, but are inappropriate when there are high levels of suspended solids, because the *Ascaris* eggs can not be sufficiently separated from the debris in the sample. The Leeds technique has a 25% efficacy consistently (Ayres, 1989). Therefore though suitable for analysis of wastewater samples as in exposed area, with predicted concentrations of over 50 *Ascaris* eggs per litre, a technique with such a low sensitivity is unsuitable for the analysis of the wastewater in semi-exposed which it was expected to have lower concentration of *Ascaris* eggs.

The Doncaster technique was designed for analysis of final effluent from waste stabilisation ponds, which has low levels of suspended solids and high levels of algae. These characteristics are similar to those of the samples in the semi-exposed area, so this technique was used to analyse samples in this area. The technique, with 80% recovery, has a higher sensitivity than the Leeds technique, and is more suited to samples where low concentrations of *Ascaris* eggs are predicted. The Doncaster technique is however unsuitable for the wastewater samples from the exposed area, with high levels of suspended solids.

Both techniques involved centrifugation of a 1 litre sample of wastewater and concentration of that sample to a pellet of sediment. The Leeds technique then uses flotation to separate the *Ascaris* eggs from the debris to assist in visualisation of the eggs following concentration of the sample, as opposed to the Doncaster technique which uses sedimentation to separate the *Ascaris* eggs and the algae (Ayres, 1989). Both techniques were evaluated through laboratory trials in London with seeded water samples (Appendix D) and then piloted during a preliminary visit to Mexico.

4.4 OUTCOME MEASURES

The main outcome measures are the prevalence and intensity of *Ascaris* reinfection, although baseline or pre-treatment prevalence and intensity levels of *Ascaris* infection were also measured. Secondary to the main outcomes is a morbidity study carried out among 5-16 year olds in the three study groups. A questionnaire was developed to collect information concerning *Ascaris*-related and general morbidity levels. A substudy of worm burden and its relation to egg counts was also carried out on a small subsample of the study population at the end of the period of *Ascaris* reinfection.

4.4.1 *Ascaris* infection levels

4.4.1.1 Study design

An individual was considered to have *Ascaris* infection if *Ascaris* eggs were detected in the stool sample. The intensity of *Ascaris* infection was estimated by the number of *Ascaris* eggs per gram of faeces.

Some authors have questioned the value of egg counts as a measure of intensity of infection (Mello 1974, Hall 1981, Croll *et al.* 1982, Hall 1982, Sinniah 1982). During the prepatent period eggs are not produced and consequently egg counts can underestimate the intensity of infection (Seo *et al.* 1979). Although the actual intensity of infection for an individual may be slightly underestimated due to the presence of prepatent worms, the study is investigating the differences in intensity between groups following reinfection. Therefore the difference in intensity of infection between groups will be unaffected. Egg production may be related to the age of the worms (Hall, 1982), although here again since everyone is treated and levels of *Ascaris* reinfection measured at 6 months following treatment, this will not affect any differences in reinfection levels between the groups. It has also been indicated that egg production may be affected by worm burden (Mello 1974, Hall 1982, Keymer 1982). While there is some evidence to suggest a density-dependent relation may affect worm burden measures at the individual level, the density-dependence is insufficient to invalidate egg counts as a measure of intensity of *Ascaris* infection in groups (Elkins *et al.* 1986).

An increase in the fibre in the host diet will cause an increase in daily stool production and a dilution in the number of *Ascaris* eggs per gram of faeces (Sinniah, 1982 and Hall, 1982). Similarly the amount of food eaten could influence egg counts. In children producing small amount of stool, abnormally high egg counts have been obtained (Hall, 1982). Consequently, the volume of the stool collected will be taken into consideration in the case of abnormally high worm burdens and diarhoeal stool samples will not be accepted for analysis.

There is an uneven distribution of the *Ascaris* eggs in a stool sample. A sample of excreta analysed from one part of the stool can give widely differing results to that analysed from another part of the stool (Hall, 1981). Collection of the whole stool from a single defecation, thorough homogenisation, and analysis of three parts of the stool sample separately will reduce these variations.

There are day to day variations in egg counts within the same person, variations that can not be related to changes in worm burden, since the prepatent period for *Ascaris* is measured in weeks and the lifespan in years (Hall, 1981). These variations in egg counts between days however are slight and will not affect groups differences, since the variations are random and have no cyclic pattern to them.

It is accepted that in an ideal situation, worm burden would be measured initially and following reinfection. This would however require the collection of stool samples for 72 hours following treatment. The logistics of such an exercise are beyond the capabilities of this study. While the points that have been raised can cause difficulties when the intensity of infection is compared between individuals, the measures described above and the nature of the study (a comparison of mean infection of intensity between groups) means that egg counts are thought to be an appropriate proxy in this study to measure the intensity of *Ascaris* infection.

A whole or complete stool sample from a single evacuation was collected from each individual at the start of the study, analysed and the concentration of *Ascaris* eggs in faeces measured to determine pre-treatment levels of infection. All individuals, with certain exceptions see 4.4.1.3 below, were treated for *Ascaris* infection with pyrantel pamoate. Treatment was given before the start of most agricultural cycles and the period of greatest wastewater reuse. Two weeks following treatment, a second whole stool sample was collected from each individual, analysed and the concentration of *Ascaris* eggs in faeces again measured. This confirmed drug efficacy and acted as a baseline from which reinfection levels of *Ascaris* were later calculated. Following a 9-10 month period of reinfection, a third whole stool sample was collected, analysed and *Ascaris* reinfection intensity levels measured in terms of the concentration of *Ascaris* eggs in faeces.

The same procedures were followed at each stool collection. However, at the first stool collection, if the majority of the household was present when the household was invited to enter the study and all agreed, then the stool pots were left on that first visit. Otherwise the stool pots were delivered on the next visit so as to allow the household time to discuss whether they wanted to enter the study.

4.4.1.2 Laboratory technique

The number of *Ascaris lumbricoides* eggs per gram of faeces has been used as the measure of intensity of infection in many studies. Two distinct techniques have been used to assess the number of *Ascaris* eggs per gram of faeces. The Kato-Katz thick smear technique (Seo *et al.*, 1979; Thein-Hlaing *et al.*, 1984; Bundy *et al.*, 1987; Forrester *et al.*, 1988) and the formol-ether concentration technique first developed by Ritchie (1948) and later modified by Ridley and Hawgood (1956) (Tedla & Ayele, 1986; Thein-Hlaing *et al.*, 1984; Thein-Hlaing *et al.*, 1987). Both the formol-ether concentration (Ridley & Hawgood 1956) and the Kato-Katz techniques are qualitative and not quantitative techniques. Two other techniques have also been used to assess the intensity of *Ascaris* infection in terms of the number of eggs per gram of faeces, the Stoll-Hausheer technique (Shield *et al.*, 1981; Croll *et al.*, 1982) and the McMaster technique. The Kato-Katz technique has been shown to be more sensitive than both these techniques (Castilho *et al.*, 1984). However a modified version of the formol-ether technique was developed by Hall (1981) producing a quantifiable technique for assessing egg counts more accurately than could previously be done. In this technique the stool sample to be analysed is weighed and the proportion of concentrate examined microscopically is measured, giving a more exact measure than previous techniques.

The modified version of the formol-ether technique developed by Hall (1981) was used for analysis of all stool samples at each stage of the study. In this study, ethyl acetate was used in place of diethyl ether. Diethyl ether is extremely flammable and explosive and studies have shown that ethyl acetate is a satisfactory substitute, being as good as or better than diethyl ether in the recovery of *Ascaris* eggs (Young *et al.,* 1979).

4.4.1.3 Chemotherapy

It was explained to the subjects before chemotherapy that (i) the drug was only effective against parasitic worm infections, (ii) it was not an experimental drug, but one that had been well tested and (iii) it had no serious side effects, but could in some individuals cause nausea and stomach ache, though these were transient. It was emphasised that should anyone suffer side effects, or have any queries regarding treatment, the doctor working with the study (see section 4.7.2) could be freely consulted if required.

Everyone in the study was treated with pyrantel pamoate for *Ascaris* infection, after the collection of the first stool sample, with the following exceptions, (i) pregnant women, who were offered treatment after the birth of their child, (ii) individuals reporting chronic gastric problems, and (iii) individuals displaying signs of severe liver disease i.e. dark urine, light coloured stools, pain just below the rib-cage or jaundice. In the case of (ii) and (iii), if any of these symptoms were reported, the doctor working with the field team examined the individual to determine whether chemotherapy could be given and the most appropriate dose.

A target dose of pyrantel pamoate of 10mg/kg of body weight was given to each person eligible for treatment. Tablets of 125 mg were given to everyone over 9 years of age and suspension of 50 mg per ml was given to children between the ages of 2 and 5 years and those that could not take tablets. A table of body weights and the relative amount of tablets or suspension to be given was drawn up. Each individual was weighed, the corresponding dose given and the medicine was taken in the presence of the fieldworker. Any recent treatment for 'worms' was noted, as this could affect the pre-treatment levels of *Ascaris* infection.

In choosing the drug to be used in this study, specificity, efficacy, side effects, number of doses, population exclusions and time to expel worms were considered. Pyrantel pamoate was chosen because it is reported to be 95% effective against *Ascaris* infection in a single dose (Elkins *et al.*, 1986) and has few side effects. Pyrantel pamoate is also effective against hookworm (though prevalence in the area were extremely low) and pinworm, although a higher dose is required for hookworm than was used for *Ascaris* infection (Desowitz *et al.*, 1970).

4.4.1.4 Collection of stool samples

Polyethylene stool pots (500ml capacity) with screw lids were delivered to each household together with a piece of thick card (8" x 12") to help the individual collect the stool sample and also a short pictorial explanation of the suggested procedure for collection provided (Appendix E). Each stool pot was labelled with the ID number, name and age of the individual (Figure 4-3), the importance of the labels was stressed and the literacy of at least one permanent member of the household checked to ensure that each individual used the correct stool pot. The details of the stool collection were explained, and the following points emphasised, (i) it must be a whole stool sample i.e. all that is evacuated during a single visit to the toilet, (ii) the stool sample should be deposited directly into the stool pot or on the card provided to avoid environmental contamination, (iii) the stool sample should be as recent as possible, (iv) the stool pot should be put in the shade once the sample has been collected (to avoid the build-up of gases), and (v) mother or other suitable adult should supervise children aged 2-12 years of age, to ensure compliance with the points listed above.

Figure 4-3 Typical stool pot label

ID : 04 0164 01

NAME : PEREZ MENDOZA JUAN

AGE : 46

Every visit to the house to deliver stool pots to the household was recorded. The stool pots were only delivered if a responsible member of the household ≥ 12 years was present in the house. This was to ensure that stool pots were not lost, although sometimes this meant it was necessary to return to the same house several times in order to deliver the stool pots. The fieldworker returned the following day to collect the stool samples. It was checked again that (i) the labels had been understood, (ii) a whole stool sample had been collected, (iii) the sample was recent (<12 hours) and (iv) no diarrhoeal samples had been collected. Generally, 2-3 visits were required to collect all the stool samples from a household. Invariably at least one person in the household 'forgot' to collect their stool sample. Every visit to the house to collect the stool pots was recorded, and the reasons for non-collection noted. Following collection, the stool samples were delivered to the laboratory for storage and subsequent analysis.

4.4.1.5 Storage of stool samples

On arrival in the laboratory each stool pot ID number was checked against the list of stool pots collected by each fieldworker. Each stool sample was weighed and then manually homogenised with a disposable wooden spatula. Three small samples (0.5-1.0 gram) were taken from each stool sample, placed on a separate piece of aluminium foil and weighed. The weight was noted and each of the three samples was placed (without the aluminium foil) in a separate storage jar containing 10 ml of 10% Formalin. The number of the tube was noted. The aluminium foil had been weighed and the mass noted.

4.4.1.6 Detection of Ascaris *eggs*

Each stool sample stored in 10% Formalin was examined using a modified version of the formol-ether technique developed by Hall (1981), using ethyl acetate instead of ether. From each stool sample collected three separate samples of 0.5 - 1.0 grams were examined and a mean concentration of eggs per gram of faeces then obtained.

4.4.2 Morbidity

4.4.2.1 Objectives

The main objective of this substudy was to identify symptoms in 5-16 year olds that could be associated with *Ascaris* reinfection and with moderate to high intensity infections. Individuals with ≥5000 eggs per gram of faeces were defined as having moderate to high intensity infections (WHO, 1987).

4.4.2.2 Study Design

The morbidity questionnaire was applied to all 5-16 year olds in the study on two occasions, before treatment and 9-10 months later following reinfection. The initial questionnaire provided comparative baseline information for the post treatment morbidity measures. The questionnaire was developed using the current WHO Guidelines for Respiratory Infections

(WHO, 1984) and taking note of standard practices employed in the field by the Department of Infectious Diseases at the National Institute of Nutrition, Mexico City.

Two forms of information were collected concerning morbidity; (i) a general statement of the health of the individual over the previous three months through several specific questions concerning major illnesses, diarrhoea and medicines taken; and (ii) a record of the individual's state of health during the 7 days preceding the visit; this included information on diarrhoea, respiratory infections, fever, asthma, skin complaints, general illnesses and medicines taken.

The symptoms that can be caused by *Ascaris* infection are listed in Table 4-4. These symptoms are not specific to *Ascaris* infection. Therefore questions were also asked about other symptoms of respiratory infection (Table 4-5). Where it was appropriate, information was collected on symptoms from both Table 4-4 and Table 4-5 in the same question to avoid duplication of questions. For children under 12 years of age, the mother was usually present when the questionnaire was applied. The questionnaire took 10-15 minutes to complete, depending on the responses given and the amount of explanation needed.

Table 4-4 *Ascaris* related morbidity symptoms

Asthma
Difficulty in breathing
Dry cough
Problems after drinking milk
Appetite increases following treatment
Skin irritations
Irritability of conjunctiva
Heartburn (wind)
Abdominal pain (stomach ache, colic)
Lethargy (less active, sleep more)
Irritability

Table 4-5 Non-*Ascaris* related symptoms of respiratory infection

Cough - dry or humid
Sore throat
Not able to drink
Blocked or runny nose
Earache or ear discharge
Sore neck glands
Fever
Wheeze
Noisy breathing
Fast breathing

4.4.3 *Ascaris* worm burden

The aim of the substudy of *Ascaris* worm burden was to investigate the relationship between egg counts and worm burden in the study area.

4.4.3.1 Study Design

Following the period of *Ascaris* reinfection, a subsample of individuals was selected so as to ensure that individuals with low, medium and high intensity infections in terms of egg counts were represented proportionately in the subsample (based on pre-treatment intensity levels of *Ascaris* infection).

The substudy was explained to each individual, it was checked that individuals would be able to collect all samples[13] and individuals were invited to enter. Individuals were excluded if they had taken any anthelmintic drugs since treatment with pyrantel pamoate 9-10 months earlier. Eligible individuals were treated with pyrantel pamoate following the same procedures as for the initial chemotherapy (section 4.4.1.3).

4.4.3.2 Collection of stool samples

Stool samples were collected for 72 hours following treatment for *Ascaris* infection (Figure 4-4). On day 1 (treatment day), a 4-litre dark polyethylene bucket with a lid and handle was left with the individual to collect all stools evacuated. The name of the individual was written on the bucket and the lid. On days 2 and 3, the household was visited to collect the 24-hour stool samples and leave clean buckets. On day 4 the final bucket was collected from the household. Any individuals who failed to collect all stools in the study buckets were excluded from the study.

Figure 4-4 Study design for worm burden substudy

Activity	Day 1	Day 2	Day 3	Day 4
Treatment given	*			
Bucket 1	Deliver	Collect		
Bucket 2		Deliver	Collect	
Bucket 3			Deliver	Collect

[13] To assist compliance rates, most individuals selected were housewives or pre-school children. Older children were only included during holiday periods.

4.4.3.3 *Detection of* Ascaris *worms*

On arrival at the laboratory, the samples were kept in a cool, shaded area until examination that afternoon. The initial separation of the worms and larvae was performed outside the laboratory to ensure adequate ventilation, and beneath a temporary structure to provide shade. The laboratory technicians wore gloves, protective coats and masks.

The entire contents of each bucket was washed through 2 sieves (300μm and 100μm) using a hosepipe with a fine nozzle. The sieves were connected by a series of pipes directly into a large septic tank rather than the town sewerage system, which discharges untreated sewage, and general run-off directly into the local river. For each sample, the worms and larvae collected in the sieves were transferred to a labelled 500ml plastic bottle, containing 10% Formalin to prevent decomposition. The adult worms and larvae were separated out. The adults worms were individually weighed, measured and sexed and the total weight of larvae recorded.

4.5 OTHER VARIABLES

4.5.1 Socioeconomic, hygiene and sanitation status

At the end of the reinfection period, every household was visited and a questionnaire applied to establish the socioeconomic status of the family, the general standard of hygiene and sanitation, the sources of water used in the home and the sources of food eaten in the household (e.g. grown locally or bought from market) and the general diet of the household over the last 24 hours and the last 7 days (including meat, vegetable and fruit consumption) (Appendix F). The questionnaire was completed with the wife of the head of the household. In houses where the wife was deceased or no longer living in the house, the person responsible for daily household activities was questioned. The time needed for completion depended on the amount of explanation required and the size of the household. The questionnaire also contained questions aimed at providing an indirect measure of peridomestic exposure to *Ascaris* infection through poor sanitation and hygiene practices both at the family and individual level.

4.5.2 Anthropometric Status

The weight of all individuals in the study was recorded, and height and mid upper arm circumference measured for people in 60% of households visited. These measurements were taken before treatment for *Ascaris* infection.

Weight was measured using a Soehnle Electronic Balance, accurate to 0.1 kg. Other types of balance were piloted before the start of the study but were either (i) accurate and precise but too heavy for the fieldworkers to carry in the field, or (ii) were light enough but were inaccurate and imprecise. Trials of this balance during fieldworker training demonstrated that this balance, though a little heavy, was consistently accurate. **Height** was measured using a 2 metre stadiometer with a level (CMS Weighing Ltd, London), accurate to 0.2cm. This was ideal for fieldwork, as it was lightweight and separated into three sections for carriage. Other height measuring instruments were considered but were either non-portable except in a van, or otherwise unsuitable, as with the microtoise, which is lightweight, but needs a vertical wall with a hook. **Mid upper arm circumference (MUAC)** was measured using a tape accurate to 0.1cm.

The fieldworkers worked in pairs. Each measurement or weighing was taken independently by each fieldworker in turn, the two values were compared, and if the difference was greater than the expected measurement variation (0.1kg for weight, 2% for height and 0.1cm for MUAC), then the measurement was repeated. Generally the older members of the household were weighed and measured first, as they were often very busy, followed by the older children and finally the youngest members of the household. It was easier to measure the younger children when they had seen their brothers and sisters being weighed and measured. If a child was very agitated they were weighed with an adult and the child's weight calculated by subtraction. In some cases it was necessary to return later the same day or the next day to measure the height, when the child was quieter.

The results of the anthropometry showed no differences between the exposure groups and no further analysis was carried out with this data.

4.6 SAMPLE SIZE CALCULATION

The sample size was chosen to give the number of individuals in each group required to detect a difference in the mean egg counts between 2 groups. The following equation was used,

$$n > \frac{2(u+v)^2 \text{ var}}{(mean_2 - mean_1)^2}$$

where n = number of individuals in the each group

u = power factor (90% power => u = 1.28)

v = significance factor (5% significance => v = 1.96)

var = variance of $mean_1$

The log transform of the data was taken, to normalise the skewed distribution of the intensity of *Ascaris* infection. The formula to calculate the sample size was accordingly transformed,

$$n > \frac{2(u+v)^2 \text{ variance}}{(\log r)^2}$$

where log = logarithm to the base 10

r = ratio of the means

variance = var / ($mean_2$ * ln10)^2

ln = logarithm to the base e

Difficulties in finding appropriate data on *Ascaris* egg counts, to estimate the required sample size, meant that data from a study where no wastewater use was reported was used to approximate to the situation in the study area. The mean difference in egg counts between 5-14 and 15+ year olds (Elkins *et al.,* 1986) was used as a proxy for the expected difference in egg counts between high exposure and low exposure to *Ascaris* infection in the study population. In 5-14 year olds, there were 16,000 - 21,000 eggs per gram (epg) and 5,000 - 12,000 epg in 15 + years. A variance was used of 200 million (Croll *et al.,* 1982). Therefore to detect a mean difference in intensity between high and low exposure groups, it was necessary to calculate the sample size required to detect a significant difference between groups with 16,000 and 12,000 epg i.e. a difference of 4000 epg.

A sample size of 75 is required, with 5 % significance and with 90 % power. A further allowance of 10% non-compliance at the start of the study and 20% non-compliance during the study and gives a sample size of 100 in each exposure area in each age group. Early results in the control area showed only 75-80% compliance i.e. 20-25% non-compliance. Consequently when the samples were selected in the exposed and semi-exposed areas, an allowance for 33% non-compliance at the start of the study was made, giving a sample size of 120 families in each area.

4.7 FIELD BASE AND PERSONNEL

4.7.1 Field Base

A field office was established in Mixquiahuala (Figure 4-2). The head office of Irrigation District 03 is in Mixquiahuala and the town was centrally located for the three study areas. Fieldworkers met at the office each morning before leaving for the field. Data entry was carried out in the office and questionnaires filed there. A project laboratory was set up in part of the laboratory attached to the head office of DR 03 in Mixquiahuala. All stool samples and wastewater samples were analysed in this project laboratory.

4.7.2 Personnel and recruitment

The personnel employed during the study are listed below,

1 Field supervisor
2 Senior fieldworkers
5 Fieldworkers
1 Doctor
2 Drivers
1 Data entry clerk
2 Laboratory technicians

Personnel were recruited locally, through word of mouth or advertisements in the municipal building, most coming from the municipality of Mixquiahuala. Fieldworkers were required to have completed sixth form or the technical college equivalent. The 2 senior fieldworkers were individuals that worked with the study from the beginning and had demonstrated good fieldwork and leadership abilities. The data entry clerk had completed a technical college course involving programming. The minimum qualification for the laboratory staff was completion of a 3-year laboratory technician course in a technical college. Only the field supervisor was recruited from outside the area. She had finished a course in social science at Mexico City Polytechnic.

A training scheme was developed, with an initial phase of basic training, followed by a one week, unpaid trial period, during which progress was closely monitored and regular feedback given. Initial training for field staff involved both office simulations of field situations (role-play) and fieldwork followed by a week's trial in the field. A similar procedure was adopted for the laboratory staff, with a one week unpaid trial period, during which procedures were explained and practised with feedback given. The data entry clerk was taught the basics of dBase III. He was given data to enter and the speed of entry and accuracy monitored over a three-day period. At the end of the trial period for field, laboratory and data entry staff, the individual's performance was assessed and it was decided whether to offer the individual a job.

4.7.3 General training of fieldworkers

Before fieldwork began, the procedures to be followed and the forms to be used were explained. Role-play was used to help in training, with fieldworkers practising explanations and interviews with each other. The fieldworkers worked in pairs, with each pair acting out the visit to the household. Then everyone discussed the problems encountered and the ways in which the visit could have been improved.

4.7.4 Wastewater contact interview training

The training for the wastewater contact interview followed similar lines to the other phases of the study, with an initial explanation of the purpose of the questionnaire, discussion of each question and role-play to assist in training. Unlike the stool collection or the general questionnaire application, the interview involved the use of initial comments by the interviewee to direct later stages of the interview i.e. the structure of the interview depended on the responses of the interviewee, with a large amount of cross-checking during the actual interview. Training was correspondingly longer than for a standard questionnaire, with several days of discussion and role-play, before field practice of the interview.

4.7.5 Observation study training

Training was begun with seven fieldworkers (three fieldworkers already recruited, trained and applying questionnaires, and four newly recruited and untrained fieldworkers). An initial explanation of the observation studies was given to all fieldworkers, including the use of a digital watch to time observations. Training was divided into two stages, (1) learning to observe people regardless of the situation or activity performed and (2) understanding of the specific definitions of movements that were to be observed in the fields.

Stage I. The fieldworkers initially practised observing people, by observing the data entry clerks at work in the field office. Two movements, "Chew side of finger" and "Finger in mouth" were recorded throughout the observation period and the following movements recorded each minute:-

Touch key	Put pen in mouth
Turn over page	Touch screen

Pick up page	Scratch eye
Scratch nose	Scratch ear
Scratch head	Scratch leg
Cross arms	
No action	

All fieldworkers observed the same data entry clerk for a period of 30 minutes. Following the period of observation, the frequency of each movement was compared between the fieldworkers and the reasons for differences discussed among the whole group. This procedure was repeated several times until the differences in the frequency of the variables between fieldworkers were minimal. Following this initial practice at observing people, the fieldworkers split into pairs and performed role-playing. One fieldworker took the part of the observer and the other the part of the observee. Again, the group discussed the problems they experienced and the ways in which these could be solved. Most of the differences detected were in the variable "hand to mouth", which was recorded continuously, unlike the other variables which were only recorded at the 1 minute time point. This was caused by confusion over the definition of the variable and by lack of concentration, which were solved by discussion and practice respectively.

During the first few observation periods, the data entry clerks were not aware that they were being observed. However knowledge that they were being watched did not seem to be affect the overall frequency of movements during a period of observation. At the start of the observation period the individual was distracted by the presence of the fieldworkers, but after less than five minutes the individual seemed to resume old behaviour patterns. Following the rehearsal in the office (stage 1), stage 2 of the training began.

Stage 2. The observation studies in the fields were explained in detail and definitions of all movements to be observed were given. No differentiation was made to the fieldworkers between actions and behaviours. There was an initial day in the field in which periods of 30-40 minutes were spent observing a variety of farmers irrigating alfalfa and chillies.

Several problems surfaced during this initial day in the field, including the definition of variables and being close enough to the farmer to observe his actions. On the return from the field, the definitions of the variables were clarified and the reasons why the fieldworker was not close enough to the farmer discussed. One of the problems identified was that there were too many people observing a single farmer. When the farmer was walking along a narrow bank not more than two or three people could be close enough to observe all the farmer's movements. This problem was resolved following the initial training phase since in the actual study there were never more than three individuals observing the same farmer in the actual study i.e. the fieldworker plus the field supervisor, when the field supervisor was duplicating the observation, and plus the author, when she was checking the accuracy of the field supervisor. Three other causes were however highlighted, which related to the fact that the team was not accustomed to (i) walking in wellington boots, (ii) walking in thick and sticky mud nor (iii) exercise.

Following the initial day in the field, the fieldworkers observed farmers performing a variety of activities,

Cleaning ditches

Damming ditches

Preparing a field

Irrigating a field

Planting

All fieldworkers observed the same farmers, so that the performance of the fieldworkers could be compared. After each day in the field there was a meeting of all fieldworkers and the field supervisor with the author to compare results and discuss any problems that had surfaced during the day, so that appropriate amendments could be made to the observation methodology in the light of any problems that had emerged. This training phase of the observation studies lasted for five days in total, during which time the differences between the fieldworkers, the field supervisor and the author, in the frequency of any one variable during the observation period, was reduced to less than or equal to 1.

Following the training period, the four newly recruited fieldworkers remained to observe and the other three fieldworkers resumed visiting the households to apply questionnaires. It had been hoped that these three fieldworkers would be able to both apply questionnaires and observe, but they were not suited to field observations. The ideal fieldworker for all other aspects of the study was someone who could communicate with all members of the family, the men, the women and children and who was easily permitted access to the house; this meant that, with few exceptions, the fieldworkers were female. However, the ideal fieldworker for the observation studies was a fit individual who could talk with the farmers, was comfortable at being left alone in the field and was not concerned about getting a little muddy. This meant that most of the fieldworkers employed for the observation studies were 18-22 year old males.

4.7.6 Anthropometry training

A nutritionist at the National Institute of Nutrition held a one-day training course for all the fieldworkers. The course provided a basic introduction to the measurement of weight, height and mid upper arm circumference. Measurement of these parameters was practised among the fieldworkers. Common errors encountered and how these can be avoided were discussed. Repeated blind measurement showed everyone that there were both intra- and inter-person variations. Reduction of intra-person variation was achieved more rapidly than inter-person variation. Further practice, prior to visiting the households in the study, was provided through a visit to a local nursery school, where children were weighed and measured. This reduced the inter-person variations to a minimum and provided all-important practice in measuring young children.

4.7.7 Laboratory training

The procedures for storage and for concentration of the stool samples were demonstrated and then practised by the laboratory technicians. The laboratory technicians were trained to identify *Ascaris* eggs by repeated observation of samples. When there was a discrepancy between the number of *Ascaris* eggs counted by the author and the technicians, both laboratory technicians rechecked the slide and the reasons for misclassification discussed. Following the initial training of the laboratory staff, quality control procedures (section 4.8.2) were maintained, together with close supervision, to deal with any problems that arose, scientific or logistical.

4.7.8 Study Management

The structure of the team is displayed in Figure 4-5. At all stages in the project, there was close supervision of the study team, to enable rapid discussion of any problems that arose during the course of the study. The responsibilities of the author included:

(i) organisation of regular meetings with all study personnel, to discuss the progress of the study and discuss any problems that have emerged,

(ii) initial explanations and training at each new phase of the study,

(iii) supervision of all field, laboratory and office personnel,

(iv) quality control checks of the work of fieldworkers and the field supervisor,

(v) revision of a proportion of all completed questionnaires and code checking prior to data entry,

(vi) quality control of laboratory work,

(vii) revision of errors in double entry of data and data inconsistencies, and

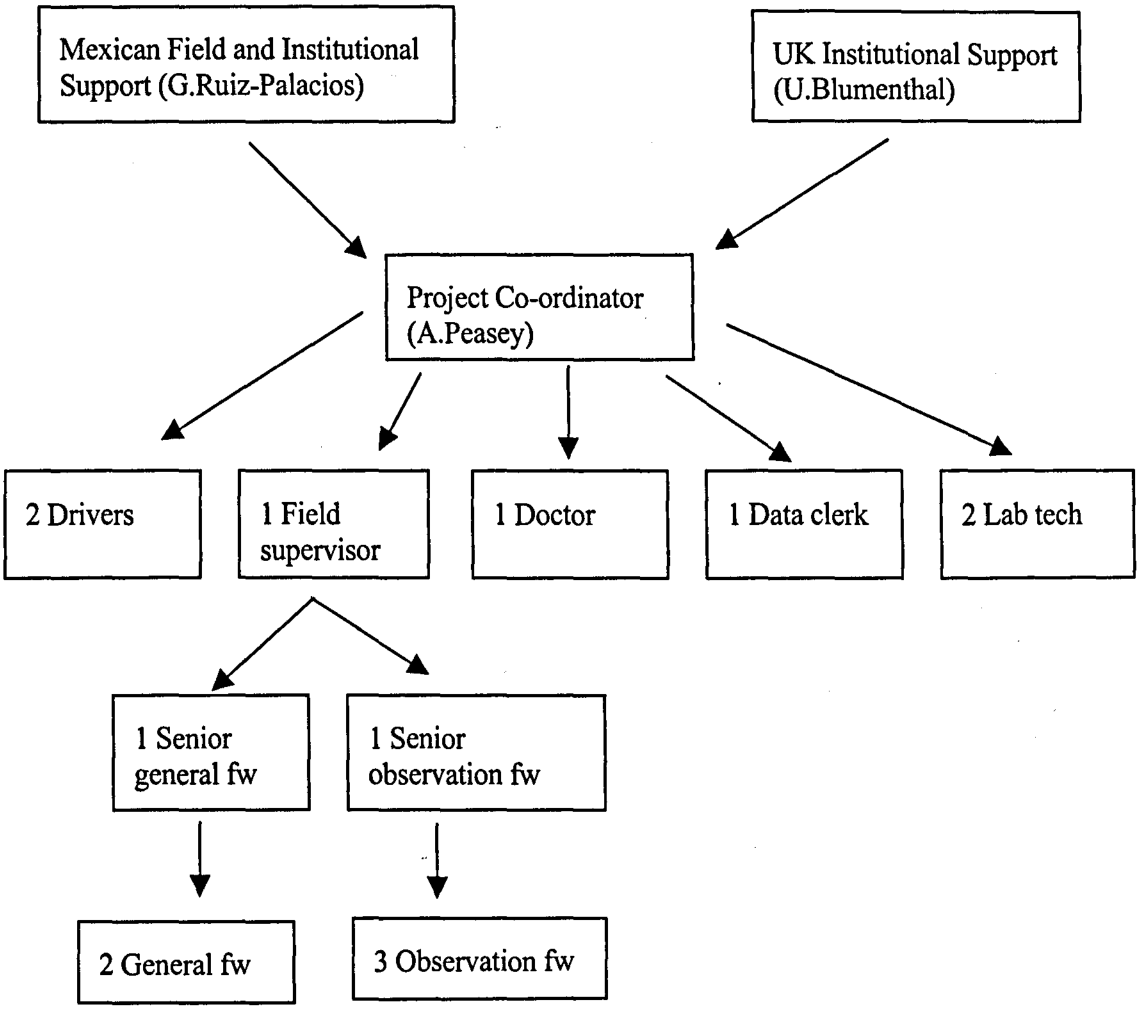
(viii) ensuring that supplies for the field and laboratory arrived when needed.

The field supervisor was responsible for the day-to-day running of the study, which included (i) organising a brief meeting each morning to discuss the day's work in the field and (ii) co-ordinating the activities of fieldworkers and drivers. The drivers had previous experience of working in the study area and provided valuable background knowledge to the author and field supervisor. Each fieldworker was assigned a separate list of houses to visit. After much consideration, it was decided that the benefits gained from having the same fieldworker always deal with a particular household outweighed the associated logistical problems. To ensure that the quality of the data was maintained, there was close supervision of the fieldworkers and regular comparisons of data collected and of compliance rates between fieldworkers. The fieldworkers were responsible for the legible recording of information on forms and the coding of questionnaires on their return from the field.

The laboratory technicians were responsible for delivery of result forms to the data entry clerk and general tidiness in the laboratory. They were directly answerable to the author, who carried out routine quality control checks.

The data entry clerk was responsible for double entry of all data from the field and laboratory and the running of programs to check the consistency of that data. He was also responsible for the filing of all data forms and answerable directly to the author.

Figure 4-5 Structure of the study team[14]



[14] Fw=fieldworker

4.8 QUALITY CONTROL AND SOURCES OF BIAS

Quality control procedures can be divided into several sections, relating to fieldwork, laboratory work, data checking and data entry. In every phase of the study, quality control procedures began once the initial training period was concluded and the supervisor, field workers, laboratory technicians or data entry clerk understood the procedures. Overall quality control was done by the author, who repeated or duplicated 10% of all forms of data collection by both staff and supervisors e.g. questionnaires, observation studies, laboratory analysis and checked 20% of all coding.

4.8.1 Field quality control

Each week, the field supervisor accompanied all fieldworkers, to check that there were no problems, gave constructive comments and repeated 10-15% of data collection procedures to check the quality of the data collected. Depending on the circumstances, the field supervisor either commented directly after the interview, while still at the house, or on the return to the office. The author spent 1-2 days each week in the field carrying out duplicate data collection with both fieldworkers and the field supervisor to further check the quality of data collected.

4.8.1.1 Questionnaires

When performing quality control, both the field supervisor and the author recorded the answers given by the respondent at the same time as the fieldworker and the two questionnaires were compared after the interview. Discrepancies encountered usually related to slight differences in understanding of the response given. In such cases the understanding of a particular question was re-emphasised by the author.

For the morbidity questionnaire, timing of the fieldwork meant that it was difficult to return to the households at a later date; also it was felt that this could affect compliance. The presence of the field supervisor during a percentage of the interviews was felt to be the best way to check the quality of the interviews, in view of the expected problems. In the socioeconomic status, sanitation and hygiene, the field supervisor, as well as duplicating questionnaires, also returned to households visited previously to ask a few selected questions from the questionnaire. Since this involved only a few questions and was directed at the wife of the head of the household, the person most often in the house and the most readily willing to participate in the study, repeat applications could be carried out with this particular questionnaire.

4.8.1.2 Wastewater contact interviews

It was not feasible to repeat this interview at a later date, because it was very difficult to find the farm workers, it was time consuming and it could have affected compliance especially among the older male age groups often prone to non-compliance. Simultaneous duplicate recording of 10% of interview responses, together with monitoring of the consistency of responses within households, were used to maintain quality control.

4.8.1.3 Observation studies

The field supervisor was present for 20% and the author for 10% of observations and they completed an observation form at the same time as the fieldworker. The percentage of observations attended was high because there is no other way to check the quality of the work done. The observation forms of the author, field supervisor and the fieldworker were later compared. Generally, only minor discrepancies were found between the fieldworker, field supervisor and the author. If large discrepancies were found, the field supervisor's observation sheet was included. Where there were clearly problems with filling in the observation form rather than a short lapse in concentration, further training was given. Subsequent observation periods were monitored until the author was satisfied with the quality of the fieldworkers' responses. When quality did not improve the fieldworker was dismissed and a new recruit trained and employed.

4.8.1.4 Anthropometry

Repeat measurements were made by both the author and field supervisor in 10-15% of households. The measurements taken were compared with those of the fieldworkers and measurements repeated where significant differences were found (see section 4.5.2).

4.8.2 Laboratory quality control

Initially, each laboratory technician carried out duplicate readings of every 12th coverslip to check the accuracy of work of the other. Then, for every 18th coverslip, a further sample of concentrate from the same sample was examined by the other laboratory technician. The concentrate not observed was stored, so that any apparent inconsistencies in the number of *Ascaris* eggs observed could be checked later. It was found that there were very few false-positives and false-negatives (< 1%). It was decided that the laboratory technicians would carry out duplicate readings of 10-15% of the positives and of none of the negatives. The author then randomly duplicated readings of 10% of all slides (positive or negative). The laboratory equipment was routinely checked every week.

4.8.3 Data checking and entry

All forms were initially revised by the fieldworker after finishing the fieldwork. The fieldworker checked that all responses were clear and readable and all coding entered. The forms were then passed to the field supervisor, who checked the data forms to ensure that (i) the date and the identification number were correct, (ii) there were no unanswered questions, (iii) the form was legible and (iv) all coding was correct. A proportion of all data forms (field and laboratory) was passed to the author for final revision prior to data entry. All data was entered then twice and the two files compared to reduce typing errors. Simple dBase programmes were used to check each variable for inconsistencies and range errors. Once identified, either the questionnaire or the database was corrected as appropriate.

4.8.4 Sources of bias

Selection bias needed to be considered. There may be differences between the families that agree to participate in all stages of the project and families that do not wish to enter the study or who dropped out during the course of the study. The characteristics of families of those included and not included in the final analysis were compared to identify any differences that could affect the outcome measures.

Information bias due to measurement error was considered when the data were analysed. It was important to consider the reproducibility and the repeatability of the techniques used, so that the amount of variation between two results that may only be due to the technique used or to the inaccuracy of the measurer was known. This applied to all data collection procedures, the stool analysis technique, the wastewater analysis techniques, and the measurements taken in the field.

Confounding bias was also important because failure to consider variables that could affect the outcome measures or other measures in the study could affect the validity and interpretation of the results of the study. Therefore it was important to measure all expected confounding variables, such as socioeconomic, sanitation and hygiene status

4.9 DATA MANAGEMENT

4.9.1 Equipment and programs employed

The study used an IBM PC compatible computer with a 40 megabyte hard disk and two 5.25" disk drives connected to an Epson LQ550 printer. The database system, dBase III was used to enter all data collected. The data were checked for inconsistencies and range errors using simple programmes written in the dBase III programming language. As well as storing the data that were to be analysed later, the database of information interacted with the needs of the field, for example the field staff were provided with updated lists of the study population, labels for stool pots, and results of laboratory analyses.

4.9.2 Data analysis

Initially descriptive analysis was done using Epi-Info5 and subsequent univariate and multivariate analyses were begun, using GLIM. However the major part of the analysis was completed using STATA 5.0.

CHAPTER 5. CHARACTERISTICS OF THE STUDY POPULATION

5.1 INTRODUCTION

All data presented in this chapter were collected by questionnaire application during the course of the study described in this thesis unless stated otherwise. For example, Hidalgo State Government census statistics are included in some sections of this chapter as comparison data. The initial sampling frame of families from which these study groups were selected was obtained from a census carried out by a large cross-sectional study in the same area (Cifuentes, 1995). However the information obtained initially from the cross-sectional study on names, addresses, ages and gender was checked and updated at the start of the study described in this thesis. All tests of association relate to the Pearson chi-squared test unless otherwise stated.

5.2 AGE AND GENDER COMPOSITION

There was a similar age distribution among both males and females (p=0.345) (Figure 5-1). The total number of individuals within each age group increased with increasing age. There was no difference in the age distribution of individuals under 15 years old between the three study populations (p=0.502) (Figure 5-2). However in the control population the proportion of 15-34 year olds was lower, than in the exposed and semi-exposed populations (p=0.004).

Figure 5-1 Age distribution according to gender

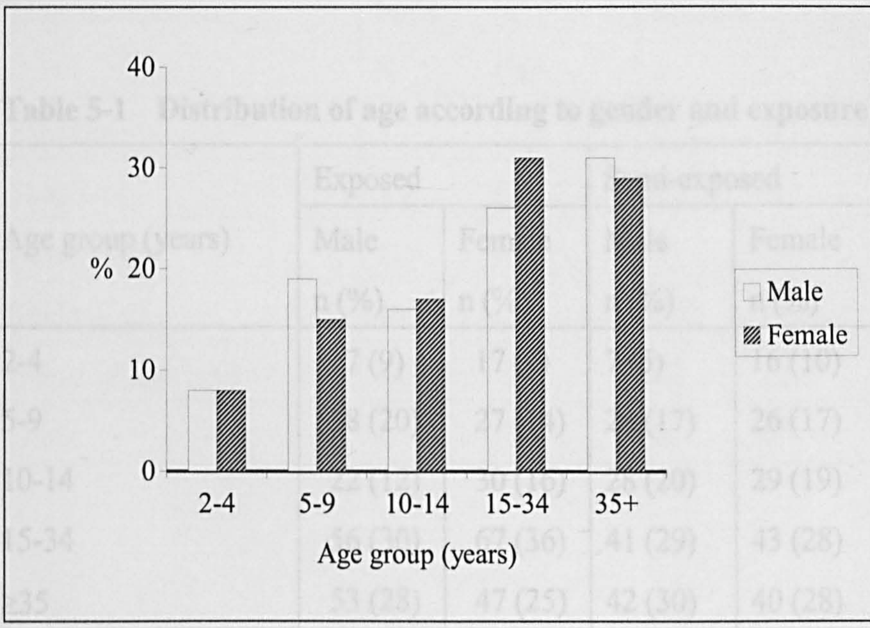
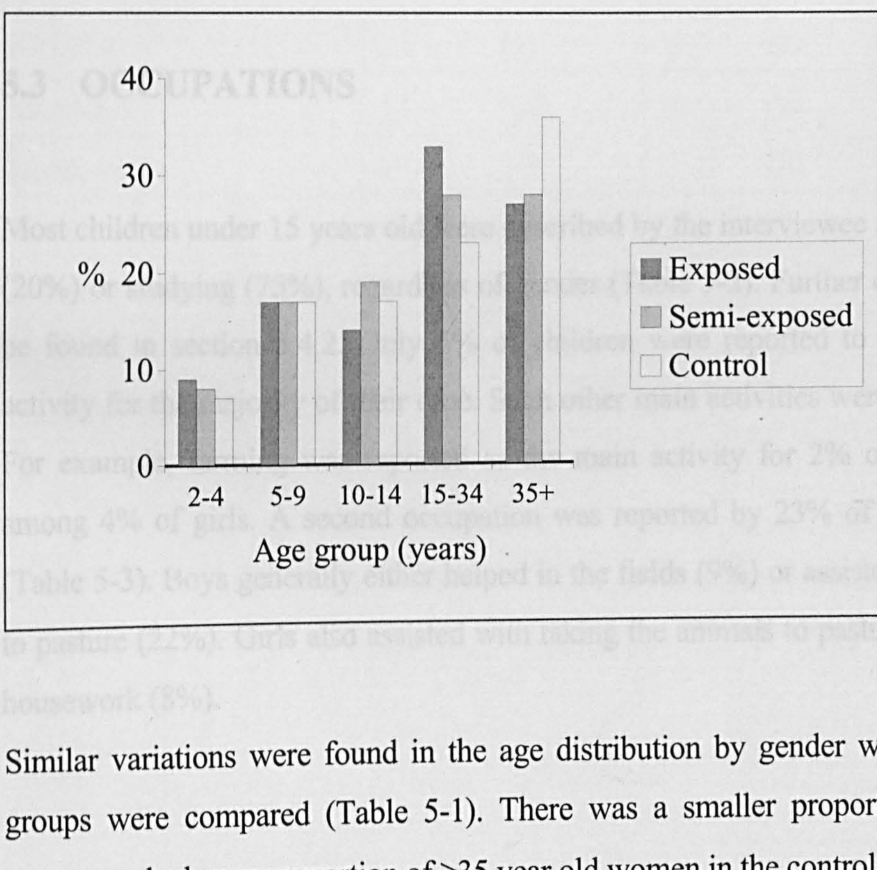


Figure 5-2 Age distribution according to exposure group



Similar variations were found in the age distribution by gender when the three exposure groups were compared (Table 5-1). There was a smaller proportion of 15-34 year old women and a larger proportion of ≥35 year old women in the control than in the exposed and semi-exposed populations (p=0.052). There were gradually decreasing proportions of 15-34 year old men and increasing proportions of ≥35 year old men, from the exposed to the semi-exposed to the control population.

Table 5-1 Distribution of age according to gender and exposure group

Age group (years)	Exposed		Semi-exposed		Control	
	Male n (%)	Female n (%)	Male n (%)	Female n (%)	Male n (%)	Female n (%)
2-4	17 (9)	17 (9)	7 (5)	16 (10)	15 (9)	7 (5)
5-9	38 (20)	27 (14)	24 (17)	26 (17)	33 (19)	19 (14)
10-14	22 (12)	30 (16)	28 (20)	29 (19)	31 (18)	22 (16)
15-34	56 (30)	67 (36)	41 (29)	43 (28)	34 (20)	37 (27)
≥35	53 (28)	47 (25)	42 (30)	40 (28)	60 (35)	52 (38)
Total	186	188	142	154	173	137
p-value (Pearson chi)	0.35		0.52		0.31	

5.3 OCCUPATIONS

Most children under 15 years old were described by the interviewee as either pre-school age (20%) or studying (75%), regardless of gender (Table 5-2). Further details of schooling can be found in section 5.4.2. Only 5% of children were reported to be engaged in another activity for the majority of their time. Such other main activities were influenced by gender. For example, farming was reported as the main activity for 2% of boys and housework among 4% of girls. A second occupation was reported by 23% of girls and 34% of boys (Table 5-3). Boys generally either helped in the fields (9%) or assisted in taking the animals to pasture (22%). Girls also assisted with taking the animals to pasture (9%) or helped with housework (8%).

Table 5-2 Occupation of under 15 year olds

Main occupation	Male		Female		Total	
	N	(%)	n	(%)	N	(%)
Farming	5	(2)	2	(1)	7	(2)
Shepherd	3	(1)	1	(1)	4	(1)
Housework	1	(1)	7	(4)	7	(2)
Student	161	(75)	143	(74)	304	(75)
Infant / Pre-school	44	(20)	38	(20)	82	(20)
Other	1	(1)	2	(1)	3	(1)

Table 5-3 Secondary occupation of under 15 year olds

Secondary occupation	Male		Female		Total	
	n	(%)	n	(%)	n	(%)
Farming	20	(9)	8	(4)	28	(7)
Shepherd	47	(22)	17	(9)	64	(16)
Housework	1	(1)	15	(8)	16	(4)
Student	0	(0)	2	(1)	2	(1)
Infant / Pre-school	1	(1)	2	(1)	3	(1)
Other	4	(2)	0	(0)	4	(1)
No other occupation	142	(66)	149	(77)	291	(71)

There was a significant difference in the main occupation between adult men and women ($p<0.001$). The most frequent main occupation for adult men was farming (71%) and for adult females was housework (82%) (Table 5-4). More farming orientated families were selected than would have been present in a cross-section of the study area population. State government census results suggest about 36% of the general population is engaged in agriculture (INEGI, 1990). Within selected households, there were a small number of adult men engaged in a variety of other occupations (Table 5-4). However, there was less diversity among adult women, with those not performing household duties either tending livestock (5%) or studying (7%). Similar numbers of young adult men and women continued studying, though percentages were low (7%).

Table 5-4 Occupation of ≥15 year olds

Main occupation	Male		Female		Total	
	n	(%)	n	(%)	n	(%)
Farming	203	(71)	2	(1)	205	(36)
Shepherd	5	(2)	15	(5)	20	(3)
Housewife	7	(2)	234	(82)	241	(42)
Student	22	(8)	20	(7)	42	(7)
Labourer	17	(6)	3	(1)	20	(4)
Professional	2	(1)	5	(2)	7	(1)
Does not work or retired	17	(6)	2	(1)	19	(3)
Sales	5	(2)	2	(1)	7	(1)
Other	8	(3)	3	(1)	11	(2)

Similar proportions of adult men (35%) and women (28%) reported having secondary or subsidiary occupations as children (Table 5-5). However, as with the children, there were differences in those occupations between the genders ($p<0.001$). Adult men were either engaged in farming (14%), tended to livestock (10%) or regarded themselves as partially retired (8%). Women were mainly involved in tending livestock (17%), with small numbers helping with farming (4%) and housework (5%).

Table 5-5 Secondary occupation of ≥15 year olds

Secondary occupation	Male		Female		Total	
	n	(%)	n	(%)	n	(%)
Farming	39	(14)	12	(4)	51	(9)
Shepherd	28	(10)	48	(17)	76	(13)
Housewife	0	(0)	13	(5)	13	(2)
Retired / do not work	23	(8)	0	(0)	23	(4)
Other	9	(3)	8	(3)	17	(3)
No other occupation	187	(65)	205	(72)	391	(68)

There were no significant differences in the occupations of children (under 15 years old) when the proportion of students, infants and other occupations was compared between the three study groups (p=0.40). There were slightly more pre-school children in the exposed population than the other two study populations, however the difference was not significant (p=0.136) (Table 5-6). There were differences in the most commonly reported secondary occupations for children in the three study populations. The numbers involved were too small to be significant (Table 5-7), but there were slightly more children helping in agriculture and tending to livestock in the semi-exposed (26%) and control (23%) than the exposed (20%) population.

Table 5-6 Effect of exposure group on occupations among under 15 year olds

Main occupation	Exposed		Semi-exposed		Control	
	n	(%)	n	(%)	n	(%)
Farming	3	(2)	3	(2)	1	(1)
Shepherd	1	(1)	1	(1)	2	(2)
Housework	3	(2)	2	(2)	3	(2)
Student	105	(70)	102	(78)	97	(76)
Infant	38	(25)	21	(16)	23	(18)
Other	1	(1)	1	(1)	1	(1)

Table 5-7 Effect of exposure group on secondary occupations among under 15 year olds

Secondary occupation	Exposed		Semi-exposed		Control	
	n	(%)	n	(%)	n	(%)
Farming	8	(5)	15	(12)	5	(4)
Shepherd	22	(15)	18	(14)	24	(19)
Housework	6	(4)	2	(2)	8	(6)
Student	2	(1)	0	(0)	0	(0)
Infant	0	(0)	3	(2)	0	(0)
Other	0	(0)	1	(1)	3	(2)
No other occupation	113	(75)	91	(70)	87	(69)

The proportion of adults engaged in the different main occupations in the three study populations was very similar (p=0.49) (Table 5-8). The majority either worked in agriculture or carried out household duties. Similar proportions of adults in the three study populations reported having a secondary occupation (p=0.27) (Table 5-9). Most of these were either agriculture-related or involved tending livestock. There were no significant differences in secondary occupation between the three study populations (p=0.13).

Table 5-8 Effect of study group on occupations among ≥15 year olds

Main occupation	Exposed		Semi-exposed		Control	
	n	(%)	n	(%)	n	(%)
Farming	79	(35)	61	(37)	65	(36)
Shepherd	7	(3)	10	(6)	3	(2)
Housework	95	(43)	64	(39)	82	(45)
Student	16	(7)	15	(9)	11	(6)
Labourer	9	(4)	5	(3)	6	(3)
Professional	1	(<1)	4	(2)	2	(1)
Does not work or retired	9	(4)	2	(1)	8	(4)
Sales	4	(2)	0	(0)	3	(2)
Other	3	(1)	5	(3)	3	(2)

Table 5-9 Effect of study group on secondary occupations among ≥15 year olds

Secondary occupation	Exposed		Semi-exposed		Control	
	n	(%)	n	(%)	n	(%)
Farming	23	(10)	9	(5)	19	(10)
Shepherd	23	(10)	29	(17)	24	(13)
Housework	4	(2)	5	(3)	4	(2)
Does not work or retired	7	(3)	6	(4)	10	(5)
Other	4	(2)	6	(4)	7	(4)
No other occupation	162	(73)	111	(67)	119	(65)

5.4 EDUCATIONAL LEVEL

5.4.1 Literacy

State Government statistics for Hidalgo reported overall literacy at 95% for 11-14 year olds and 79% for adults (INEGI, 1990). However levels of literacy between municipalities ranged from 48-90% for adults, with the more remote regions having much lower levels of literacy (INEGI, 1990). Vehicular access to the three study populations was reasonable for the region and this was clearly reflected in their literacy levels. Municipal literacy levels for the study populations ranged from 81-90% for adults (INEGI, 1990).

Among 11-14 year olds literacy levels were nearly at 100% in all three study populations and there were no significant differences between boys and girls (p=0.50) (Table 5-10).

Table 5-10 Reading and writing ability of 11-14 year olds

Skill	Gender	Exposed n/N (%)	Semi-exposed n/N (%)	Control n/N (%)	Total n/N (%)
Reading	Male	19/19 (100)	22/22 (100)	22/22 (100)	63/63 (100)
	Female	26/26 (100)	24/25 (96)	18/18 (100)	68/69 (99)
	Total	45/45 (100)	46/47 (98)	40/40 (100)	131/132 (99)
Writing	Male	19/19 (100)	22/22 (100)	22/22 (100)	63/63 (100)
	Female	26/26 (100)	24/25 (96)	18/18 (100)	68/69 (99)
	Total	45/45 (100)	46/47 (98)	40/40 (100)	131/132 (99)

Unlike literacy levels for 11-14 year olds, those for adults were different in the three study populations and there were differences between genders (Table 5-11). There was a decreasing trend of ability to read from the exposed (89%) to the semi-exposed (83%) to the control population (78%) ($p<0.01$). Overall, in the three study populations, women had consistently lower reading ability than men ($p=0.06$), though only in the semi-exposed area was this effect significant ($p=0.002$).

Similar trends were seen for adult writing ability, with differences between the study populations and a marked gender effect. Writing ability decreased from the exposed (87%), to the semi-exposed (81%) to the control (78%) ($p<0.01$). Overall less women could write than men ($p<0.001$), this effect was significant in the adult exposed ($p=0.06$) and semi-exposed ($p=0.003$) populations.

Table 5-11 Reading and writing ability of adults (≥15 year olds)

Skill	Gender	Exposed n/N (%)	Semi-exposed n/N (%)	Control n/N (%)	Total n/N (%)
Reading	Male	101/109 (93)	76/ 83 (92)	76/94 (81)	253/286 (88)
	Female	98/114 (86)	61/ 83 (73)	67/89 (75)	226/286 (79)
	Total	199/223 (89)	137/166 (83)	143/183 (78)	484/572 (85)
Writing	Male	100/109 (92)	75/83 (90)	76/94 (81)	251/286 (88)
	Female	95/114 (83)	60/83 (72)	66/89 (74)	221/286 (77)
	Total	195/223 (87)	135/166 (81)	142/183 (78)	472/572 (83)

5.4.2 Schooling

In the State as a whole, between 64% and 92% of 5-14 year olds attend school depending on the municipality (INEGI, 1990), with overall slightly more boys than girls in school (84% versus 82%). Levels of school attendance for 5-14 year olds ranged from 85-90% in the municipalities that included the study groups (INEGI, 1990).

There were no significant differences between the three study populations in schooling level attained by boys or girls aged 11-14 years ($p>0.10$) (Table 5-12 and Table 5-13 respectively). There were no significant differences between boys and girls ($p>0.1$); apparent differences observed were due to slight differences in the age structure between genders and areas.

Table 5-12 Level of schooling for 11-14 year old males

Level of schooling	Exposed (N=19)		Semi-exposed (N=22)		Control (N=22)		Total (N=63)	
	n	(%)	n	(%)	n	(%)	n	(%)
Did not go to school	0	(0)	1	(5)	0	(0)	1	(2)
Primary not completed	9	(47)	6	(27)	10	(45)	25	(40)
Only primary completed	2	(11)	4	(18)	1	(5)	7	(11)
Further studies completed	8	(42)	11	(50)	11	(50)	30	(48)

Table 5-13 Level of schooling for 11-14 year old females

Level of schooling	Exposed (N=26)		Semi-exposed (N=25)		Control (N=18)		Total (N=69)	
	n	(%)	n	(%)	n	(%)	n	(%)
Did not go to school	0	(0)	2	(8)	0	(0)	2	(3)
Primary not completed	7	(27)	8	(32)	6	(33)	21	(30)
Only primary completed	6	(23)	1	(4)	0	(0)	7	(10)
Further studies completed	13	(50)	14	(56)	12	(67)	39	(57)

State census results for adults showed 19% had never attended school, 26% failed to complete primary school, 19% completed primary school only and 34% had some level of post-primary tuition (INEGI, 1990). Within the study population municipalities, 45-65% of adults had completed at least primary school (INEGI, 1990).

Study results indicated the three study populations had higher levels of schooling than the State as a whole. There were no significant differences between the level of schooling of men in the three study populations (p=0.17) (Table 5-14).

Table 5-14 Level of schooling of males aged ≥15 years

Level of schooling	Exposed (N=109)		Semi-exposed (N=83)		Control (N=94)		Total (N=286)	
	n	(%)	n	(%)	N	(%)	N	(%)
Did not go to school	7	(6)	4	(5)	11	(12)	22	(8)
Primary not completed	51	(47)	38	(46)	49	(52)	138	(48)
Only primary completed	28	(26)	15	(18)	15	(16)	58	(20)
Further studies completed	23	(21)	26	(31)	19	(20)	68	(24)

The level of schooling for women was different in the three study populations (p=0.08) (Table 5-15). The proportion of women with no schooling gradually increased from the exposed to the semi-exposed to the control population (p_T=0.01). More women continued their education after primary school in the semi-exposed than in the exposed (p=0.04).

Differences in the level of schooling between men and women were apparent (p=0.01). However there was only a significant difference seen in the semi-exposed (p=0.05), where the effect was due primarily to a higher proportion of women than men not having received any schooling.

Table 5-15 Level of schooling of females aged ≥15 years

Level of schooling	Exposed (N=114)		Semi-exposed (N=83)		Control (N=89)		Total (N=286)	
	n	(%)	n	(%)	n	(%)	n	(%)
Did not go to school	11	(10)	13	(16)	20	(22)	44	(15)
Primary not completed	60	(53)	43	(52)	41	(46)	144	(50)
Only primary completed	25	(22)	9	(11)	11	(12)	45	(16)
Further studies completed	18	(16)	18	(22)	17	(19)	53	(19)

5.5 CHARACTERISTICS OF HEAD OF HOUSEHOLD AND WIFE[15]

Literacy levels of the head of the household (HH) and his wife (Table 5-16) were similar to those for adults in general (Table 5-11). A higher proportion of heads of household than their wives reported to be able to read or write a short message ($p<0.0001$) and literacy levels were higher in the exposed, than the semi-exposed or control population ($p=0.004$).

Table 5-16 Reading and writing ability

Skill	Individual	Exposed		Semi-exposed		Control		Total	
		n	(%)	n	(%)	n	(%)	n	(%)
Reading	HH	353	(94)	259	(88)	261	(84)	873	(89)
	Wife	307	(83)	211	(71)	224	(73)	742	(76)
Writing	HH	345	(93)	252	(85)	261	(84)	858	(88)
	Wife	296	(80)	203	(69)	221	(72)	720	(74)

[15] Missing data for wife for one HH in exposed (n=372) and one in control group (n=308). Total for wife variables =976.

The level of schooling reached by the head of the household was significantly lower than the adult men in general in the study population (Table 5-14 and Table 5-17 respectively). Only 20-28% had completed at least primary school, compared with 36-49% among adult men in the general study population. There were differences between the three study groups, with slightly more heads of household completing primary school in the semi-exposed than the exposed and control areas (p=0.008). The situation was similar for the wives of the heads of household (Table 5-18). Only 18-22% of individuals were in households where the main female had completed at least primary school, compared to 31-38% of the adult female study population as a whole (refer to Table 5-15). There were significant differences between the schooling of heads of households and their wives. Wives achieved a significantly lower level of schooling than their husbands (p<0.001).

Table 5-17 Level of Schooling of Head of Household

Level of schooling	Exposed (N=374)		Semi-exposed (N=296)		Control (N=310)		Total (N=980)	
	n	(%)	n	(%)	N	(%)	n	(%)
Did not go to school	19	(5)	34	(11)	26	(8)	79	(8)
Primary not completed	270	(72)	179	(60)	223	(72)	672	(69)
Only primary completed	73	(20)	71	(24)	51	(16)	195	(20)
Further studies	12	(3)	12	(4)	10	(3)	34	(3)

Table 5-18 Level of Schooling of Wife

Level of schooling	Exposed (N=372)		Semi-exposed (N=296)		Control (N=308)		Total (N=976)	
	n	(%)	n	(%)	N	(%)	n	(%)
Did not go to school	49	(13)	44	(15)	69	(22)	162	(17)
Primary not completed	242	(65)	200	(68)	182	(59)	624	(64)
Only primary completed	70	(19)	38	(13)	46	(15)	154	(16)
Further studies	11	(3)	14	(5)	11	(4)	36	(4)

For more than 55% of individuals in each group, the head of household was over 40 years old (Table 5-19). The age of the head of household was different in the three study populations. Heads of households were younger in the semi-exposed group compared with the exposed and control groups ($p=0.001$). The wives of the heads of households were also younger in the semi-exposed group compared to the exposed and control groups ($p<0.001$).

Table 5-19 Age of Head of household and wife

Indiv	Age	Exposed		Semi-exposed		Control		Total	
	(years)	n	(%)	N	(%)	n	(%)	n	(%)
HH	15-30	47	(13)	59	(20)	26	(8)	132	(13)
	31-40	83	(22)	68	(23)	84	(27)	235	(24)
	41-90	244	(65)	169	(57)	200	(65)	613	(63)
Wife	15-30	73	(20)	94	(32)	64	(21)	231	(24)
	31-40	137	(37)	80	(27)	125	(41)	342	(35)
	41-90	162	(44)	122	(41)	119	(39)	403	(41)

5.6 SOCIOECONOMIC CHARACTERISTICS

5.6.1 Housing conditions

Census results for the State indicate that over 80% of households own their home, 88-94% have electricity and 64% of homes have at least three rooms (INEGI, 1990). Brick, breeze block or stone are used for house walls in over 70% of homes and 45% of roofs are concrete or brick (flat roof). Census results show variations in floor materials between study areas. Earth floors were present in 5% of homes in the municipalities in the exposed areas, 24% in the semi-exposed and 15% in the control, with the remainder of floors being either concrete or mosaic (INEGI, 1990).

The results from the study presented here indicated that 94-99% of individuals lived in households with electricity, and most owned their homes (91-94%) (Table 5-20). Length of residency within the village, not necessarily in the same house, varied slightly between study populations. The proportion of individuals in households with less than 6 years in the village was lower in the control area than the exposed and semi-exposed groups ($p<0.01$). There were also more individuals in households with over 20 years in the village in the exposed and control areas, than in the semi-exposed ($p<0.01$). The proportion of individuals in homes with earth floors was greatest in the semi-exposed population (31%) and lowest in the exposed (11%). Walls were more often strongly built with brick or breeze block and roofs more durable in the exposed and control areas than the semi-exposed area ($p<0.01$). The size of houses was similar in the three study areas. The proportion of individuals in households using wood, or wood and gas as a fuel for cooking was lower in the exposed than the semi-exposed and control groups ($p<0.01$).

Table 5-20 Housing conditions

Variable	Category	Exposed		Semi-exposed		Control	
		n	(%)	n	(%)	n	(%)
Residence in Village (years)	1-5	38	(10)	38	(13)	15	(5)
	6-10	59	(16)	69	(23)	44	(14)
	11-20	99	(26)	96	(32)	121	(39)
	>20	178	(48)	93	(31)	130	(42)
Own house		340	(91)	273	(92)	291	(94)
Floor Covering	Earth	41	(11)	91	(31)	53	(17)
	Concrete	333	(89)	205	(69)	257	(83)
Wall material	Mud bricks	45	(12)	54	(18)	28	(9)
	Stone	50	(13)	63	(21)	41	(13)
	Red brick	57	(15)	8	(3)	134	(43)
	Breeze block	222	(59)	171	(58)	107	(35)
Roof material	Concrete/brick	221	(59)	106	(36)	181	(58)
	Corrugated[16]	144	(39)	187	(63)	112	(36)
	Other	9	(2)	3	(1)	17	(5)
No. rooms (excluding kitchen & bathroom)	1	64	(17)	61	(21)	34	(11)
	2	68	(18)	82	(28)	100	(32)
	3	143	(38)	107	(36)	111	(36)
	≥4	99	(26)	46	(16)	65	(21)
Crowding (individuals per room)	<2	93	(25)	75	(25)	128	(41)
	2-2.9	139	(37)	87	(29)	68	(22)
	3-3.9	70	(19)	60	(20)	67	(22)
	≥4	72	(19)	74	(25)	47	(15)
Electricity supply to house		369	(99)	279	(94)	308	(99)
Fuel for Cooking	Wood	34	(9)	83	(28)	72	(23)
	Gas	220	(59)	68	(23)	105	(34)
	Wood & gas	120	(32)	145	(49)	133	(43)

16 Corrugated cardboard (heavy duty), asbestos or metal

5.6.2 Domestic animal ownership

There were no general patterns regarding domestic animal ownership in the three study populations, but there were some differences when individual animals were considered (Table 5-21). In the exposed area, the number of individuals in households owning dogs was lower ($p=0.005$) compared with the semi-exposed and control areas. The proportion of individuals with cats was lower in the control area than the other two study areas ($p<0.01$).

Having chickens demonstrated a highly significant trend, with least kept in the exposed area and most kept in the control area ($p_T<0.001$). There were more cows and sheep in the semi-exposed area, though this was only significant for cows ($p<0.001$). Fewest sheep were kept in the exposed area ($p<0.001$). The control area had more donkeys and horses than the exposed and semi-exposed areas ($p<0.001$). There were no significant differences in ownership of pigs.

Table 5-21 Households with domestic animals owned or cared for

Variable	Exposed (N=374)		Semi-exposed (N=296)		Control (N=310)	
	n	(%)	n	(%)	n	(%)
Dog	314	(84)	273	(92)	274	(88)
Cat	196	(52)	176	(59)	123	(40)
Domestic fowl	230	(61)	256	(86)	287	(93)
Cow	185	(49)	201	(68)	157	(51)
Pig	246	(66)	186	(63)	199	(64)
Sheep	226	(60)	220	(74)	217	(70)
Donkey	113	(30)	81	(27)	227	(73)
Horse	84	(22)	27	(9)	142	(46)

5.6.3 Household possessions

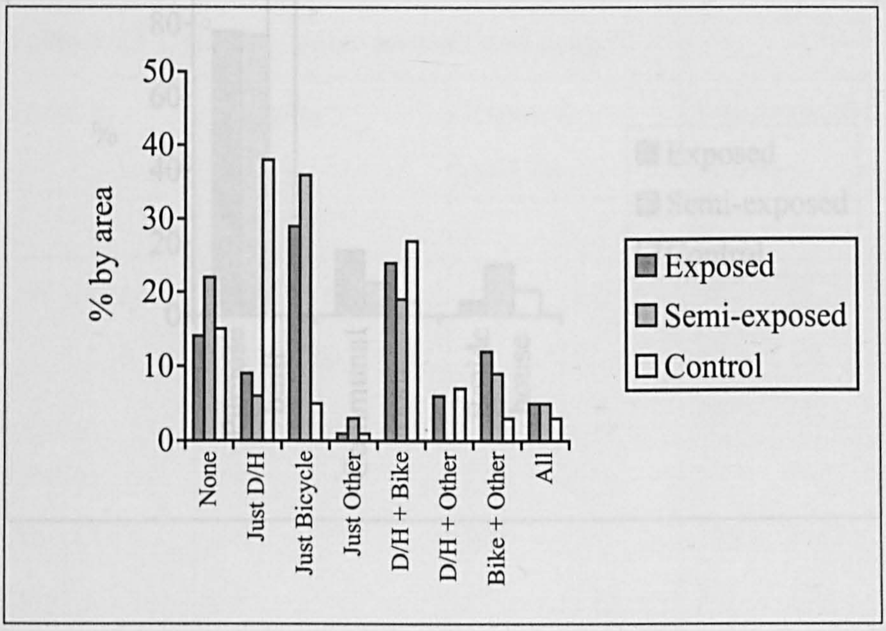
Individuals had greatest access to radios, televisions, stereos or video players in the exposed group, and least in the semi-exposed group (Table 5-22). Most individuals had access to at least a radio (80-99%). The proportion with televisions was lower (54-82%). Cookers were present in the homes of over 75% of individuals, with the highest proportion in the exposed group ($p<0.001$). The proportion of individuals with a refrigerator in their home was under 25%. Refrigerators were more common in the control than either the exposed or the semi-exposed groups ($p<0.001$).

Table 5-22 Household possessions

Variable	Exposed (N=374)		Semi-exposed (N=296)		Control (N=310)	
	n	(%)	n	(%)	n	(%)
Radio	371	(99)	238	(80)	304	(98)
TV	308	(82)	159	(54)	237	(76)
Stereo	76	(20)	35	(12)	46	(15)
Video player	41	(11)	1	(<1)	7	(2)
Cooker	357	(95)	241	(81)	242	(78)
Fridge	47	(13)	44	(15)	71	(23)
Bicycle	262	(70)	205	(69)	121	(39)
Motorbike	3	(1)	8	(3)	0	(0)
Car	38	(10)	23	(8)	10	(3)
Truck	51	(14)	26	(9)	31	(10)
Tractor	27	(7)	8	(3)	10	(3)

One of the most striking differences between the study populations was in the possible modes of transport available to households (Figure 5-3). The main difference was that in the control group, more individuals lived in households with horses or donkeys as the only means of transport (p<0.001) and less had bicycles (p<0.001) than in either the exposed or the semi-exposed areas.

Figure 5-3 Modes of transport[17]

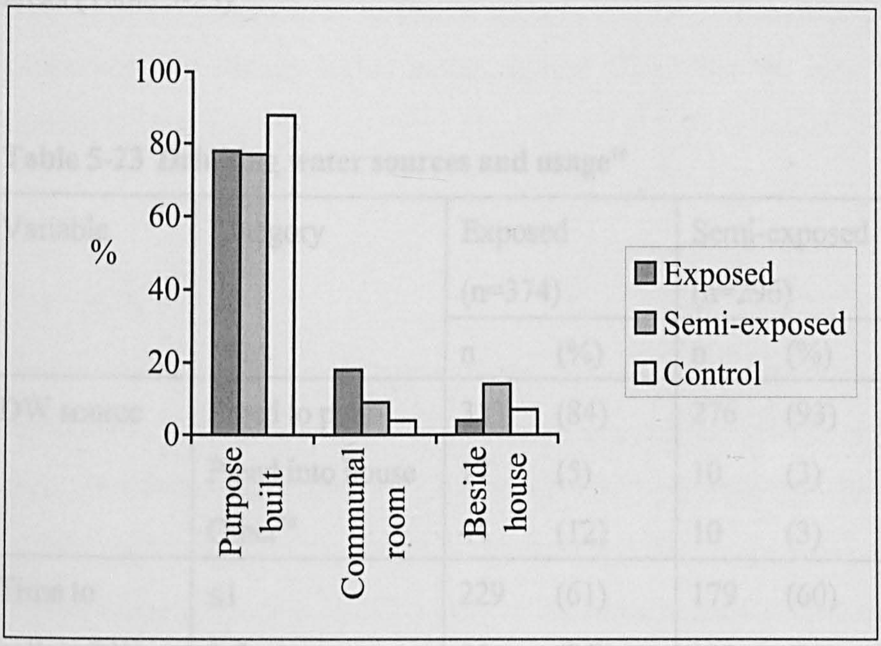


5.7 HOUSEHOLD HYGIENE

There was no significant difference in the distance that individuals in the exposed and semi-exposed populations lived from the irrigation channels in the area. There was a trend in the proportion of household yards which were swept at least 5 times per week, from the control (183/310, 59%) to the semi-exposed (191/296, 65%) to the exposed population (280/374, 78%) ($p_T<0.001$). More individuals in the control area lived in homes with a purpose built room for cooking (Figure 5-4), with walls and roof of the same materials as the rest of the house (p<0.001). There was a decreasing trend, from the exposed to the semi-exposed to the control population, in the proportion of individuals in the homes where a communal room or bedroom was used for cooking ($p_T<0.001$).

[17] D/H=donkey and/or horse, Other includes motorbike, car, tractor or truck

Figure 5-4 Location of the kitchen[18]



State Census results indicated that 70% of households in the State received piped drinking water (INEGI, 1990). Census results for the municipalities within the study areas showed higher percentages of coverage, with 84-92% in the exposed, 90-93% in the semi-exposed and 90% in the control areas.

Actual results from the study population indicated similarly high percentages, with coverage of drinking water pipes to the property at 89% in the exposed, 96% in the semi-exposed and 89% in the control area (Table 5-23). The time required to go and collect drinking water was similar in the three study areas, although there were slightly more individuals in households in the exposed, where collection took over 6 minutes because the drinking water was not piped to the property (Table 5-23). Although the drinking water system reached a similar proportion of individuals in the three areas, actual supply was less reliable in the control

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[18] Purpose built = room just for cooking built of same materials as rest of house. Communal room = lounge, dining room or bedroom. Beside house = outside or lean-to usually constructed of natural materials

area. As a result, significantly higher numbers stored drinking water in the control than the exposed and semi-exposed populations. The proportion boiling the drinking water, whether stored or direct from the tap was lower in the exposed than the semi-exposed and control areas (Table 5-23).

Table 5-23 Drinking water sources and usage[19]

Variable	Category	Exposed (n=374)		Semi-exposed (n=296)		Control (n=310)	
		n	(%)	n	(%)	n	(%)
DW source	Piped to patio	313	(84)	276	(93)	248	(80)
	Piped into house	17	(5)	10	(3)	29	(9)
	Other[20]	44	(12)	10	(3)	33	(11)
Time to collect DW (min)	≤1	229	(61)	179	(60)	184	(59)
	2-5	95	(25)	112	(38)	99	(32)
	≥6	50	(13)	5	(2)	27	(9)
Storage of DW	Do not store	209	(56)	184	(62)	73	(24)
	Closed container	115	(31)	105	(35)	191	(62)
	Open container	50	(13)	7	(2)	46	(15)
Boil DW before drinking		57	(15)	85	(29)	86	(28)
Boil DW for less than 5 min[21]		3	(5)	14	(16)	14	(16)

The proportion of individuals with no access to a toilet of any description was greater in the exposed and semi-exposed populations than in the control population (p=0.004) (Table 5-24). Among those with access to a toilet facility of some description, the exposed and semi-exposed populations were significantly more likely to have their toilet facility close to the house than the control population (p=0.005). The proportion of individuals aged at least 12 years old that always used the toilet facility was similar in the three study areas (33-42%),

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[19] DW=drinking water

[20] Other drinking water sources were river, well, spring, or lake

despite differences in the distance of the toilet from the house. The proportion fell to 29-36% among 4-12 year olds and, not surprisingly, to only 10-13% in 1-3 year olds. If the family was considered as a whole, then the proportions of individuals in households where there was open defecation near to the house was similar in the three populations, though the proportion was slightly higher in the exposed (57%) than the semi-exposed (53%) and the control (52%) ($p=0.04$).

5.8 DIET

Red meat and chicken were eaten at least once a week by more individuals in the exposed population than the semi-exposed or control populations ($p<0.001$) (Table 5-25). Eggs and fruit were eaten more often (3-7 times per week) by more individuals in the exposed and control populations than in the semi-exposed ($p<0.001$ and $p=0.043$ respectively). There was an increasing proportion of individuals eating beans at least 3 times per week, from the semi-exposed (77%) to the exposed (86%) to the control (96%) population ($p_T<0.001$). In contrast vegetables were eaten a minimum of 3 times per week by more individuals in the semi-exposed than the control ($p=0.008$) and by more in the control than the exposed ($p<0.001$). The percentage of individuals eating pasta soup increased significantly from the control to the semi-exposed to the exposed population ($p_T<0.001$). In all three study areas, 98-100% of individuals ate tortillas at least 5 times per week.

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[21] Denominator = those who boiled DW before drinking

Table 5-24 Sanitation facilities and usage

Variable	Category	Exposed		Semi-exposed		Control	
		n/N	(%)	n/N	(%)	n/N	(%)
Toilet	Flush toilet	113/374	(30)	41/296	(14)	20/310	(6)
	Septic tank	9/374	(2)	21/296	(7)	28/310	(9)
	Pit latrine	32/374	(9)	51/296	(17)	106/310	(34)
	None	220/374	(59)	183/296	(62)	156/310	(50)
Distance of toilet from house[22]	Inside	19/154	(12)	20/113	(18)	17/154	(11)
	1-4m	39/154	(25)	33/113	(29)	25/154	(16)
	5-9m	31/154	(20)	19/113	(17)	24/154	(16)
	≥10m	65/154	(42)	41/113	(36)	88/154	(57)
Defecation by ≥12 yr olds In HH	Always in toilet	138/374	(37)	99/296	(33)	131/310	(42)
	Open+far from house	95/374	(25)	113/296	(38)	133/310	(43)
	Open+near to house	141/374	(38)	84/296	(28)	46/310	(15)
Defecation by 4-12 yr olds in HH[23]	Always in toilet	100/334	(30)	68/238	(29)	91/253	(36)
	Open+far from house	82/334	(25)	80/238	(34)	72/253	(28)
	Open+near to house	152/334	(46)	90/238	(38)	90/253	(36)
Defecation by ≤3 yr olds in HH[24]	Always in toilet	20/186	(11)	14/134	(10)	16/126	(13)
	Open+far from house	22/186	(12)	7/134	(5)	0/126	(0)
	Open+near to house	144/186	(77)	113/134	(84)	110/126	(87)
Defecation by household in general	Always in toilet	97/374	(26)	67/296	(23)	87/310	(28)
	Open+far from house	62/374	(17)	72/296	(24)	62/310	(20)
	Open+near to house	215/374	(57)	157/296	(53)	161/310	(52)

[22] Only includes those individuals in households with a toilet

[23] Only includes those individuals in households where at least one household member is 4-12 yrs old

[24] Only includes those individuals in households where at least one household member is ≤3 yrs old

Table 5-25 General weekly diet[25]

Food	Frequency (0-7/wk)	Exposed (n=374)		Semi-exposed (n=296)		Control (n=310)	
Red meat	0	48	(13)	80	(27)	70	(23)
	1-2	278	(74)	181	(61)	190	(61)
	3-7	48	(13)	35	(12)	50	(16)
Chicken	0	41	(11)	64	(22)	81	(26)
	1-2	278	(74)	207	(70)	193	(62)
	3-7	55	(15)	25	(8)	36	(12)
Eggs	0	36	(10)	28	(9)	32	(10)
	1-2	141	(38)	133	(45)	109	(35)
	3-7	197	(53)	135	(46)	169	(55)
Beans	1-2	49	(13)	70	(24)	12	(4)
	3-7	325	(87)	226	(76)	298	(96)
Vegetables	0	44	(12)	16	(5)	27	(9)
	1-2	191	(51)	93	(31)	120	(39)
	3-7	139	(37)	187	(63)	163	(53)
Fruit	0	30	(8)	29	(10)	46	(15)
	1-2	154	(41)	164	(55)	87	(28)
	3-7	190	(51)	103	(35)	177	(57)
Pasta broth[26]	0	21	(6)	30	(10)	61	(20)
	1-2	162	(44)	152	(51)	110	(35)
	3-7	184	(50)	114	(39)	139	(45)

[25] Dietary questions were directed to the mother and information requested on household consumption over last 7 days

[26] Data missing for one household in exposed group, N=367.

Due to the very different growing conditions, not surprisingly there were significant differences in the types of produce consumed from local fields in the rain-fed control area compared to the other two wastewater irrigated study areas ($p<0.001$) (Table 5-26). Within the wastewater irrigated areas, there were also differences in crop consumption between the exposed and semi-exposed populations. The semi-exposed population ate significantly more vegetables and wild greens from local fields than the exposed population ($p<0.001$).

Table 5-26 Local crop consumption

Variable	Exposed (n=374)	Semi-exposed (n=296)	Control (n=310)
Any crops from local fields	239 (64)	262 (89)	153 (49)
Salad crops grown locally	8 (2)	0 (0)	5 (2)
Vegetables grown locally	239 (64)	257 (87)	153 (49)
Greens grown locally	223 (60)	253 (85)	95 (31)
Herbs grown locally	2 (1)	9 (3)	5 (2)

5.9 LOCAL BELIEFS CONCERNING PARASITE INFECTIONS

The reasons behind a mother thinking her child had "worms" were numerous, but they can be divided into five types of response,

a) Confirmed evidence - for example, worms had been seen in the child's stools or the child had been diagnosed as having worms by the doctor

b) Poor hygiene-related behaviours that the mother felt would result in the child having worms

c) Symptoms that the mother felt were related to having a worm infestation

d) Eating or drinking behaviour by the child, that the mother felt were a sign that the child had worms

e) Do not know why believe child has worms

A more detailed account of the response given by each mother questioned (n=448) can be seen in Table 5-27. Comparison of the responses of mothers in the three study areas (Figure 5-5), indicated that the only difference was in the proportion of mothers reporting stomach problems as their reason for believing their child had worms, which was highest in the exposed group (20%), then 14% in the semi-exposed group and 13% in the control (p_T=0.07).

Figure 5-5 Mother's reasons for believing child has worms

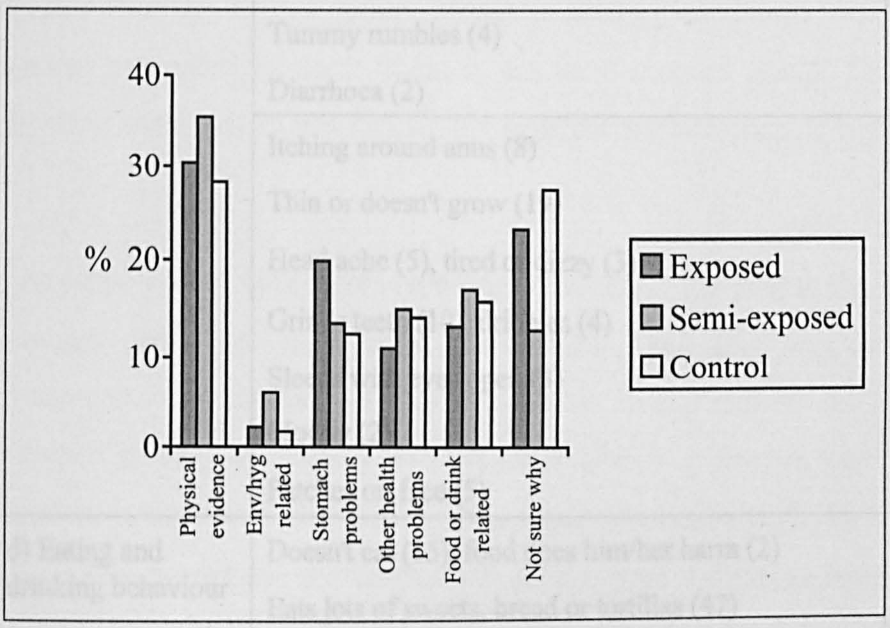


Table 5-27 Mothers' perceptions regarding worm infestation

Why do you think your child has worms (n)?		Response type frequency (N=448)	
		N	(%)
a) Physical evidence	Expelled or vomited worms (126) Has or has not received treatment (10) Doctor said child or siblings had worms (5)	141	(31)
b) Environmental or hygiene-related	Type of water (4) Lack of hygiene (2), don't wash hands (1) Eats worms (1), eats or ate earth (3) Because of the earth (4)	15	(3)
c) Symptoms	Stomach ache (59) Inflated or enlarged tummy (6) Tummy rumbles (4) Diarrhoea (2)	71	(16)
	Itching around anus (8) Thin or doesn't grow (19) Head ache (5), tired or dizzy (3) Grinds teeth (10), dribbles (4) Sleeps with eyes open (3) Moody (2) Patches on face (5)	59	(13)
d) Eating and drinking behaviour	Doesn't eat (15), food does him/her harm (2) Eats lots of sweets, bread or tortillas (47) Drinks a lot of atole[27] (1) Doesn't drink milk or drinks a lot of milk (3)	68	(15)
f) Just think so	All children have worms (1) Don't know or just think so - no reason (93)	94	(21)

[27] Atole is a hot drink made from sugar, corn flour and milk

5.10 SUMMARY

There was a slightly lower proportion of young adults in the control group. Households in the control group often mentioned individuals as being absent because they worked in Mexico City during the week and came home only at weekends. Literacy levels and schooling were very similar in the three groups, reflecting a nation-wide drive to encourage children to finish primary school and to promote adult literacy. Only in the control group were adult literacy levels slightly lower. School levels were similar, except for the control group, where the proportion of women never to attend school was higher.

There were slightly more recently arrived households in the exposed and semi-exposed groups than the control group. However housing conditions were similar, except for a greater proportion of earth floors in the semi-exposed group. The coverage of a piped drinking water system was similar, but supply was more irregular in the control group.

The proportion of households reporting open defecation near to the house was similar. The exposed group ate more meat and chicken, and less vegetables. There was more consumption of greens and vegetables from local fields in the semi-exposed than the exposed group. This reflects both the prohibition of widescale vegetable production in the exposed group, due to the quality of the wastewater and a slight feeling in the semi-exposed group that the sedimented wastewater did not pose a threat to health as did the untreated wastewater. Fewer crops were eaten from local fields or the family plot in the control group, probably reflecting the lack of vegetables or wild crops growing in this more arid rain-fed area, as compared to a wastewater irrigated area.

The domestic animals and possessions owned by households perhaps most clearly reflected the differences between the three study groups, with fewer households keeping chickens in the exposed group, and more households owning horses or donkeys in the control group, whereas in the exposed and semi-exposed more had bicycles. This was possibly an indication of a general trend towards urbanisation and the mechanisation of agriculture in the exposed and semi-exposed groups, rather than a statement relating to the availability of grazing land. Generally there was also greater wealth (in terms of possessions) in the exposed group and least in the control group, with the exception of refrigerators which were more common in the control group.

The differences between the three study groups that were identified in this chapter and that have been summarised above will be adjusted for in the final multivariate analyses in Chapter 8.

CHAPTER 6. EXPOSURE TO *ASCARIS* THROUGH WASTEWATER REUSE IN AGRICULTURE

6.1 INTRODUCTION

The aim of this chapter is to identify and describe the principal sources of wastewater contact in the exposed and semi-exposed groups. There was no wastewater contact reported by individuals in the control group. The data described in this chapter come from two sources, from observation studies of farmers (not necessarily included in the study population) carried out in the exposed and semi-exposed areas and from structured in-depth interviews conducted with all individuals included in the study and reporting to have had contact with wastewater during the 9-10 month re-infection period. Details of the methodology of the wastewater contact interviews and the observation studies can be found in Chapter 4, sections 4.3.1 and 4.3.2 respectively. In this chapter all tests of association refer to the Pearson chi-squared test unless otherwise specified. The non-parametric Wilcoxon rank-sum unpaired test (or Mann-Whitney test) was used to compare the frequency distributions of wastewater contact variables between groups and to determine whether the distributions were significantly different. When more than two groups were compared the Kruskal-Wallis equality of populations rank test was used.

In both the exposed and semi-exposed areas, farmers cultivate mainly maize, beans, alfalfa and a range of grain crops, all of which are flood irrigated i.e. the irrigation water is allowed to spread across the whole field, touching the stems of the crop, rather than going along previously defined furrows. Irrigation can often take several hours, depending on the size of the field and there can be long periods when the farmer is just watching the water move across the field.

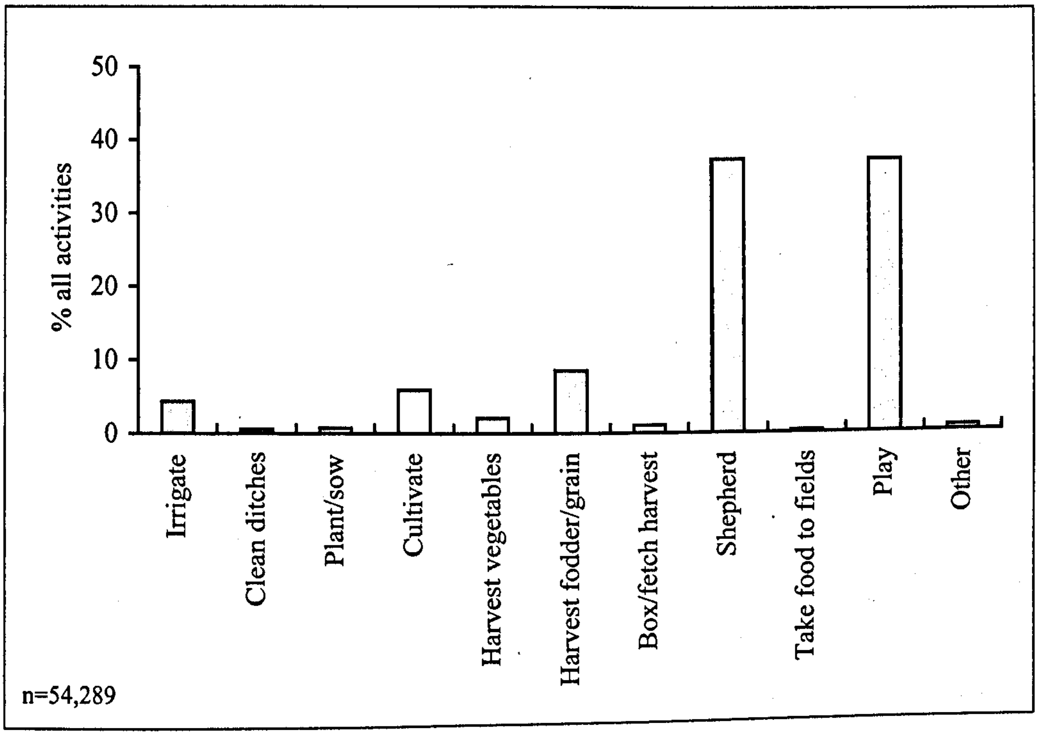
There are also chillies, red and green tomatoes and courgettes grown in the region. Most chilli growing occurred in the exposed area. Tomato and courgette production was small-scale compared with chilli production, consequently observations of irrigation of these crops were limited. Chillies, red and green tomatoes and courgettes are irrigated using furrow irrigation, in which the irrigation water flows along pre-defined furrows to avoid contact with the crop. This method of irrigation was adopted to protect the plant from being swept away and to reduce the risk of the crop coming into contact with the wastewater. Traditionally, chillies are initially sown on small raised beds of soil. The seedlings are transplanted immediately after the field has been irrigated, when the wastewater is still lying on the field. This facilitates planting of the seedlings, though may obviously result in prolonged hand contact with the wastewater for those involved in planting chillies. Unlike irrigation, chilli planting is a labour intensive activity with few moments to rest. Work related to crop production, whether planting, cultivating, irrigating or crop picking (termed 'agricultural work' in this thesis) is done mostly by men, although women often work during the crop-picking season.

Many households in the study groups have a small number of livestock, mainly milking cows, beef cattle or sheep, that provide an additional source of income and food. Livestock are taken to pasture (also termed 'shepherding' in this thesis), which can occur daily. The livestock are herded along the roads, beside irrigation channels and beside other fields to an appropriate field where the crop has recently been removed. The livestock will remain in the field from a couple of hours to the whole day. Whilst in the fields the livestock are watched to ensure they do not stray into neighbouring fields or fall into irrigation ditches. Livestock are taken to pasture by men, women and children, although most shepherding is done by women. Often the younger children in the family will accompany the women to the fields. Children will also take livestock to pasture, either before school or on their return from school (the school day is divided into two shifts, either 7-1pm or 1pm-7pm).

6.2 ACTIVITIES INVOLVING CONTACT WITH WASTEWATER

During the 9-10 month reinfection period, a total of 54,289 activities (events) that could potentially involve wastewater contact were reported during in-depth interviews with the exposed (n=374) and semi-exposed groups (n=296) (Figure 6-1). Tending livestock taken to pasture and playing were the two most common activities reported during the re-infection period. A total of 28,206 activities were reported by the control group (n=310), but none had the potential for wastewater contact because all were carried out within the rain-fed control area.

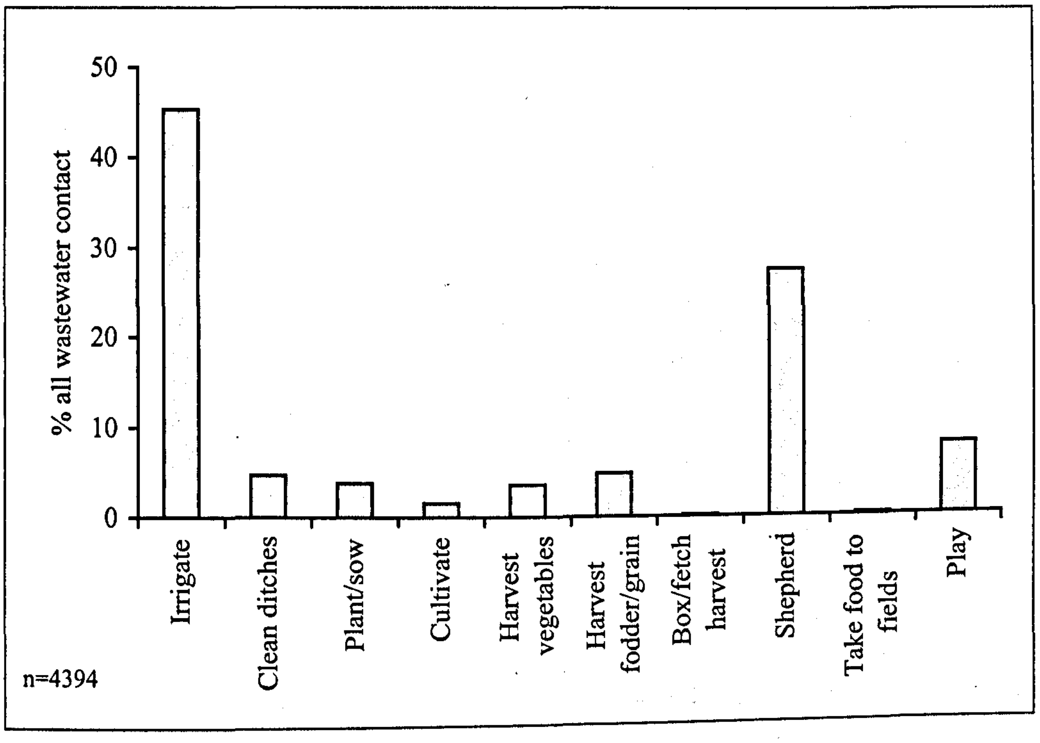
Figure 6-1 Activities reported during 9-10 month re-infection period that potentially involved wastewater contact



6.2.1 Frequency of wastewater contact activities

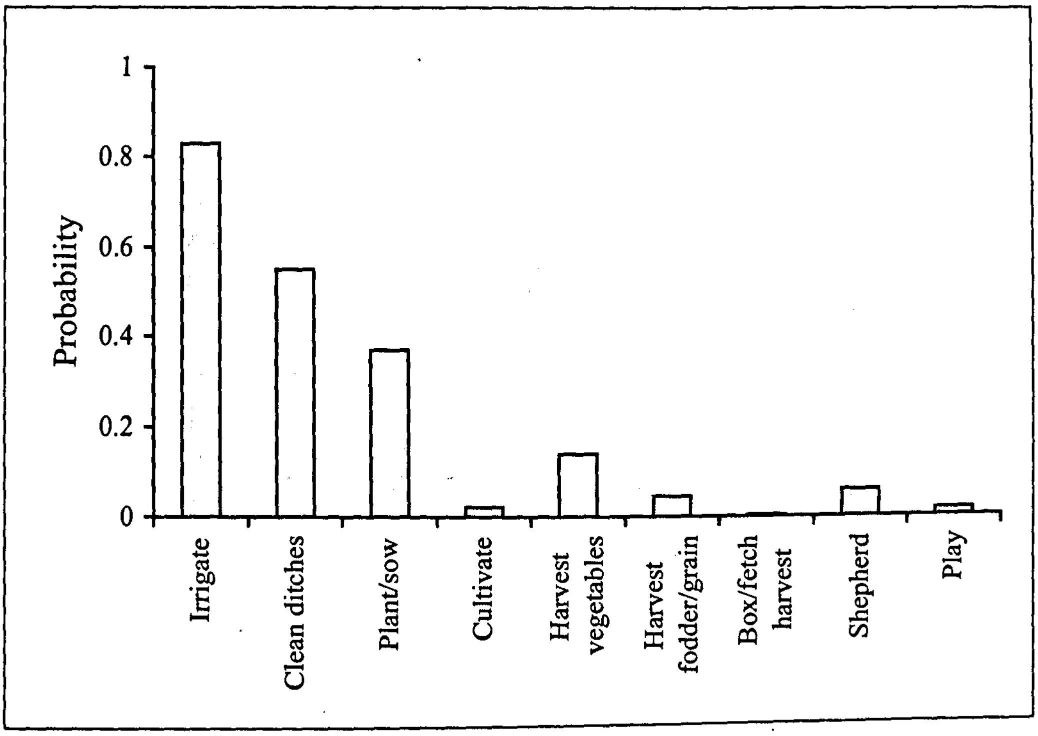
Individuals in the exposed or semi-exposed groups reported 4394 activities which resulted in contact with wastewater (8% of all activities reported by these two groups). No activities involving wastewater contact were reported by the control group. Over 70% of reported wastewater contact in the exposed and semi-exposed groups occurred during crop irrigation (n=1990) or while individuals were tending to livestock that had been taken to pasture (n=1215) (Figure 6-2). Children at play were responsible for 8% of the wastewater contact reported during the reinfection period.

Figure 6-2 Activities involving contact with wastewater



As well as identifying the most common activities which involved wastewater contact, it is also important to ascertain the likelihood of each particular activity involving wastewater contact (Figure 6-3). This will enable high-risk activities to be pin-pointed whether or not they occur frequently. Irrigation and the cleaning of ditches (a preparatory process prior to irrigation) are the two activities most likely to involve contact with wastewater. Then come other activities related to crop production, such as planting, sowing, cultivation and harvest. The chance of wastewater contact occurring while taking livestock to pasture is only 6%, although this activity accounted for 36% of all reported activities and 28% of all reported contacts with wastewater (Figure 6-2). Children's play accounted for 36% of all reported activities and 8% of all activities involving wastewater contact. The proportion of play reported to involve wastewater contact was 1.7% .

Figure 6-3 Probability of wastewater contact occurring during each activity



6.2.2 Influence of crop on wastewater contact activities

It has been shown in section 6.2.1 that, as well as irrigation, there are a number of other agricultural activities that can result in wastewater contact. The work done during the agricultural cycle of each crop is slightly different. Some crops are sown, while other crops are initially grown on raised beds, and then planted out. The frequency of irrigation and the type of irrigation employed are also dependent on the crop grown (Table 6-1). Furrow irrigation is often more labour intensive than flood irrigation. There is more earth moving required to direct the water along the appropriate furrow. If the water is allowed to enter the field too fast then furrows can be destroyed, and at the beginning of the growing season small plants can be swept away. Irrigation of a field can take between 1-7 hours depending on the crop, the stage in the agricultural cycle and the size of the field.

Table 6-1 Agricultural cycle for maize, chilli and alfalfa

	Maize	Chilli	Alfalfa
Growing season	Feb-Aug	Feb-Jul	All year
Type of irrigation	Flood	Furrow	Flood
Date of 1st irrigation	Late Feb	Late Feb	-
Total no. irrigation sessions	6	5	12
No. days between irrigation sessions	30-40	14-21	30
Plant or sow	Sow	Plant	Sow (every 5-6 yr.)

The different activities carried out, depending on the crop grown, are clearly illustrated by comparing two farmers from the study population; farmer A grew maize and farmer B grew chillies (Table 6-2). The production of chillies was much more labour intensive than maize over the course of the year. More irrigation sessions are required, as well as constant weeding and cultivation to ensure a healthy chilli crop.

Table 6-2 Case histories comparing maize and chilli production

Month	Farmer A - maize production	Farmer B - chilli production
January		Irrigate twice
February	Prepare soil and irrigate in preparation for sowing	Irrigate twice
March	Sow	Prepare field, irrigate and transplant seedlings
April	Weed and irrigate	Weed, cultivate and irrigate
May	Irrigate	Weed, cultivate and irrigate
June	Clean ditches and irrigate	Weed, cultivate and irrigate
July	Irrigate	Weed, cultivate and irrigate
August	Irrigate	Cut chillies
September	Cut maize	
October	Cut maize	
November	Collect harvest	Prepare and level soil for sowing
December	Collect harvest	Irrigate and sow

6.2.3 Assessing the amount of wastewater contact

It is important to understand the amount of contact as well as the frequency of contact with wastewater when assessing wastewater contact. Both the activity and the crop grown help to determine the amount of wastewater contact. The amount of wastewater contact was assessed by observation and by structured interview (see Chapter 4, sections 4.3.1 and 4.3.2 for details).

The observation studies were performed between September 1990 and May 1991. 529 male farmers were observed in the fields and nearly 1430 hours of observation were completed with over 65% of farmers being observed for at least 3 hours (mean observation period = 2 hours 40 minutes, standard deviation = 37 min). After the observation period, a summary report was made by the observer (see Appendix C: Structured observation form). Structured interviews were done with all individuals in the study who reported to have had contact with wastewater (n=879). From these interviews detailed reports were obtained of 1519 activities that resulted in wastewater contact (see Appendix B: Wastewater contact interview form).

Farmers were observed, for example, irrigating crops or cleaning the irrigation channels surrounding the field (see Photo 6-1 - Photo 6-7). Over 80% of the observational periods began in the morning between 7am and 10am. Farmers generally cleaned irrigation channels and transplanted seedlings during the daylight hours. However crop irrigation sessions could start at any time day or night, depending on when they were allocated the wastewater.

Photo 6-1 Farmer cleans out an irrigation ditch

A farmer uses a spade to clear out the irrigation ditch in preparation for irrigating chillies

Photo 6-2 Farmer creates a small dam across the irrigation ditch

A farmer uses large stones, clods of grass and an old plastic bag to enable him to re-direct the wastewater over the field of chillies

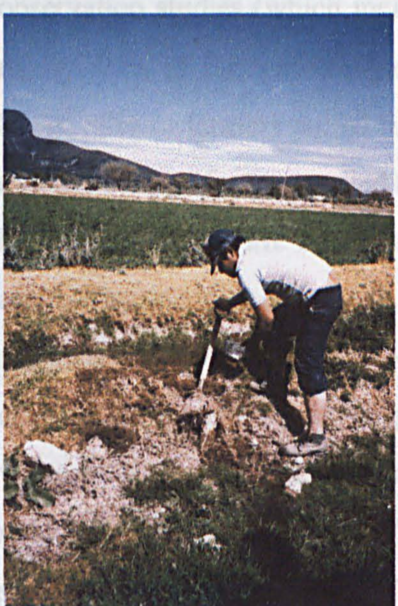
Photo 6-3 Farmer directs the wastewater as it moves down the field

A farmer has now irrigated about half the field. He can be seen moving earth with a spade to direct the water along the furrows between the rows of chillies. A youth with a spade is helping him.

Photo 6-4 Farmer clears debris from an irrigation ditch

A farmer clears debris with a spade from the irrigation ditch to help the wastewater flow

Photo 6-5 Farmer cuts through the bank to allow the wastewater to flow onto the field



A farmer cuts through the bank between the irrigation ditch and the field with a spade to allow the wastewater to flow on to alfalfa

Photo 6-6 Farmer carries pasture to dam an irrigation ditch

A farmer uses a spade to carry a clod of pasture to help dam the irrigation ditch and re-direct the wastewater onto the field of alfalfa

Photo 6-7 Farmer dams an irrigation ditch

A farmer dams the irrigation ditch using stones and clods of pasture, allowing wastewater to flow onto the alfalfa to his left. A previously irrigated field of alfalfa can be seen on the right

Very little purpose-made protective clothing was worn by the farmers, though some measures were taken by the farmer when in contact with the wastewater. For example, in the observation studies (which included crop irrigation, planting chillies and cleaning ditches), nearly 40% of farmers worked barefoot, 27% wore everyday shoes and only 34% used boots. 80% of farmers pulled their trousers up over their knees to stop them getting wet and muddy. 60% of farmers pulled their sleeves above their elbows, to protect their shirt sleeves. No gloves were worn. In Table 6-3, footwear and the extent of wastewater contact during crop irrigation are compared between the observer summary report and the interview reported data. Footwear was similar for observed and reported incidents of wastewater contact. There was a high degree of correlation between the reported and observed mean wastewater contact during crop irrigation (r^2=0.95) but more wastewater contact was reported than observed (p<0.001). The proportion of children was greater in the reported sample than in the observed sample, but exclusion of the children did not reduce these sample than in the observed sample, but exclusion of the children did not reduce these differences; nor did allowing for the crop irrigated. Individuals may have been more likely to remember activities involving greater wastewater contact than activities where wastewater contact was minimal.

Table 6-3 The extent of wastewater contact during irrigation: a comparison of observer summary and interview reported contact

Variable	Category	Observation studies No/Total (%)		Reported during wastewater contact interview No/Total (%)	
Footwear	Barefoot	131/436	(30)	180/654	(28)
	Shoes	130/436	(30)	193/654	(30)
	Boots	175/436	(40)	281/654	(43)
Upper limbs	Hands wet	368/435	(85)	744/768	(97)
	Lower arm wet	225/435	(52)	582/768	(76)
	Upper arm wet	68/435	(16)	*	
Lower limbs	Feet wet	405/435	(93)	735/768	(96)
	Shins wet	349/435	(80)	674/768	(88)
	Thighs wet	*		170/768	(22)
Whole body wet		0/435	(0)	63/768	(8)

* In the interview, lower and upper arm were not considered separately. In the observation studies, shin and thigh were not recorded separately.

Eating, drinking and smoking were recorded during the observation studies. 42% (223/529) of those observed ate at least once during the observation period, 12% (65/529) drank and 3% smoked. There was no significant relationship between eating, drinking or smoking and the activity performed or the duration of the activity.

Two composite variables have been created from the observational data set, by summing the frequency of related variables. This ensured wastewater contact frequencies were high enough to enable comparison of the amount of contact with wastewater for a variety of activities and crops.

"*Foot contact with wastewater*" is defined as the number of contacts per hour of one foot or both feet with wastewater (whether or not footwear was used). It is a composite of two variables :- foot in wastewater, leg in wastewater (shin and thigh were not considered separately). Although transmission of *Ascaris* infection is not possible by foot contact with wastewater, it was used as a proxy measure of general exposure during a particular activity.

"*Hand contact with wastewater*" is defined as the number of contacts per hour by one or both hands with wastewater as a direct result of the agricultural activity. It is a composite of the following 6 variables :- puts hand in wastewater, puts hand on wet soil, puts hand on wet plant, puts hand on wet clod of earth, puts hand on foot or footwear, puts arm in wastewater and washing hands in wastewater.

All the variables combined, except for washing hands, were the result of structured observations of the farmers, in which every 60 seconds, all activities that occurred during a 5-second period were recorded. The "washing hands" variable was recorded continuously during the observation period (see Chapter 4, section 4.3.2 for details).

The frequency distribution of the variable "*foot contact with wastewater*" varied according to the crop and the activity (Kruskal-Wallis equality of populations rank test, $p_{KW}=0.042$). The distribution was slightly skewed for maize and grain crop irrigation (Figure 6-4) and was strongly skewed for chilli planting ($p<0.001$) (Figure 6-5). Foot contact with wastewater was greater for maize irrigation (median=27.7 contacts per hour) than for alfalfa irrigation (median=21.7 contacts per hour, Wilcoxon test, $p_W=0.029$) (Figure 6-4). Foot contact with wastewater was also greater for courgette irrigation (median=39 contacts per hour) than for chilli irrigation (median=27.5 contacts per hour, $p_W=0.023$) (Figure 6-5). Foot contact was significantly less frequent during chilli planting than during chilli irrigation ($p_W<0.001$). Overall, more than 95% of farmers had some foot contact with wastewater during crop irrigation irrespective of the crop, compared to only 77% for those planting chillies (p=0.004).

Figure 6-4 Foot contact with wastewater during irrigation of grain and fodder crops

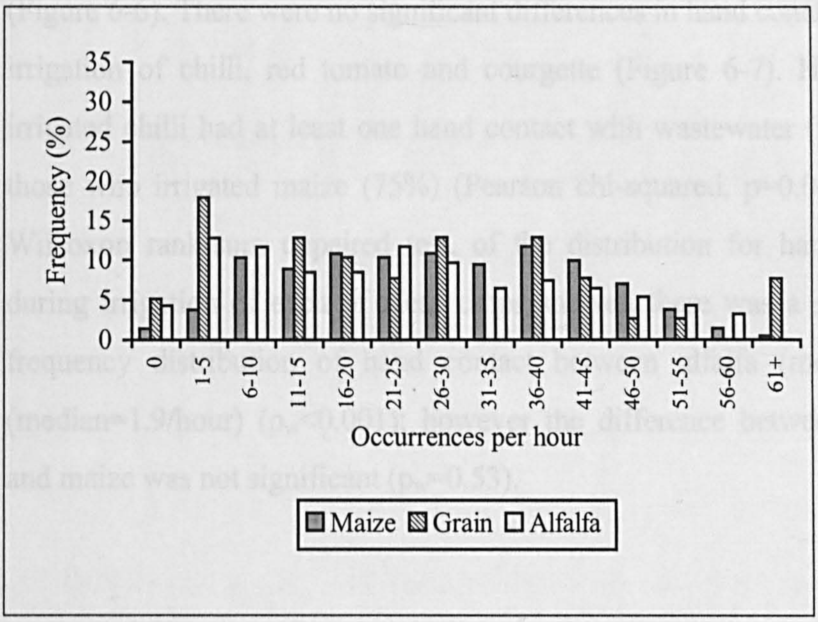
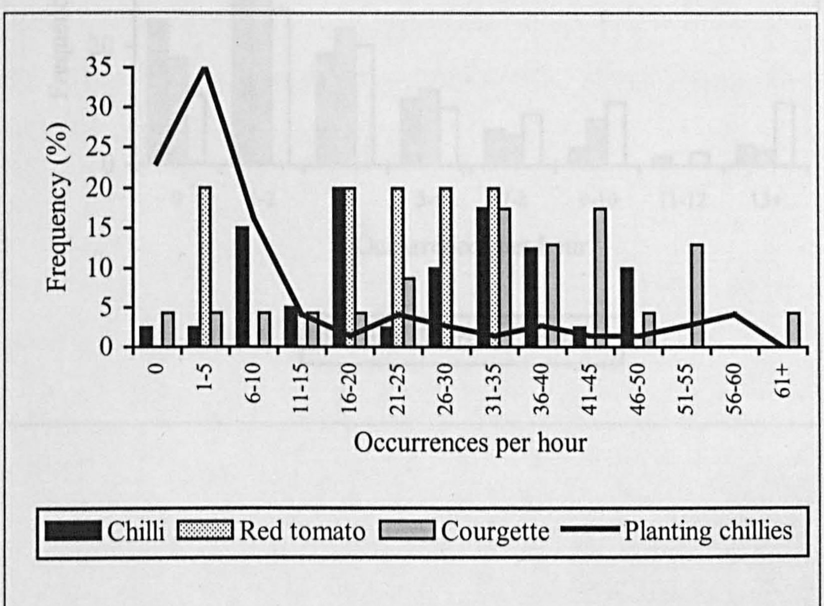


Figure 6-5 Foot contact with wastewater during irrigation and planting of vegetables



The frequency distribution of the variable "*hand contact with wastewater*" during irrigation of maize, grain crops, alfalfa, chilli, red tomato and courgette (Figure 6-6 and Figure 6-7) was positively skewed. Over 89% of farmers who irrigated alfalfa had at least one hand contact with wastewater compared with only 76% of those who irrigated maize (p=0.008) (Figure 6-6). There were no significant differences in hand contact with wastewater between irrigation of chilli, red tomato and courgette (Figure 6-7). However more farmers who irrigated chilli had at least one hand contact with wastewater (90%), when compared with those who irrigated maize (75%) (Pearson chi-squared, p=0.045). Comparison, using the Wilcoxon rank-sum unpaired test, of the distribution for hand contact with wastewater during irrigation of each of these crops showed there was a significant difference in the frequency distribution of hand contact between alfalfa (median=3.3/hour) and maize (median=1.9/hour) (p_w<0.001); however the difference between chilli (median=2.0/hour) and maize was not significant (p_w=0.53).

Figure 6-6 Hand contact with wastewater during irrigation of grain and fodder crops

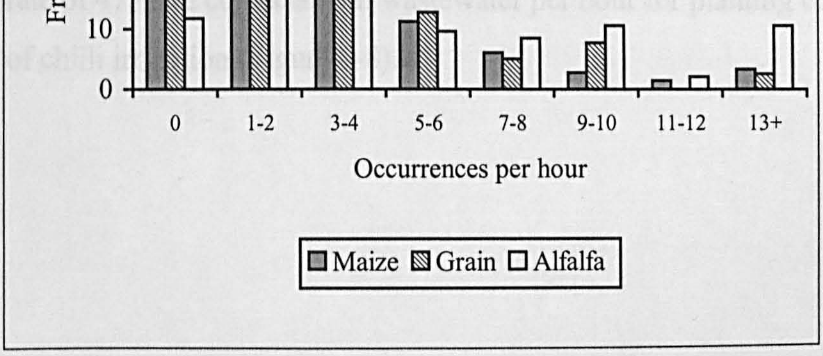
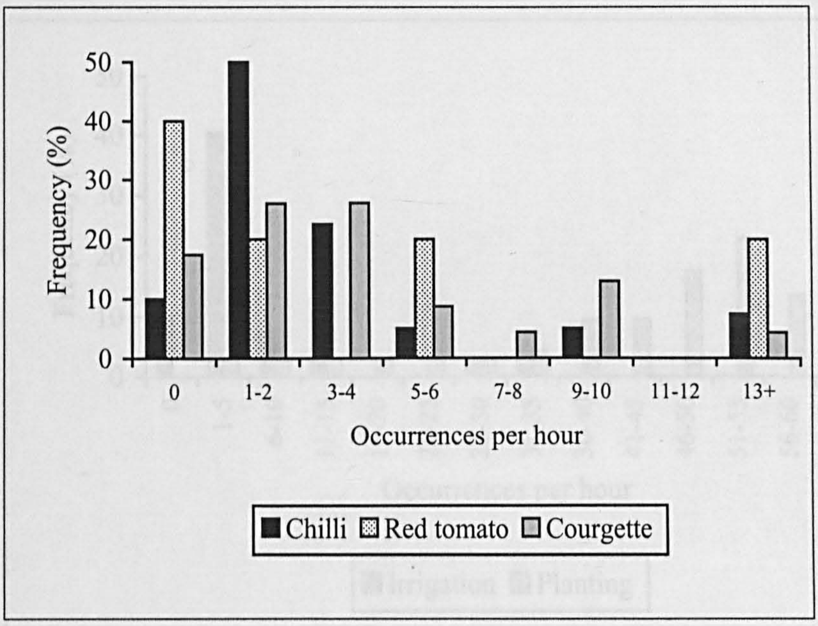
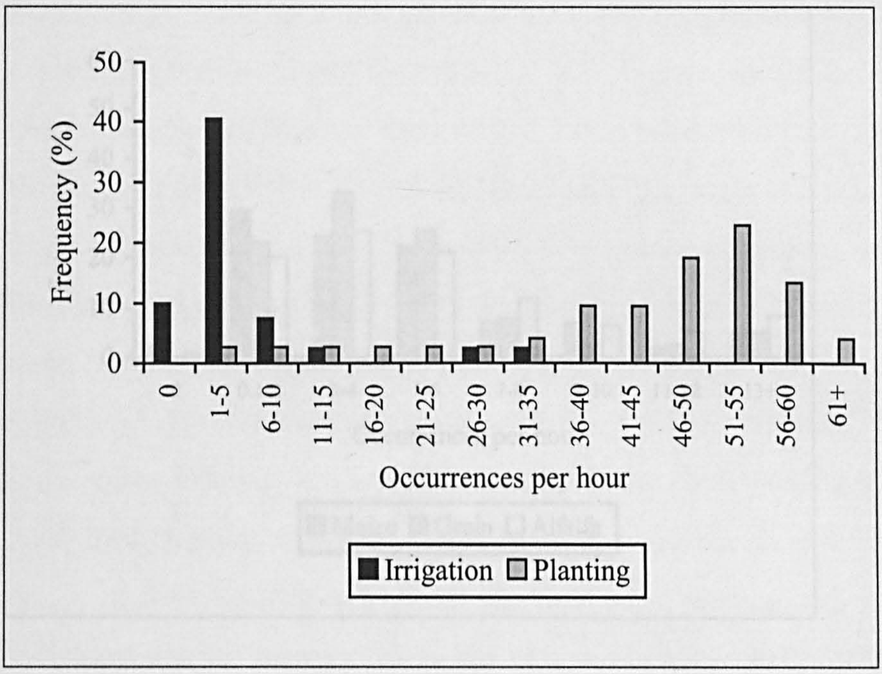


Figure 6-7 Hand contact with wastewater during irrigation of vegetables



All farmers had at least one hand contact with wastewater during the planting of chillies compared with 90% during chilli irrigation (Pearson chi-squared p=0.006). The frequency distribution of hand contact with wastewater for chilli planting and irrigation was significantly different (Wilcoxon rank-sum unpaired test, $p_w<0.001$); the distribution was negatively skewed for planting (coefficient of skew = -1.2) and positively skewed for irrigation (coefficient of skew = 3.5) (Figure 6-8). The majority of farmers planting chillies had significantly more hand contact with wastewater than during irrigation, with a median rate of 47 hand contacts with wastewater per hour for planting compared with just 2 per hour of chilli irrigation (Figure 6-8).

Figure 6-8 Hand contact with wastewater during chilli irrigation and planting



The frequency distribution of the behaviour "hand touches mouth or lips", recorded continuously during the observation period, is displayed in Figure 6-9 and Figure 6-10. There were no significant differences in the frequency of the variable "hand to mouth" during irrigation of the six crops studied. There were however significant differences between chilli irrigation and planting. Over 95% of individuals touched their mouth with their hand at least once during crop irrigation, compared with only 68% during chilli planting (Pearson chi-squared $p=0.006$). The median hourly rate for touching their mouth was 4.0 for irrigation and 0.9 for planting (Wilcoxon rank-sum unpaired test $p<0.001$).

Figure 6-9 "Hand touches mouth" during irrigation of grain and fodder crops

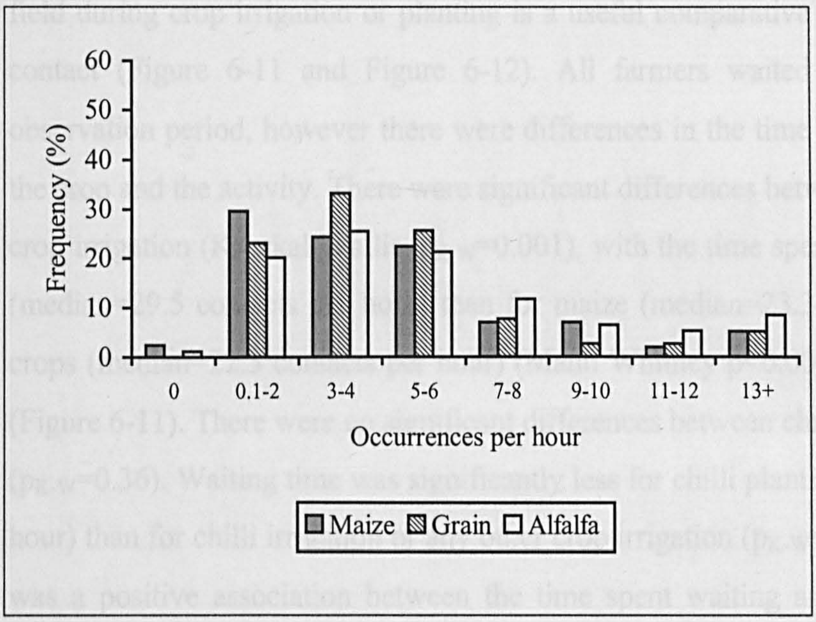
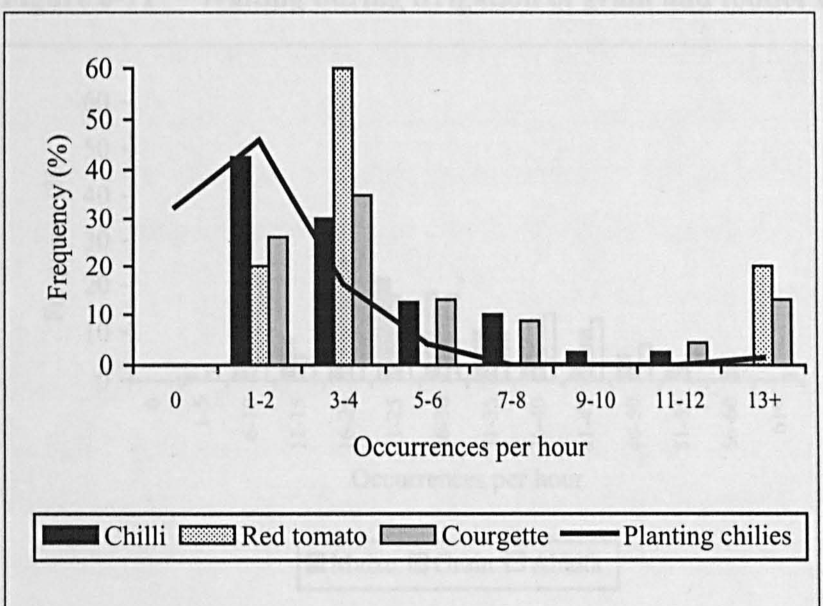


Figure 6-10 "Hand touches mouth" during irrigation of vegetables



The time spent either resting for a moment or waiting for the wastewater to move across the field during crop irrigation or planting is a useful comparative measure for hand to mouth contact (Figure 6-11 and Figure 6-12). All farmers waited at some point during the observation period, however there were differences in the time spent waiting depending on the crop and the activity. There were significant differences between alfalfa, maize and grain crop irrigation (Kruskal-Wallis, $p_{K-W}=0.001$), with the time spent waiting greater for alfalfa (median=29.5 contacts per hour) than for maize (median=23.3 contacts per hour) or grain crops (median=22.3 contacts per hour) (Mann Whitney $p<0.001$ and $p=0.027$ respectively) (Figure 6-11). There were no significant differences between chilli, red tomato and courgette ($p_{K-W}=0.36$). Waiting time was significantly less for chilli planting (median=7.7 contacts per hour) than for chilli irrigation or any other crop irrigation ($p_{K-W}<0.001$) (Figure 6-12). There was a positive association between the time spent waiting and the number of times the individual touched their mouth or lips with their hand ($p<0.001$). The longer an individual had to spend waiting, the greater the hourly rate of "hands touches mouth".

Figure 6-11 Waiting during irrigation of grain and fodder crops

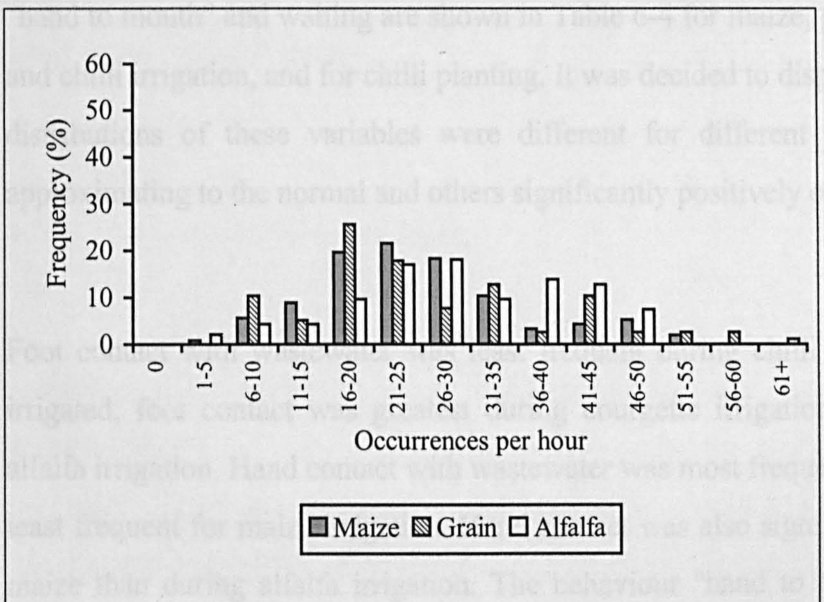
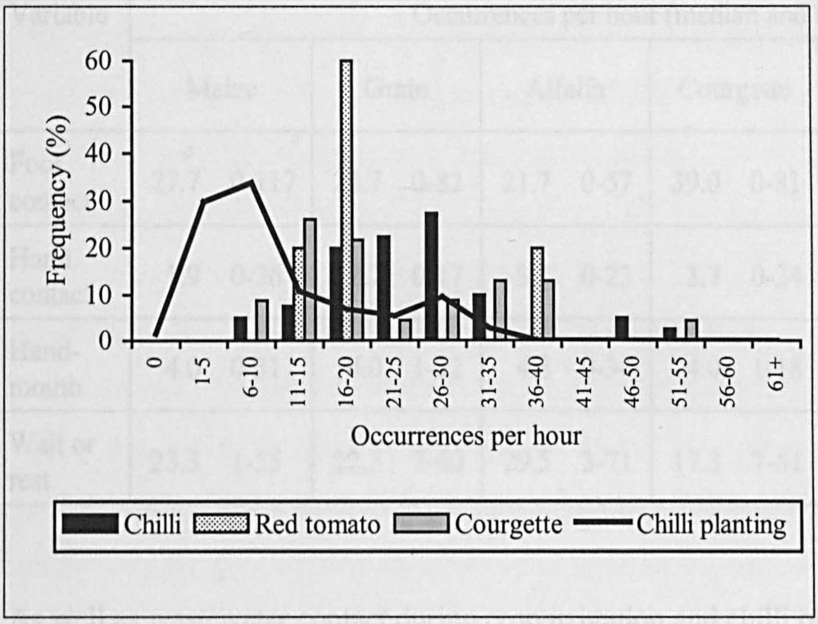


Figure 6-12 Waiting during planting and irrigation of vegetables



To summarise the differences that were found in wastewater contact during the observation studies, depending on the crop and the activity performed, the median, minimum and maximum hourly rate for "*foot contact with wastewater*", "*hand contact with wastewater*", "hand to mouth" and waiting are shown in Table 6-4 for maize, grain crop, alfalfa, courgette and chilli irrigation, and for chilli planting. It was decided to display the median, because the distributions of these variables were different for different crops and activities, some approximating to the normal and others significantly positively or negatively skewed.

Foot contact with wastewater was least frequent during chilli planting. Among the crops irrigated, foot contact was greatest during courgette irrigation and least frequent during alfalfa irrigation. Hand contact with wastewater was most frequent during chilli planting and least frequent for maize irrigation. Hand contact was also significantly less frequent during maize than during alfalfa irrigation. The behaviour "hand to mouth" was more frequent during irrigation than planting. There were no differences in this behaviour among the crops irrigated. The time spent waiting or resting was least frequent during chilli planting. Waiting time was greater during alfalfa than during maize irrigation.

Table 6-4 Summary of wastewater contact during irrigation and planting

Variable	Occurrences per hour (median and range)											
	Maize		Grain		Alfalfa		Courgette		Chilli		Chilli planting	
Foot contact	27.7	0-117	24.7	0-82	21.7	0-57	39.0	0-81	27.5	0-49	2.5	0-57
Hand contact	1.9	0-26	2.7	0-17	3.3	0-23	3.7	0-34	2.0	0-33	47.3	1-109
Hand-mouth	4.0	0-31	4.0	1-22	4.8	0-34	4.0	1-18	2.8	1-12	0.8	0-20
Wait or rest	23.3	1-55	22.3	7-60	29.5	3-71	17.3	7-51	23.7	7-51	7.7	0-33

As well as wastewater contact during crop irrigation and chilli planting, there were also other agricultural activities where wastewater contact was reported (Figure 6-2). When crops were sprayed, the farmer often used water from the irrigation ditch to prepare the pesticide concentrate. Courgette flowers and chillies were cut soon after crop irrigation, and the pickers had to walk through a wet and muddy field, and often picked flowers or chillies when they were wet. After picking, courgettes and cucumbers were sometimes washed by hand in the irrigation ditch.

6.2.4 Non-agricultural activities

Several non-agricultural activities resulted in wastewater contact (Figure 6-2), but the probability of wastewater contact during these activities was lower than for crop irrigation and such occurrences of wastewater contact were sporadic and unplanned. As a result the descriptions here relate to reported accounts of wastewater contact. However there was a good correlation between the reported and observed data for agricultural activities.

The main non-agricultural activities related to wastewater contact were tending to livestock in the fields and playing. As well as assessing the amount of wastewater contact occurring during non-agricultural activities, it is important to understand how this contact occurs, when there would appear to be no obvious need for wastewater contact. Descriptions of wastewater contact during tending to animals (Table 6-5) and play (Table 6-6) reveal a great deal about why the wastewater contact is occurring. The reasons reported for having had wastewater contact while tending animals, can be divided into three categories; activities resulting in accidental wastewater contact (e.g. a person slipped while stepping over an irrigation ditch), activities that were necessary to tend to the animals (e.g. a person had to a push cow out of an irrigation ditch) and thirdly activities designed to while away the time whilst animals were feeding and that intentionally resulted in wastewater contact (e.g. playing with wastewater). Similarly, activities during play that resulted in wastewater contact can be divided into accidental and intentional wastewater contact. For example, a child could have slipped and fallen while crossing an irrigation ditch as compared to a child making mud pies with water from the irrigation ditch. The influence of the age and gender of the individual on whether there is wastewater contact while tending animals or during play is described in section 6.3.

Table 6-5 Descriptions of wastewater contact during shepherding activities

Amount of wastewater contact	Responses
All body	Getting animals out of irrigation ditch Fell in, while jumping across irrigation ditch Had wash in irrigation ditch
Thighs and arms / hands	Getting animal out of irrigation ditch
Thighs only	Crossing irrigation ditch (too wide to jump)
Shins and arms / hands	Fell in, while jumping across irrigation ditch Get animal out of irrigation ditch Cutting feed for animals Fell while playing beside irrigation ditch Play in irrigation ditch while watching animals
Shins only	Slipped while jumping across irrigation ditch Walking through irrigation ditch (too wide to jump) Crossed recently irrigated field
Feet and hands /arms	Get animal out of irrigation ditch Fell in, while jumping across irrigation ditch
Feet only	Water from irrigation ditch spilled onto path Animals feed in field recently irrigated Livestock pass field recently irrigated
Arms only	Break open irrigation ditch, so animals can drink Playing boats in irrigation ditch Playing with mud and water from irrigation ditch
Hands only	Wash hands Playing with mud

Table 6-6 Descriptions of wastewater contact during play

Amount of wastewater contact	Responses
All body wet	Wash in irrigation ditch Fell in irrigation ditch Playing with wastewater Playing in recently irrigated field
Thighs and arms / hands	Running in wastewater Fell in irrigation ditch
Thighs only	Put feet in irrigation ditch
Shins and arms / hands	Fell in irrigation ditch Play with mud / wastewater Float toys in irrigation channel Played jumping over irrigation ditches
Shins only	Walk in irrigation ditch Crossed irrigated field Played "getting objects out of irrigation ditch"
Feet and hands /arms	Jumping in irrigated field Retrieve object that fell in irrigation ditch Playing with wastewater
Feet only	Slipped into irrigation ditch Walk in irrigation ditch Wastewater on path
Arms or hands only	Play with wastewater Play with toys in irrigation ditch / channel

Table 6-7 summarises the amount of wastewater contact on those occasions when there was some degree of contact with the wastewater. There was foot contact with wastewater during over 90% of shepherding-related wastewater contact and 78% of play related wastewater contact. Hand contact with the wastewater occurred during nearly 60% of shepherding-related wastewater contact and 87% of play-related wastewater contact. There was also a proportion, though small, of individuals who reported having had full body contact with wastewater (shepherding 5.9% and play 16.8%).

Wastewater contact was also reported when someone took the farmer's lunch to the field and during collection of the sap from the maguey cactus for pulque production. This was either just foot contact with the wastewater caused by walking through an irrigated field, or accidental hand and foot contact caused by slipping into an irrigation ditch.

Table 6-7 Extent of wastewater contact during shepherding and play

Variable	Category	Shepherding (N =221)		Play (N=113)	
		N	%	n	%
Upper limbs	Hands	127	57.5	98	86.7
	Arms	67	30.3	64	56.6
Lower limbs	Feet	204	92.3	89	78.8
	Shins	124	56.1	67	59.3
	Thighs	23	10.4	28	24.8
Whole body		13	5.9	19	16.8

6.3 DEMOGRAPHIC FACTORS AFFECTING WASTEWATER CONTACT

6.3.1 Factors affecting frequency of wastewater contact

In the study area, certain activities were carried out almost exclusively by a particular gender or age group. Nearly 100% of crop planting and irrigation was carried out by males, 95% of whom were over 14 years old. Shepherding was done by both males (45%) and females (55%), a third of whom were under 15 years old. Most play that resulted in wastewater contact occurred in the semi-exposed group. As a consequence of these age and gender differences, the activities that resulted in wastewater contact in each age and gender group, in the exposed and semi-exposed groups are different (Figure 6-13 and Figure 6-14 respectively). More than 50% of all wastewater contact among males aged over 9 years old occurred during crop irrigation in both the exposed and semi-exposed groups. Among 5-9 year old boys in the exposed group most wastewater contact occurred while tending to animals at pasture, whereas in the semi-exposed most contact occurred during play (Figure 6-13). Shepherding and harvest activities accounted for most wastewater contact for females aged over 9 years old in the exposed, while in the semi-exposed group most contact occurred during shepherding (Figure 6-14).

Figure 6-13 Activities involving wastewater contact in the exposed group

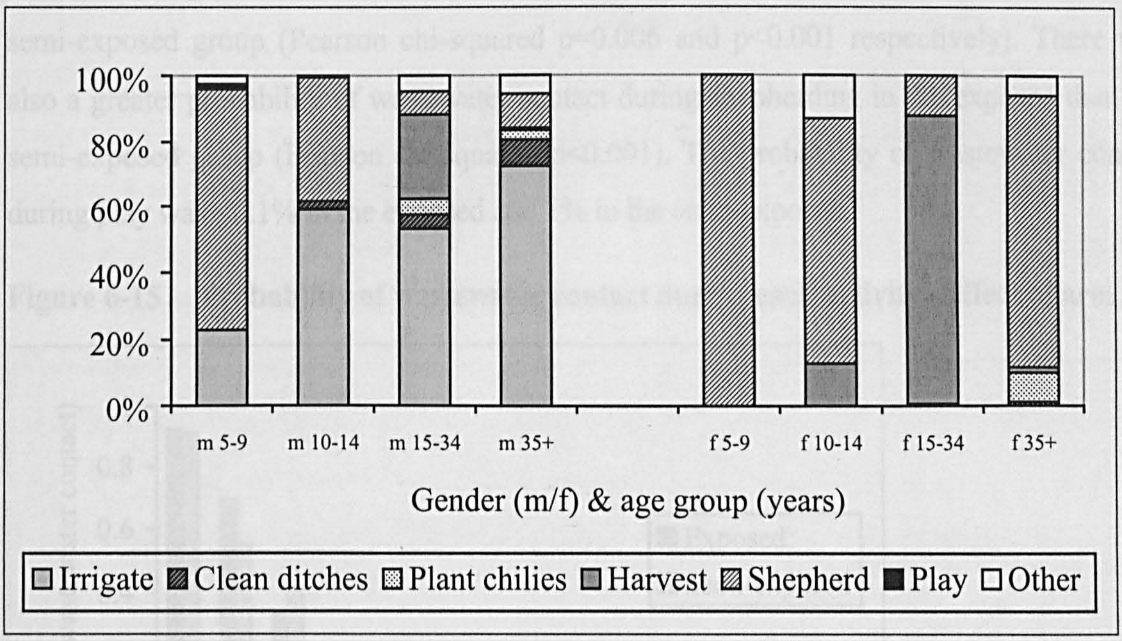
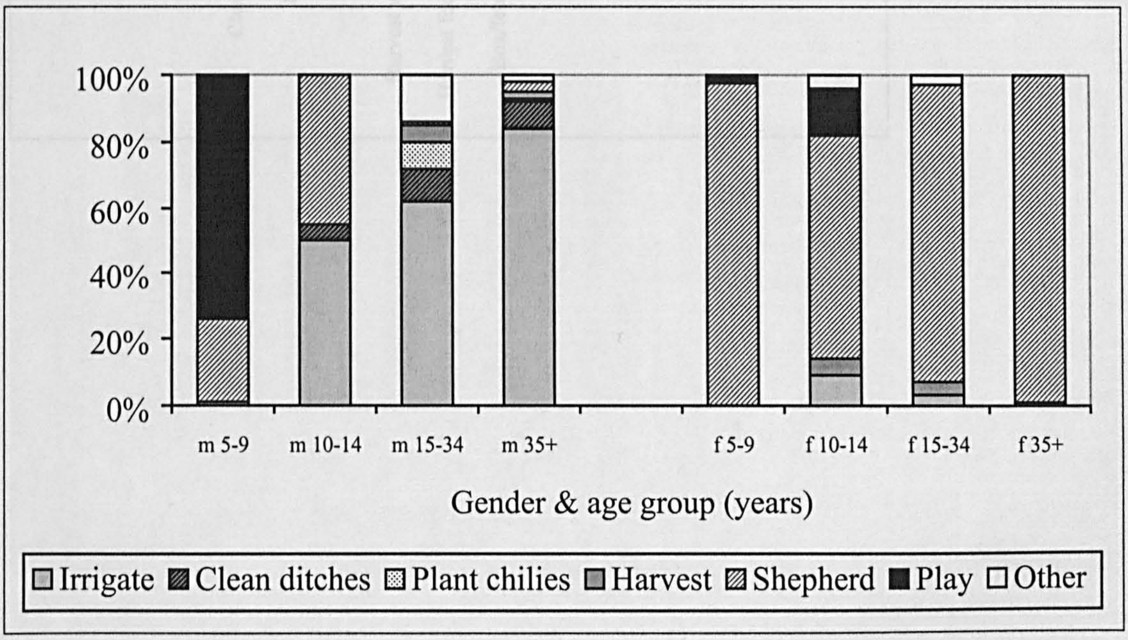
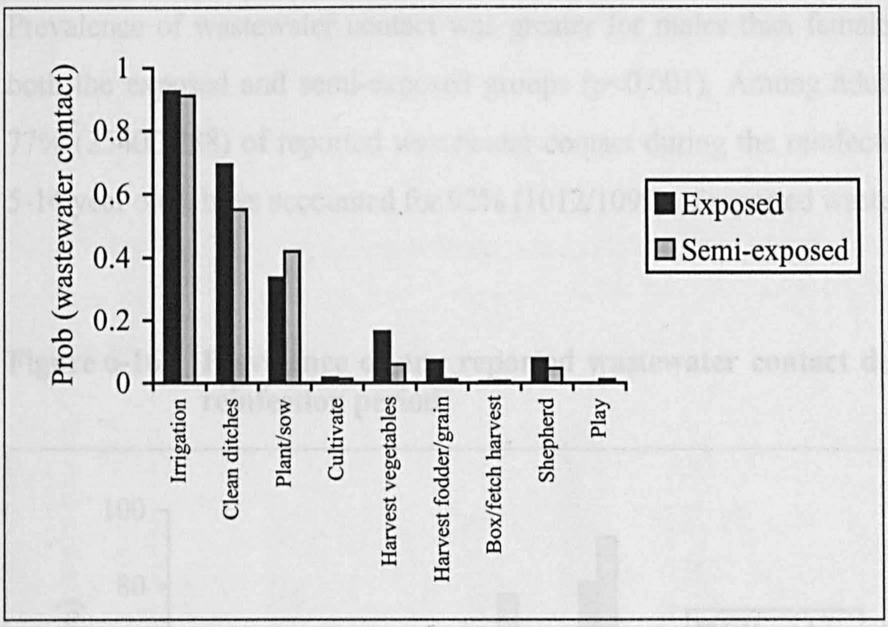


Figure 6-14 Activities involving wastewater contact in the semi-exposed group



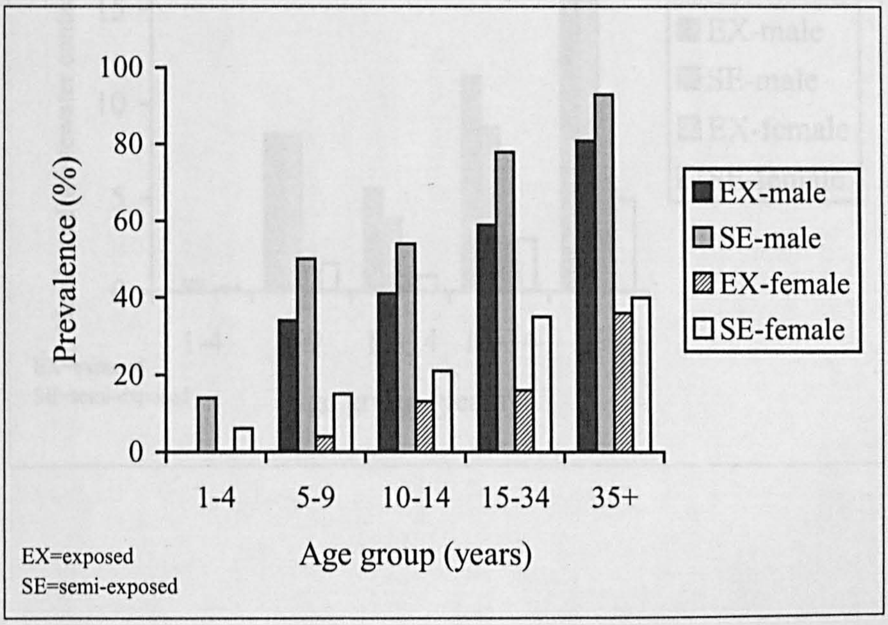
There was no difference in the probability of wastewater contact during irrigation between the exposed and semi-exposed groups (Figure 6-15). The probability of wastewater contact during cleaning ditches and during harvesting vegetables was greater in the exposed than the semi-exposed group (Pearson chi-squared $p=0.006$ and $p<0.001$ respectively). There was also a greater probability of wastewater contact during shepherding in the exposed than the semi-exposed group (Pearson chi-squared $p<0.001$). The probability of wastewater contact during play was <0.1% in the exposed and 1% in the semi-exposed.

Figure 6-15 Probability of wastewater contact during each activity - effect of area



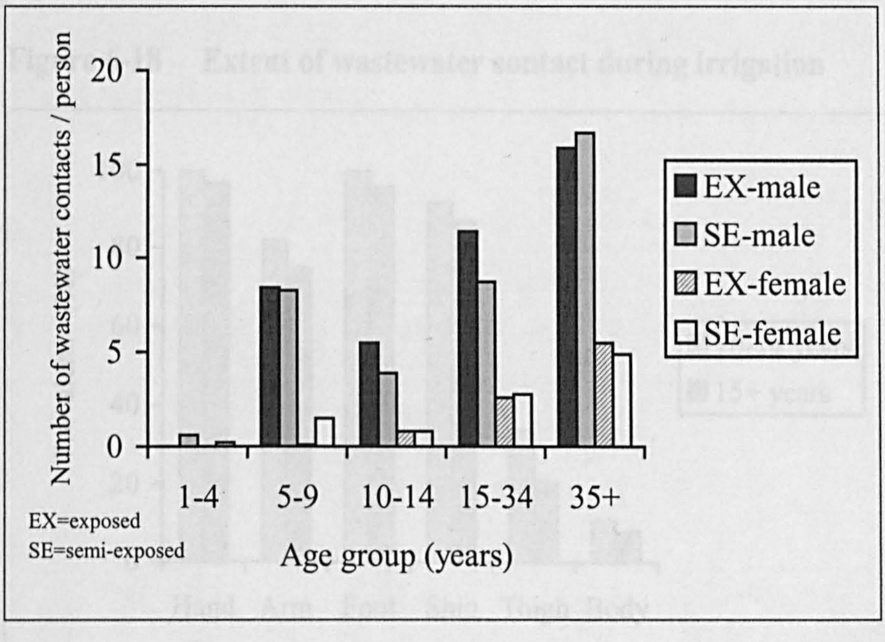
In the exposed group, 35% of individuals reported wastewater contact during the reinfection period (i.e. prevalence of wastewater contact = 35%), compared to 48% in the semi-exposed group (p=0.001). No wastewater contact was reported in the control group. Prevalence of wastewater contact was greater among adults and children in the semi-exposed than the exposed group. This group effect was significant among males and females (p=0.002 and p=0.031 respectively). In both the exposed and semi-exposed groups, the prevalence of wastewater contact increased with age among males and females (p<0.001) (Figure 6-16). Prevalence of wastewater contact was greater for males than females in all age groups in both the exposed and semi-exposed groups (p<0.001). Among adults, men accounted for 77% (2540/3288) of reported wastewater contact during the reinfection period, and among 5-14 year olds, boys accounted for 92% (1012/1099) of reported wastewater contact.

Figure 6-16 Prevalence of any reported wastewater contact during the 9-10 month reinfection period



The mean number of wastewater contacts per person during the reinfection period (Figure 6-17) was not significantly different in the exposed and semi-exposed groups (6.4 and 6.8 respectively, $p=0.77$), nor was there a significant difference between the exposed and semi-exposed groups by gender or age group. It was greater among males than females, both overall (10.8 and 2.5 respectively, $p<0.001$) and by age group from 5-9 years onwards. Among males in the exposed and semi-exposed groups, the mean number of wastewater contact per person decreased slightly between 5-9 year olds and 10-14 year olds, after which there was a trend with age ($p<0.001$). In females, from 10-14 years, there was a significant increase in the mean number of wastewater contact per person with age ($p<0.001$).

Figure 6-17 Number of wastewater contacts during the reinfection period

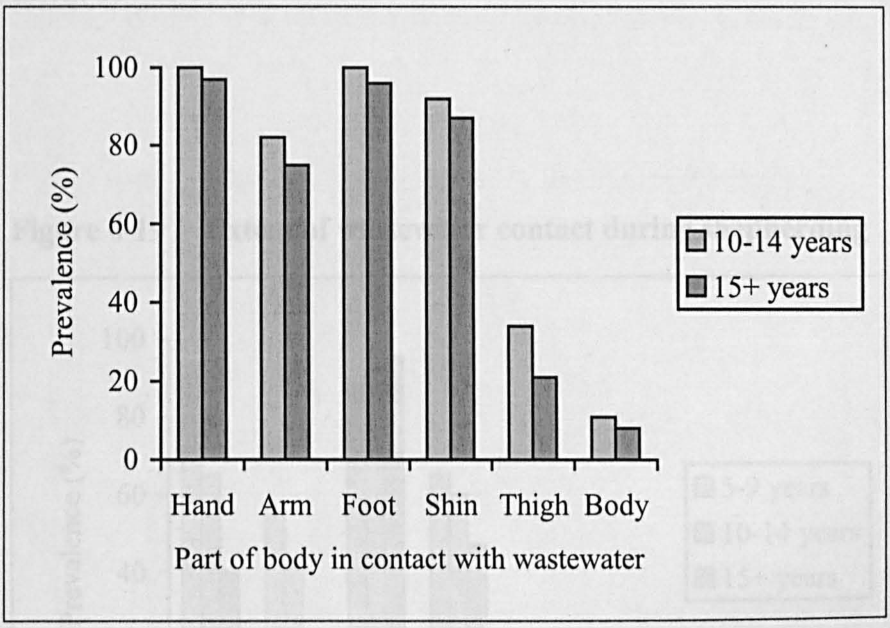


6.3.2 Factors affecting the extent of wastewater contact

6.3.2.1 Agricultural activities

The overall extent of body contact with wastewater by men during irrigation was 10% less than by 10-14 year old boys (Pearson chi-squared, p=0.064). For each part of the body, wastewater contact was consistently less among 15+ year olds than 10-14 year olds (Figure 6-18), however the difference was only significant for 'thigh' (p=0.054). There were no significant differences in wastewater contact during chilli planting reported between children and adults, nor between males and females.

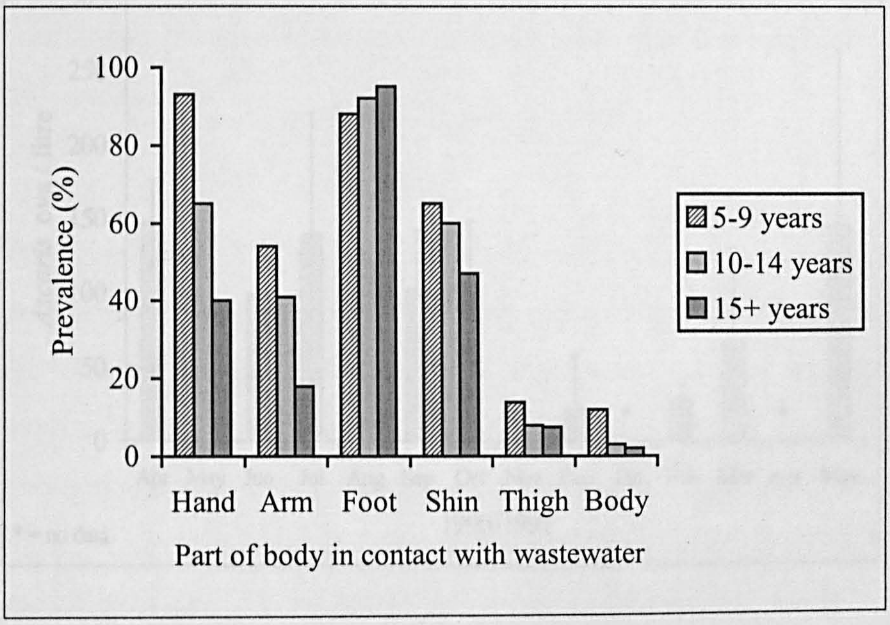
Figure 6-18 Extent of wastewater contact during irrigation



6.3.2.2 *Non-agricultural activities*

The overall extent of body contact with wastewater during shepherding fell by 18% between 5-9 year olds and 10-14 year olds and by a further 30% between 10-14 year olds and adults (trend $p=0.001$). Examining the wastewater contact with each part of the body individually (Figure 6-19), hand and arm contact with wastewater showed a significant decreasing trend with increasing age ($p<0.001$). There was no difference in foot contact between age groups. There was a trend of reducing shin contact with wastewater with age ($p=0.04$). There was significantly less full body contact with wastewater among adults than under 15 year olds ($p=0.013$). There were no significant differences between males and females in wastewater contact during shepherding. 90% of wastewater contact during play occurred among 5-11 year olds; however within this group, there was no significant effect of age or gender on the extent of wastewater contact.

Figure 6-19 Extent of wastewater contact during shepherding



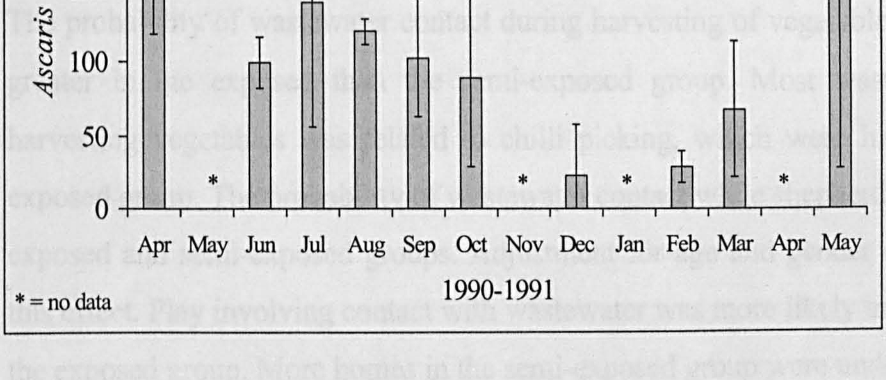
6.4 QUALITY OF THE WASTEWATER IN THE STUDY AREA

The seasonal variations in the quality of the wastewater used by the exposed group are shown in Figure 6-20. The number of *Ascaris* ova per litre peaked between April and July, then fell slightly between December and February, only to increase again from March to May. In the exposed area, four principal irrigation channels were monitored in duplicate (Rio Salado, Tlamaco-Juando, Dendho and Requena) (see Chapter 3,

Figure 3-4). The mean concentration of *Ascaris* ova per litre was 96 (95% confidence interval 79-113) in the four sites in the exposed area. In the semi-exposed area, 4 sites were sampled in duplicate along the length of the Endho channel (see Chapter 3,

Figure 3-4). One *Ascaris* ova was detected on two occasions during the monitoring period. The concentration of *Ascaris* ova per litre in the wastewater in the semi-exposed area was consequently <1 ova per litre throughout the 12 month period of monitoring.

Figure 6-20 Quality of wastewater in the exposed area[28]



[28] Bars show 95% confidence intervals for mean concentrations of *Ascaris* ova/litre

6.5 SUMMARY

The majority of wastewater contact occurred during crop irrigation, chilli planting, play or while tending to animals at pasture. Crop irrigation was a male dominated activity, in which most men and all 10-14 year old boys reported hand contact with wastewater. Men may have been more skilled than the 10-14 year olds at avoiding contact with the wastewater. Shepherding or tending to animals at pasture was carried out more by women than men. Women were often accompanied by small children. Children aged 5-9 years helped to care for the livestock and also played while the animals grazed. Children aged 10-14 years often took the livestock to pasture on their own. 5-9 year olds also reported a variety of play activities which involved wastewater and, consequently contact with the wastewater.

The probability of wastewater contact during irrigation was over 80%. The probability of wastewater contact during shepherding was only 6%, but shepherding was a much more frequent activity than crop irrigation. Crop irrigation was done every 21- 40 days depending on the crop, though one individual would often own or work several fields at any one time. In comparison, livestock were taken to pasture on an almost daily basis. Only 1-2% of play was reported to involve wastewater contact, but play was again an almost daily activity for 5-9 year olds.

The probability of wastewater contact during harvesting of vegetables and shepherding was greater in the exposed than the semi-exposed group. Most wastewater contact whilst harvesting vegetables was related to chilli picking, which were harvested mostly by the exposed group. The probability of wastewater contact while shepherding was different in the exposed and semi-exposed groups. Adjustment for age and gender did not help to explain this effect. Play involving contact with wastewater was more likely in the semi-exposed than the exposed group. More homes in the semi-exposed group were under 3 minutes walk from an irrigation channel than in the exposed group (24% and 13% respectively, $p<0.001$), therefore wastewater was more accessible to children in the semi-exposed group.

Prevalence of wastewater contact during the 9-10 month reinfection period was greater in the semi-exposed (47%) than the exposed group (35%). This difference was observed in adults and children, and in both males and females. Prevalence of wastewater contact increased with age. The mean number of wastewater contacts per person during the reinfection period (contacts/person) was not significantly different when the exposed and semi-exposed groups were compared (6.4 and 6.8 contacts/person, $p=0.77$). It did however increase with age, and was greater for males than females (10.8 and 2.4 contacts/person, $p<0.001$). This reflects the fact that many of the activities involving wastewater contact were agricultural in nature, and were found to be carried out principally by adult males.

The extent of the wastewater contact varied depending on the activity being performed and the age of the individual. There was more wastewater contact during chilli planting than during crop irrigation, and more contact during crop irrigation than during shepherding. In both crop irrigation and shepherding, the younger an individual, the greater the amount of body in contact with the wastewater. The reasons behind wastewater contact also varied depending on the activity. During crop irrigation and chilli planting most contact could be described as an essential part of the job, however during shepherding much of the contact was not necessary, but resulted from accidental contact or activities to while away the time.

Frequency of hand to mouth contact per hour during activities that involved contact with wastewater was negatively correlated with how labour intensive the activity was. The decrease in hand to mouth contact in chilli planting as compared with irrigation was probably because planting was very labour intensive whereas in irrigation, for much of the time the farmer must wait and watch the water as it enters the field. There also appears to be a downward trend in frequency of hand to mouth contact from alfalfa to maize to chillies. This again may relate to the intensity of the work involved in irrigating each crop.

Obtaining information on wastewater contact through structured observation studies and a detailed contact interview with each individual resulted in the collection of large volumes of data. In this thesis, the main differences in wastewater contact according to age, sex, activity and crop are described. However additional investigation of the data collected may further enhance understanding of the process of wastewater contact in this community.

The wastewater quality in the study area would suggest a greater risk of *Ascaris* infection from wastewater contact in the exposed than the semi-exposed area (96 ova/litre and <1 ova/litre respectively).

The components of exposure described in this chapter are considered further in Chapter 8, where their effects on the prevalence and intensity of *Ascaris* infection are assessed.

CHAPTER 7. *ASCARIS* INFECTION LEVELS IN THE STUDY POPULATION

The results presented in Chapter 7 describe *Ascaris* infection prevalence and intensity at the start of the study (prior to treatment) and 9-10 months later following anthelmintic treatment. A substudy of *Ascaris* worm burden is also described. In this chapter, crude odds ratios (OR), rather than crude relative risks (RR) are displayed. Although it could be argued that it is more appropriate in a prospective study to display crude RRs, it was decided to use crude ORs, because the final multivariate analysis was based on multiple logistic regression (hence adjusted ORs) rather than Mantel-Haenszel relative risks. All ORs reported in this chapter are unadjusted for other variables and are shown with 95% confidence intervals. The analyses done and the statistical tests performed in each section are described at the beginning of each section.

7.1 PREVALENCE OF *ASCARIS* INFECTION

In section 7.1 logistic regression is used to calculate crude odds ratios and 95% confidence intervals. All p-values and p_T-values[29] come from the likelihood ratio test performed after logistic regression, unless stated otherwise.

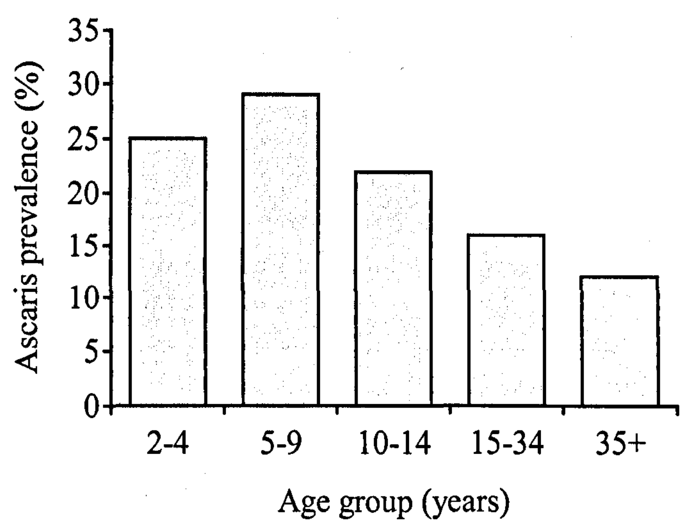
[29] p_T = trend p-value

7.1.1 Pretreatment prevalence of *Ascaris* infection

7.1.1.1 Age-related effects

The overall prevalence of *Ascaris* infection at the start of the study, prior to treatment was 18% (173/940)[30]. The prevalence of *Ascaris* infection was age-related. It was slightly lower in 2-4 year olds (25%) than among 5-9 year olds (29%) and then gradually declined in the older age groups (Figure 7-1). There was a significant trend of decreasing prevalence with increasing age (p_T<0.001) (Table 7-1).

Figure 7-1 Age distribution of pre-treatment prevalence of *Ascaris* infection



[30] Pre-treatment prevalence sample size was 940. Data were not available for 40 individuals who were absent at the time samples were collected. However it was decided to include these individuals in the study, since post-treatment prevalence data (the main outcome measure) and all other necessary information were available for these individuals.

Table 7-1 Pre-treatment prevalence of *Ascaris* infection - relationship with age

Age Group (years)	No/Total	(%)	OR	95% confidence interval
2-4	17/67	(25)	1.00	
5-9	46/161	(29)	1.18	(0.62,2.25)
10-14	33/152	(22)	0.82	(0.42,1.60)
15-34	42/271	(15)	0.54	(0.28,1.02)
35+	35/289	(12)	0.41	(0.21,0.78)
Total	173/940	(18)	$p_T<0.001$	

The significance of this relationship with age was very different in each of the three exposure groups (Figure 7-2). There was a significant trend of decreasing prevalence of *Ascaris* infection with increasing age, in both the exposed ($p_T<0.001$) and semi-exposed ($p_T=0.001$) groups (Table 7-2). There was no age-related trend for *Ascaris* prevalence in the control group ($p_T=0.55$).

Figure 7-2 Pre-treatment prevalence of *Ascaris* infection - relationship with age for each exposure group

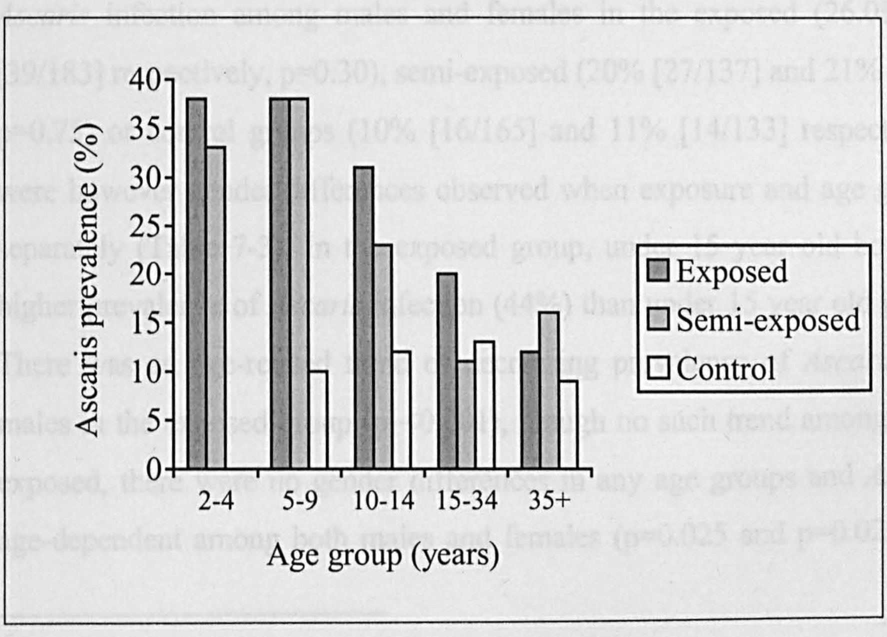


Table 7-2 Pre-treatment prevalence of *Ascaris* infection - relationship with age for each exposure group

Age Group (yr.)	Exposed (n=364)		Semi-exposed (n=278)		Control (n=298)	
	Total (%)	OR (CI)	Total (%)	OR (CI)	Total (%)	OR[31] (CI)
2-4	29 (38)	1.00	18 (33)	1.00	20 (0)	
5-9	64 (38)	0.98 (0.40,2.43)	45 (38)	1.21 (0.38,3.84)	52 (10)	1.00
10-14	49 (31)	0.72 (0.27,1.90)	53 (23)	0.59 (0.18,1.89)	50 (12)	1.83 (0.53,6.35)
15-34	122 (20)	0.40 (0.17,0.96)	82 (11)	0.25 (0.07,0.82)	67 (13)	2.08 (0.66,6.56)
35+	100 (12)	0.22 (0.09,0.58)	80 (16)	0.39 (0.12,1.22)	109 (9)	1.35 (0.44,4.14)
p value	<0.001		0.005		0.59	
OR_T	0.66 (0.55,0.80)		0.68 (0.54,0.87)		1.08 (0.84,1.39)	
P_T value	< 0.001		0.001		0.55	

7.1.1.2 Gender-related effects

The overall prevalence of *Ascaris* infection was not significantly different between males (18.6%) and females (18.2%) (p=0.85). There was no significant difference in prevalence of *Ascaris* infection among males and females in the exposed (26.0% [47/181] and 21% [39/183] respectively, p=0.30), semi-exposed (20% [27/137] and 21% [30/141] respectively, p=0.75) or control groups (10% [16/165] and 11% [14/133] respectively, p=0.81). There were however gender differences observed when exposure and age groups were examined separately (Table 7-3). In the exposed group, under 15 year old boys had a significantly higher prevalence of *Ascaris* infection (44%) than under 15 year old girls (26%) (p=0.026). There was an age-related trend of decreasing prevalence of *Ascaris* infection among all males in the exposed group (p_T<0.001), though no such trend among females. In the semi-exposed, there were no gender differences in any age groups and *Ascaris* prevalence was age-dependent among both males and females (p=0.025 and p=0.022 respectively). There

[31] 2-4 and 5-9 years olds combined in control group for OR calculations due to very low prevalence

were no significant gender differences nor was there an age-dependent effect for *Ascaris* prevalence in the control group.

Table 7-3 Pre-treatment prevalence of *Ascaris* infection - effect of age, gender and exposure group

Age Group (years)	Exposed (n=364)		Semi-exposed (n=278)		Control (n=298)[32]	
	Males	Females	Males	Females	Males	Females
	No/Total (%)	No/Total (%)	No/Total (%)	No/Total (%)	No/Total (%)	No/Total (%)
2-4	7/15 (47)	4/14 (29)	1/6 (17)	5/12 (42)	3/46 (7)	2/26 (8)
5-9	16/37 (43)	8/27 (30)	9/22 (41)	8/23 (35)		
10-14	9/21 (43)	6/28 (21)	7/27 (26)	5/26 (19)	3/31 (10)	3/19 (16)
15-34	10/55 (18)	14/67 (21)	4/41 (10)	5/41 (12)	5/30 (17)	4/37 (11)
35+	5/53 (9)	7/47 (15)	6/41 (15)	7/39 (18)	5/58 (9)	5/51 (10)
P value	<0.001	0.61	0.048	0.12	0.56	0.86
OR_T	0.56	0.80	0.67	0.70	1.11	1.03
P_T value	<0.001	0.12	0.025	0.022	0.53	0.87

7.1.1.3 Differences between exposure groups

There were significant differences in prevalence between exposure groups (p<0.001). The overall prevalence of *Ascaris* infection in the exposed was 24% (86/364), in the semi-exposed 21% (57/278) and in the control 10% (30/298). Comparison of the pre-treatment prevalence of *Ascaris* infection between age and exposure groups (Table 7-4) reveals no significant differences between the exposed and semi-exposed groups for 2-4 year olds (p=0.75) nor for 5-9 year olds (p=0.98). Prevalence of *Ascaris* infection among 2-4 and 5-9 year olds in the control group was significantly lower than in both the semi-exposed and exposed groups (p<0.001). There was a trend of decreasing prevalence among 10-14 year

[32] 2-4 and 5-9 years olds are combined in the control group due to very low prevalence

olds from the exposed, to the semi-exposed to the control (p_T=0.024). Among adults there were no significant differences in pre-treatment prevalence between the exposure groups.

Table 7-4 Comparison of *Ascaris* infection prevalence between exposure groups

Age group (years)	Study group	Pre-treatment			Reinfection		
		Total (%)	OR	(CI)	Total (%)	OR	(CI)
2-4	Exposed	29 (38)	1.00		29 (48)	1.00	
	Semi-exposed	18 (33)	0.82	(0.24,2.81)	18 (22)	0.31	(0.08,1.16)
	Control	20 (0)	-	-	20 (0)	-	-
	p-value	0.75			0.07		
5-9	Exposed	64 (38)	1.00		64 (34)	1.00	
	Semi-exposed	45 (38)	1.01	(0.46,2.22)	45 (33)	0.95	(0.43,2.14)
	Control	52 (10)	0.18	(0.06,0.51)	52 (2)	0.04	(0.00,0.29)
	p-value	<0.001			<0.001		
10-14	Exposed	49 (31)	1.00		49 (27)	1.00	
	Semi-exposed	52 (23)	0.66	(0.27,1.61)	53 (32)	1.31	(0.55,3.08)
	Control	50 (12)	0.31	(0.11,0.88)	50 (4)	0.12	(0.02,0.54)
	p-value	0.071 (p_T=0.024)			<0.001		
15-34	Exposed	122 (20)	1.00		122 (28)	1.00	
	Semi-exposed	82 (11)	0.50	(0.22,1.15)	82 (6)	0.17	(0.06,0.45)
	Control	67 (13)	0.63	(0.28,1.46)	67 (1)	0.04	(0.01,0.30)
	p-value	0.21			<0.001		
35+	Exposed	100 (12)	1.00		100 (11)	1.00	
	Semi-exposed	80 (16)	1.42	(0.61,3.32)	80 (6)	0.54	(0.18,1.62)
	Control	109 (9)	0.74	(0.31,1.80)	109 (4)	0.31	(0.09,1.00)
	p-value	0.34			0.11 (p_T=0.04)		

7.1.2 Reinfection prevalence of *Ascaris* infection

7.1.2.1 Age-related effects

In the general study population, the reinfection prevalence of *Ascaris* infection, 9-10 months after treatment, was 17% (165/980) (Table 7-5). The prevalence of *Ascaris* infection was age-related. It was greatest among 2-4 year olds (30%) and lowest among adults aged 35+ years (7%) (Figure 7-3). There was no significant difference in prevalence between 2-4 year olds and 5-9 year olds (p=0.62) nor with 10-14 year olds (p=0.55). However prevalence was significantly lower among adults than children (15-34 years, p=0.07; 35+ years, p=0.009). Despite there not being a significant difference in prevalence between the younger age groups, overall there was a significant trend in decreasing prevalence with increasing age (p_T<0.001) (Table 7-5).

Figure 7-3 Age distribution of reinfection prevalence of *Ascaris* infection

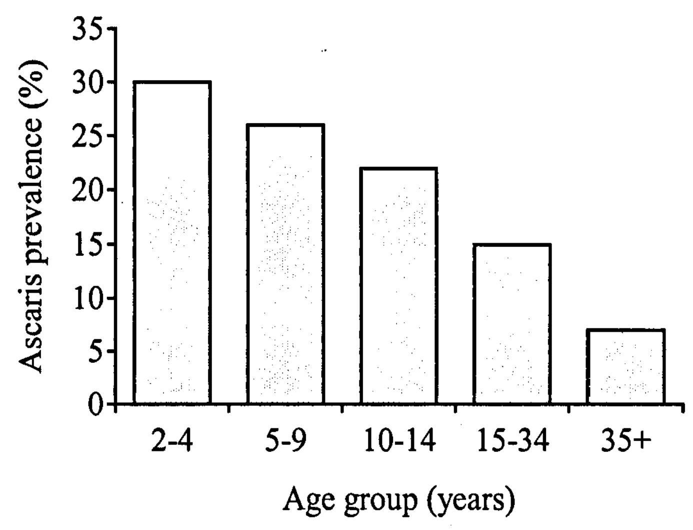


Table 7-5 Reinfection prevalence of *Ascaris* infection - effect of age

Age group (years)	No/Total	(%)	Crude OR	95% confidence interval
2-4	24/79	(30)	1.00	
5-9	43/167	(26)	0.79	(0.44,1.44)
10-14	35/162	(22)	0.63	(0.34,1.16)
15-34	42/278	(15)	0.41	(0.23,0.73)
35+	21/294	(7)	0.18	(0.09,0.34)
Total	165/980	(17)	p_T<0.001	

The age-dependent effect was different for each of the exposure groups (Figure 7-4). There was a trend of decreasing prevalence with age in the exposed group (p<0.001) (Table 7-6). Children in the semi-exposed group displayed no reduction in *Ascaris* prevalence with increasing age. *Ascaris* prevalence among adults in the semi-exposed group was significantly lower than among children (p<0.001). Prevalence of *Ascaris* infection was lower in the control group and no age effect was discernible.

Figure 7-4 Reinfection prevalence of *Ascaris* infection - effect of age for each exposure group

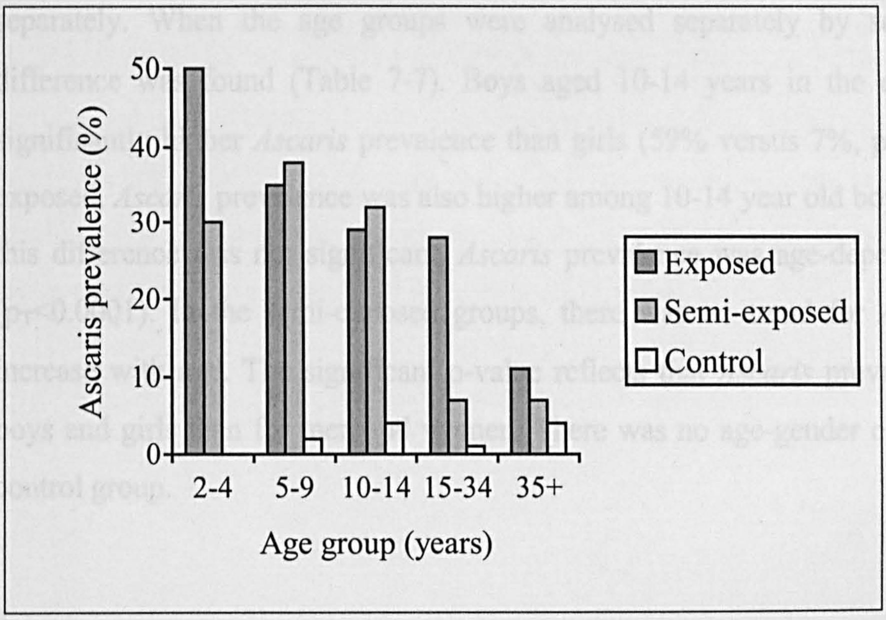


Table 7-6 Reinfection prevalence of *Ascaris* infection – effect of age for each exposure group

Age group (years)	Exposed (n=374)			Semi-exposed (n=296)			Control (n=310)		
	Total (%)	OR	(CI)	Total (%)	OR	(CI)	Total (%)	OR[33]	(CI)
2-4	34 (50)	1.00		23 (30)	1.00		22 (0)	-	-
5-9	65 (35)	0.55	(0.24,1.27)	50 (38)	1.40	(0.49,4.03)	52 (2)	-	-
10-14	52 (29)	0.41	(0.16,1.00)	57 (32)	1.05	(0.37,3.01)	53 (4)	-	-
15-34	123 (28)	0.40	(0.18,0.87)	84 (7)	0.18	(0.05,0.59)	71 (1)	-	-
35+	100 (11)	0.12	(0.05,0.31)	82 (7)	0.18	(0.05,0.61)	112 (4)	-	-
P value	< 0.001			< 0.001			0.67		
OR_T	0.66			0.55			-		
P_T value	<0.001			< 0.001			-		

7.1.2.2 Gender-related effects

There was no overall difference in reinfection *Ascaris* prevalence between males and females (p=0.43), nor was there a difference observed when exposure groups were examined separately. When the age groups were analysed separately by sex, only one gender difference was found (Table 7-7). Boys aged 10-14 years in the exposed group had a significantly higher *Ascaris* prevalence than girls (59% versus 7%, p<0.001). In the semi-exposed, *Ascaris* prevalence was also higher among 10-14 year old boys than girls, however this difference was not significant. *Ascaris* prevalence was age-dependent in the exposed (p_T<0.0001). In the semi-exposed groups, there was no trend for *Ascaris* prevalence to increase with age. The significant p-value reflects that *Ascaris* prevalence was higher for boys and girls than for men and women. There was no age-gender effect detectable in the control group.

[33] Odds ratios not calculated because of small numbers

Table 7-7 Reinfection prevalence of *Ascaris* infection - effect of age, gender and exposure group

Age group (years)	Exposed (n=374)		Semi-exposed (n=296)		Control (n=310)[34]	
	Males	Females	Males	Females	Males	Females
	No/Total (%)	No/Total (%)	No/Total (%)	No/Total (%)	No/Total (%)	No/Total (%)
2-4	9/17 (53)	8/17 (47)	3/7 (43)	4/16 (25)	0/15 (0)	0/7 (0)
5-9	14/38 (37)	9/27 (33)	10/24 (42)	9/26 (35)	0/33 (0)	1/19 (5)
10-14	13/22 (59)	2/30 (7)	11/28 (39)	7/29 (24)	2/31 (6)	0/22 (0)
15-34	15/56 (27)	20/67 (30)	3/41 (7)	3/43 (7)	0/34 (0)	1/37 (3)
35+	4/53 (8)	7/47 (15)	4/42 (10)	2/40 (5)	1/60 (2)	3/52 (6)
P value	< 0.001	0.004	<0.001	0.004	-	-
OR_T	0.59	0.76	0.50	0.57	-	-
P_T value	<0.001	0.04	<0.001	<0.001	-	-

7.1.2.3 *Differences between exposure groups*

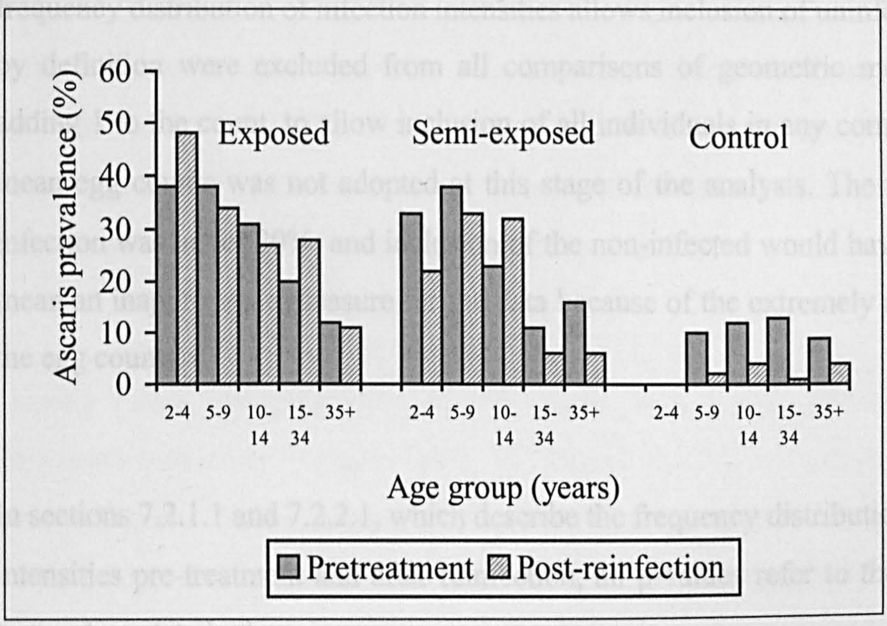
There was a significant difference in *Ascaris* prevalence between exposure groups (p<0.001) and between the exposed and semi-exposed groups (p=0.014). *Ascaris* prevalence was 27% (101/374) in the exposed, 19% (56/296) in the semi-exposed and 3% (8/310) in the control. Among 2-4 year olds and adults, *Ascaris* prevalence was higher in the exposed than the semi-exposed (Table 7-4), though this effect was not significant in the older adults (≥35 years) (p=0.27). For all age groups, *Ascaris* prevalence was significantly lower in the control group than both the exposed and semi-exposed groups.

[34] OR and p values not calculated because of very small numbers

7.1.3 Comparison of Pre-treatment and Reinfection Prevalence of *Ascaris* Infection

The prevalence of *Ascaris* infection in the exposed group had returned to pre-treatment levels 9-10 months after treatment (Figure 7-5). There were no significant differences between pre-treatment and reinfection *Ascaris* prevalence in the exposed group (Pearson chi-squared p>0.10). In the semi-exposed group, prevalence of *Ascaris* infection reached pre-treatment levels in the children, but not in the adults, where the reinfection *Ascaris* prevalence was lower (6%) than pre-treatment levels (14%) (Pearson chi-squared p=0.059). In the control group, reinfection *Ascaris* prevalence levels were lower than pre-treatment prevalence levels for all age groups (Pearson chi-squared p<0.001).

Figure 7-5 Comparison of pre-treatment and reinfection prevalence of *Ascaris* infection



7.2 INTENSITY OF *ASCARIS* INFECTION

Intensity of *Ascaris* infection is approximated by the number of eggs per gram of faeces (epg). It is described below in terms of the frequency distribution of low, medium and high egg counts and then as the geometric mean egg counts. To avoid continual referral to "egg counts", the term "intensity" is adopted in the text. The geometric mean abundance (GMA) of *Ascaris* infection is defined as the geometric mean intensity of *Ascaris* infection for the infected individuals. The GMA rather than the arithmetic mean abundance is presented because of the skewed distribution of the intensity data within the infected proportion of the study population.

It was decided that a fuller interpretation of the data could be achieved if both the frequency distribution and the geometric mean abundance of *Ascaris* infection were included. Since the frequency distribution of infection intensities allows inclusion of uninfected individuals, who by definition were excluded from all comparisons of geometric means. The practice of adding 1 to the count, to allow inclusion of all individuals in any comparisons of geometric mean egg counts was not adopted at this stage of the analysis. The prevalence of *Ascaris* infection was under 20%, and inclusion of the non-infected would have made the geometric mean an inappropriate measure for the data because of the extremely skewed distribution of the egg counts.

In sections 7.2.1.1 and 7.2.2.1, which describe the frequency distribution of *Ascaris* infection intensities pre-treatment and after reinfection, all p-values refer to the Pearson chi-squared test, unless stated otherwise.

In sections 7.2.1.2 and 7.2.2.2, which describe the geometric mean abundance of *Ascaris* infection (epg), all p-values originate from the one-tailed Student t-test for comparison of means assuming equal variances, unless stated otherwise. Bartlett's test for equal variances was performed. If variances were significantly different ($p<0.10$), the one-tailed Student t-test for comparison of means assuming unequal variances was used. Tests for trend were done using the F-statistic in analysis of variance.

7.2.1 Pretreatment

7.2.1.1 Frequency distribution of Ascaris *infection intensity*

The distribution of *Ascaris* infection intensity was not normal, but highly over-dispersed, with the majority of individuals having low egg counts, and just a small number of individuals having high egg counts.

The proportion of individuals with higher intensity *Ascaris* infections decreased with increasing age, in both the exposed and semi-exposed groups (Table 7-8). Children under 15 years old, in both the exposed and semi-exposed groups, had a larger proportion of individuals with higher intensity infections than adults in those groups ($p<0.001$ and $p=0.006$ respectively). The distribution of infection intensity could not be assessed in the control group due to the low numbers of infected individuals. The control group is excluded from all subsequent comparisons in this section (7.2.1.1).

Among males, the proportion of individuals with higher intensity *Ascaris* infections decreased with increasing age (Table 7-9). Boys aged 2-4, 5-9 and 10-14 years old each had proportionately higher intensity infections than men ($p=0.02$, $p<0.001$ and $p=0.002$ respectively). For females, the only difference in the distribution of *Ascaris* infection intensity was between girls under 10 years old and women aged ≥35 years ($p=0.076$).

Table 7-8 Pre-treatment intensity of *Ascaris* infection - effect of age and exposure group

Exposure group	Age group (years)	Total	Frequency distribution of egg counts (epg) (%)[35]			
			0	1-999	1000-9999	10,000+
Exposed	2-4	29	62	10	24	3
	5-9	64	63	14	16	8
	10-14	49	69	14	16	0
	15-34	122	80	13	6	1
	35+	100	88	10	2	0
Semi-exposed	2-4	18	67	22	11	0
	5-9	45	62	22	13	2
	10-14	53	77	13	9	0
	15-34	82	89	5	6	0
	35+	80	84	14	3	0
Control	2-4	20	100	0	0	0
	5-9	52	90	10	0	0
	10-14	50	88	12	0	0
	15-34	67	87	12	1	0
	35+	109	91	8	1	0

[35] Row percentages are shown

Table 7-9 Pre-treatment intensity of *Ascaris* infection - effect of gender and age

Gender[36]	Age group (years)	Total	Frequency distribution of egg counts (epg) (%)[37]			
			0	1-999	1000-9999	10,000+
Males	2-4	21	62	19	19	0
	5-9	59	58	17	17	8
	10-14	48	67	15	19	0
	15-34	95	85	9	5	0
	35+	94	88	11	1	0
Females	2-4	26	65	12	19	4
	5-9	50	68	18	12	2
	10-14	54	80	13	7	0
	15-34	108	82	10	6	1
	35+	86	84	13	3	0

Analysis of the effect of age, gender and exposure group reveals that boys aged 2-4, 5-9 and 10-14 years old had greater proportions of higher intensity infections than 15-34 year olds ($p=0.03$, $p=0.004$ and $p=0.017$ respectively). Differences were also observed among males, when each of the younger age groups were compared with ≥35 year olds ($p<0.001$). The differences in distribution of *Ascaris* infection intensity were less marked among females in the exposed group, where the only differences observed were between 2-4 year olds and adults ($p=0.02$). In the semi-exposed group, the distribution of *Ascaris* infection intensity was similar among males and females irrespective of age group. Only between 5-9 year old boys and adults was there a difference in the distribution of *Ascaris* infection intensity, with higher intensity infection among the boys ($p=0.029$). There were no significant differences in the distribution of *Ascaris* infection intensity between genders in any age groups. Children

[36] Control group excluded

[37] Row percentages shown

in the exposed had a greater proportion of higher intensity infections than the semi-exposed ($p=0.045$).

Using the negative binomial regression, the value of alpha (α), a measure of overdispersion was obtained. In the conventional notation the overdispersion parameter k, an inverse measure of overdispersion is equal to $1/\alpha$. The overdispersion parameter k displayed a linear relationship with intensity of infection, such that k decreased as the degree of overdispersion increased ($p<0.001$).

Overall, the pre-treatment intensity of *Ascaris* infection in terms of eggs per gram of faeces was significantly overdispersed ($n=940$, $k=0.15$, LRT $p<0.001$). Egg counts were most overdispersed in the exposed and least in the control group. There was an association with age, with egg counts more overdispersed in children than adults. There was no significant gender dependency in overdispersion ($p>0.10$). The effects of age, gender and exposure group are summarised in Table 7-10. Adjustment for intensity in the model, removed the age-dependency observed ($p=0.34$), however the association between overdispersion and exposure group remained significant ($p<0.001$).

Table 7-10 Overdispersion in pre-treatment *Ascaris* intensity

Variable		Unadjusted k	Adjusted coefficient of regression for overdispersion (α)	Overall LRT p-value
Exposure group	Exposed	0.14	Baseline	<0.001
	Semi-exposed	0.16	-0.39 (-0.57, -0.21)	
	Control	0.21	-0.70 (-0.90, -0.49)	
Age (years)	<15	0.15	Baseline	<0.001
	≥15	0.16	-0.39 (-0.55, -0.22)	
Gender	Male	0.14	Baseline	0.13
	Female	0.16	-0.12 (-0.27, 0.03)	

7.2.1.2 Geometric mean abundance of Ascaris *infection*

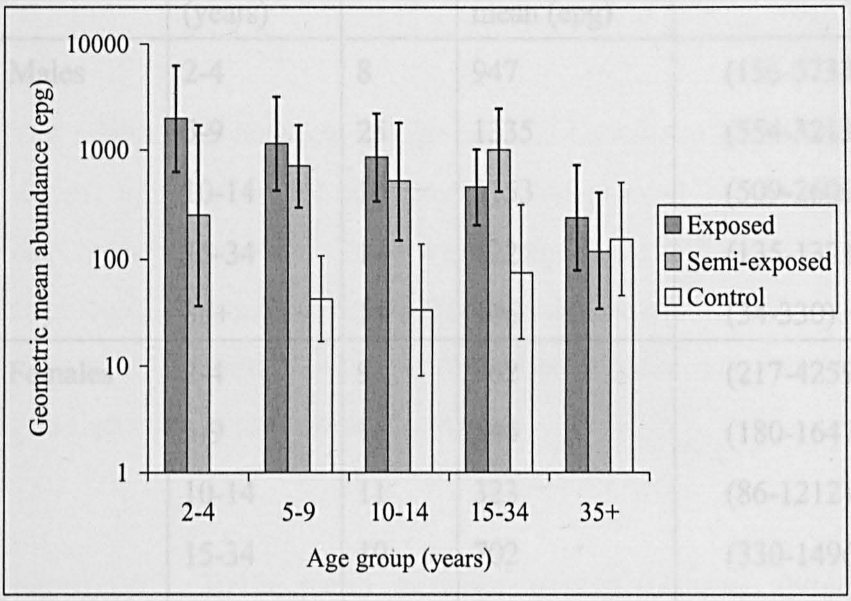
The geometric mean abundance (GMA) of *Ascaris* infection was higher in the exposed [727 epg, 95% CI (470,1126)] than the semi-exposed group [419 epg, 95% CI (254,693)] ($p=0.053$). There was an age effect on GMA in the exposed group, with GMA decreasing with increasing age ($p_F=0.065$) (Figure 7-6). Children aged 2-4 years in the exposed group had higher intensity infections than adults aged 15-34 and ≥35 years ($p=0.017$ and $p=0.004$ respectively) (Table 7-11). In the exposed group, both 5-9 and 10-14 year olds had higher intensity infections than older adults ($p=0.017$ and $p=0.035$ respectively). There were however no significant differences in the intensity of *Ascaris* infection between the younger age groups, or between the 15-34 and ≥35 year olds.

In the semi-exposed group there were no significant differences in intensity of infection between the younger age groups. The GMA in the 15-34 year olds was higher overall than all other age groups ($p=0.02$). No comparisons were made with the GMA for the control group due to the low numbers of *Ascaris* infected individuals. The control group is excluded from all subsequent comparisons in this section (7.2.1.2).

Table 7-11 Pre-treatment geometric mean abundance of *Ascaris* infection - effect of age and exposure group

Exposure group	Age group (years)	Total	Geometric mean (epg)	95% CI
Exposed	2-4	11	1973	(623-6253)
	5-9	24	1148	(418-3157)
	10-14	15	859	(335-2206)
	15-34	24	456	(205-1013)
	35+	12	241	(80-727)
Semi-exposed	2-4	6	252	(37-1703)
	5-9	17	710	(294-1714)
	10-14	12	518	(149-1802)
	15-34	9	1007	(413-2458)
	35+	13	119	(35-406)
Control	2-4	0	-	-
	5-9	5	43	(17-108)
	10-14	6	34	(8-138)
	15-34	9	76	(18-314)
	35+	10	154	(47-503)

Figure 7-6 Age and exposure intensity profile of pre-treatment *Ascaris* infection



Note : 95% CI displayed

The GMA decreased with increasing age among males (p_F=0.007)[38] (Table 7-12). Under 15 year old boys had significantly higher intensity infections than adults (p<0.001). There were no age-dependent trends for GMA among females. The only differences observed were among ≥35 year old women, where GMA was slightly lower than the 2-4 and 15-24 year olds (p=0.05).

Analysis of the combined effect of age, gender and exposure group (Figure 7-7) revealed that one of the largest differences in GMA related to under 10 year old boys, who had higher infection intensities in the exposed than the semi-exposed group (p=0.021). This effect remained significant when all under 15 year olds are considered together (p=0.033). The trend of decreasing GMA in the 10-14 year olds was not significant.

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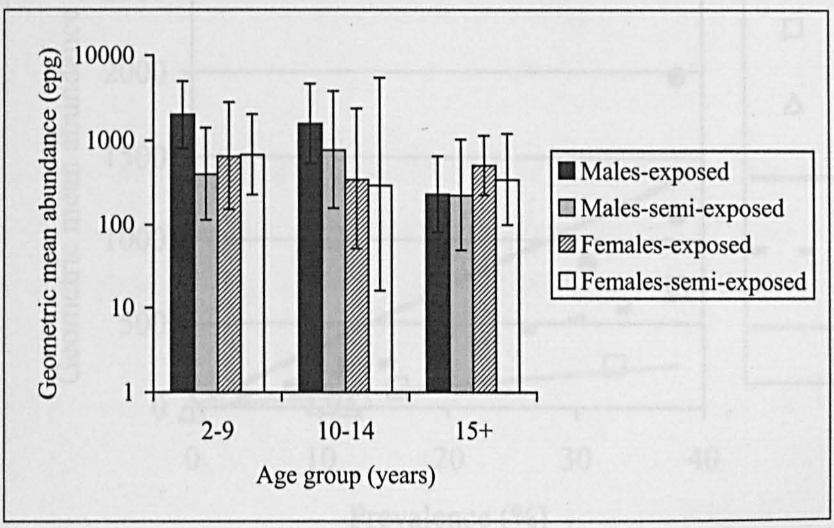
[38] p_F = p-value calculated from F-statistic

Table 7-12 Pre-treatment geometric mean abundance of *Ascaris* infection - effect of gender and age group

Gender[39]	Age group (years)	Total	Geometric mean (epg)	95% CI
Males	2-4	8	947	(156-5733)
	5-9	25	1335	(554-3213)
	10-14	16	1153	(509-2609)
	15-34	14	422	(135-1321)
	35+	11	106	(34-330)
Females	2-4	9	962	(217-4259)
	5-9	16	544	(180-1647)
	10-14	11	323	(86-1212)
	15-34	19	702	(330-1496)
	35+	14	239	(74-767)

Figure 7-7 Age, gender and exposure intensity profile of pre-treatment *Ascaris* infection

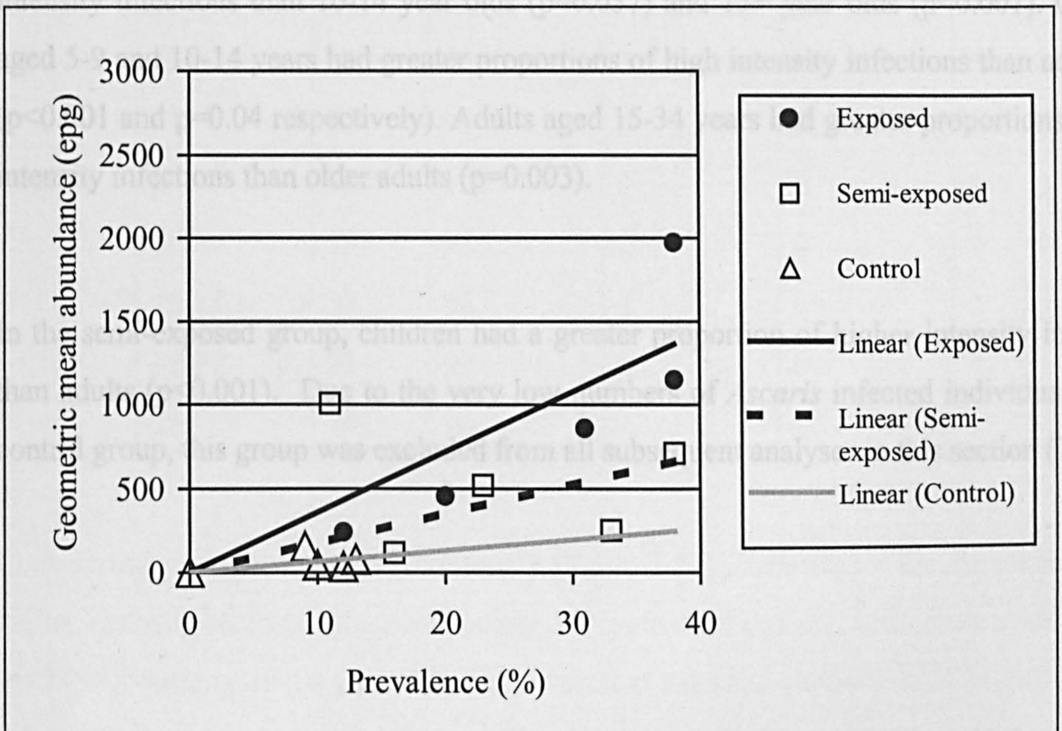


39 Control group excluded

7.2.1.3 *Relationship between prevalence and intensity of* Ascaris *infection*

The relationship between the age-specific prevalence and geometric mean abundance of *Ascaris* infection for all age groups in the exposed, semi-exposed and control groups is displayed in Figure 7-8. There was no significant difference between the exposed and the semi-exposed groups (p=0.85). The correlation coefficients were, exposed r=0.82, semi-exposed r=0.77 and control r=0.39 (this latter value reflected the narrow range of *Ascaris* prevalence found throughout this group).

Figure 7-8 Relationship between prevalence and abundance of pre-treatment *Ascaris* infection



7.2.2 Reinfection

7.2.2.1 Frequency distribution of Ascaris *infection intensity*

The distribution of *Ascaris* infection intensity (epg) was highly over-dispersed, as was seen with pre-treatment intensity. There were just a few individuals with high intensity infections while the majority had low intensity infections. All p-values in this section refer to the Pearson chi-squared test, unless stated otherwise.

The proportion of individuals with higher intensity *Ascaris* infections decreased with increasing age in the exposed group. The 2-4 year olds had greater proportions of high intensity infections than 10-14 year olds ($p=0.037$) and 15+ year olds ($p<0.001$). Children aged 5-9 and 10-14 years had greater proportions of high intensity infections than adults did ($p<0.001$ and $p=0.04$ respectively). Adults aged 15-34 years had greater proportions of high intensity infections than older adults ($p=0.003$).

In the semi-exposed group, children had a greater proportion of higher intensity infections than adults ($p<0.001$). Due to the very low numbers of *Ascaris* infected individuals in the control group, this group was excluded from all subsequent analyses in this section (7.2.2.1).

Table 7-13 Reinfection intensity of *Ascaris* infection - effect of age and exposure group

Exposure group	Age group (years)	Total	Frequency distribution[40] of intensity of *Ascaris* infection (epg) (%)			
			0	1-999	1000-9999	10,000+
Exposed	2-4	34	50	15	26	9
	5-9	65	65	8	18	9
	10-14	52	71	10	17	2
	15-34	123	72	19	8	2
	35+	100	89	7	4	0
Semi-exposed	2-4	23	60	22	9	0
	5-9	50	62	14	14	10
	10-14	57	68	12	14	5
	15-34	84	93	2	5	0
	35+	82	93	6	1	0
Control	2-4	22	100	0	0	0
	5-9	52	98	2	0	0
	10-14	53	96	4	0	0
	15-34	71	99	0	0	1
	35+	112	96	1	2	1

Considering males and females separately (Table 7-14), there was a greater proportion of higher intensity *Ascaris* infections among 2-9 year old boys and girls than among adults ($p<0.001$). Among 10-14 year olds there was also a greater proportion of higher intensity infections than among adults; these differences were greater among boys ($p<0.001$) than girls ($p=0.035$). There were however no significant differences in the distribution of *Ascaris* infection intensities between the younger age groups for boys or for girls, nor between 15-34

[40] Row percentages are shown

and ≥35 year olds. Only between 10-14 year olds was there a gender difference in the distribution of *Ascaris* infection intensities (p=0.001).

Table 7-14 Reinfection intensity of *Ascaris* infection - effect of gender

Gender	Age group (years)	Total	Frequency distribution[41] of intensity of *Ascaris* infection (epg) (%)			
			0	1-999	1000-9999	10,000+
Males	2-4	24	50	17	29	4
	5-9	62	61	8	23	8
	10-14	50	52	22	22	4
	15-34	97	81	11	6	1
	35+	95	92	6	2	0
Females	2-4	33	54	18	12	6
	5-9	53	66	13	9	11
	10-14	59	85	2	10	3
	15-34	110	79	13	7	1
	35+	87	90	7	3	0

Separate consideration of age, gender and exposure group revealed that among males and females in the exposed and semi-exposed groups, children had a greater proportion of higher intensity infections than adults (p<0.001). Among children, there were less higher intensity infection among 10-14 year old girls than 2-4 year olds girls in the exposed and semi-exposed groups. There were no significant differences in the proportions of higher intensity infections between males and females in the exposed or semi-exposed groups, except for the 10-14 year olds in the exposed, where boys had greater proportions of higher intensity infections than girls. The proportion of higher intensity infections was greater in the exposed than the semi-exposed group for males aged 15-34 years (p=0.072) and for all adult women (p=0.007).

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[41] Control group not included

As previously mentioned in section 7.2.1.1, by using the negative binomial regression, the value of alpha (α), a measure of overdispersion can be obtained. From this, k, the conventional parameter for overdispersion can be calculated, $k=1/\alpha$. Overdispersion parameter k decreased with increasing intensity of infection ($p<0.001$).

The reinfection intensity data were significantly overdispersed in the exposed and semi-exposed groups(n=670, k=0.14, LRT $p<0.001$). The control group was excluded from these comparisons because of small sample size. There was no significant difference in k between the exposed and semi-exposed groups nor between the sexes. There was an association with age, with egg counts more overdispersed in children than adults ($p=0.01$). The effects of exposure group, age and gender for the exposed and semi-exposed groups are summarised in Table 7-15. Adjustment for intensity in the model, removed the age-dependency observed ($p=0.78$). There was a slight association between exposure group and overdispersion after adjustment for intensity ($p=0.043$), with egg counts more overdispersed in the exposed than the semi-exposed group.

Table 7-15 Overdispersion in reinfection *Ascaris* intensity

Variable		Unadjusted k	Adjusted coefficient of regression for overdispersion	LRT p-value
Exposure	Exposed	0.15	Baseline	0.89
	Semi-exposed	0.13	0.01 (-0.16, 0.19)	
Age group (years)	<15	0.14	Baseline	0.01
	≥ 15	0.15	-0.25 (-0.43, -0.06)	
Gender	Male	0.14	Baseline	0.92
	Female	0.13	0.01 (-0.16, 0.18)	

7.2.2.2 Geometric mean abundance of Ascaris *infection*

The two adult age groups (15-34 and ≥35 years) have been combined in this section, because low numbers of infected individuals made it difficult to make meaningful comparisons. Geometric mean abundance (GMA) of *Ascaris* infection was slightly higher in the 5-9 year olds than the 2-4 year olds, then GMA decreased with increasing age in the exposed group (Trend p=0.003) (Figure 7-9). Children aged 2-4, 5-9 and 10-14 years each had higher intensity infections than adults in the exposed group (p=0.024, p<0.001 and p=0.04 respectively) (Table 7-16). There were no significant differences in the intensity of *Ascaris* infection between the younger age groups in the exposed group. Infection intensities were higher among the 5-9 and 10-14 year olds than among adults in the semi-exposed (p<0.001 and p=0.04 respectively). There was however no overall age-related trend for GMA in this group.

Figure 7-9 Reinfection geometric mean abundance of *Ascaris* infection

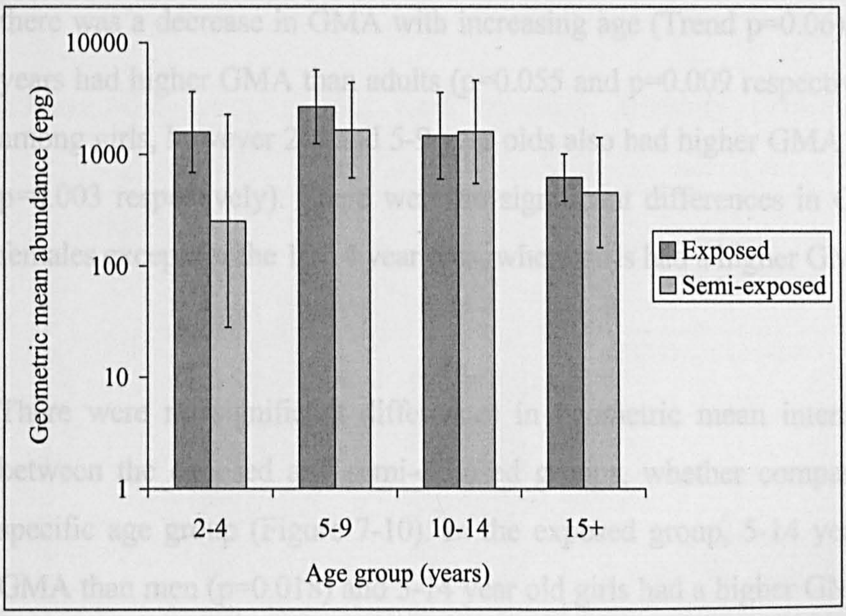


Table 7-16 Reinfection geometric mean abundance of *Ascaris* infection

Exposure group[42]	Age group (years)	Total	Geometric mean (epg)	95% CI
Exposed	2-4	17	1576	(688-3613)
	5-9	23	2659	(1246-5673)
	10-14	15	1466	(603-3564)
	15+	46	622	(384-1008)
Semi-exposed	2-4	7	252	(28-2269)
	5-9	19	1643	(613-4404)
	10-14	18	1590	(550-4601)
	15+	12	456	(147-1413)

Among males, the GMA was also lower in the 2-4 year olds than in the 5-9 year olds, then there was a decrease in GMA with increasing age (Trend p=0.06). Boys aged 2-4 and 5-9 years had higher GMA than adults (p=0.055 and p=0.009 respectively). There was no trend among girls, however 2-4 and 5-9 year olds also had higher GMA than adults (p=0.046 and p=0.003 respectively). There were no significant differences in GMA between males and females except for the 10-14 year olds, where girls had a higher GMA than boys (p=0.033).

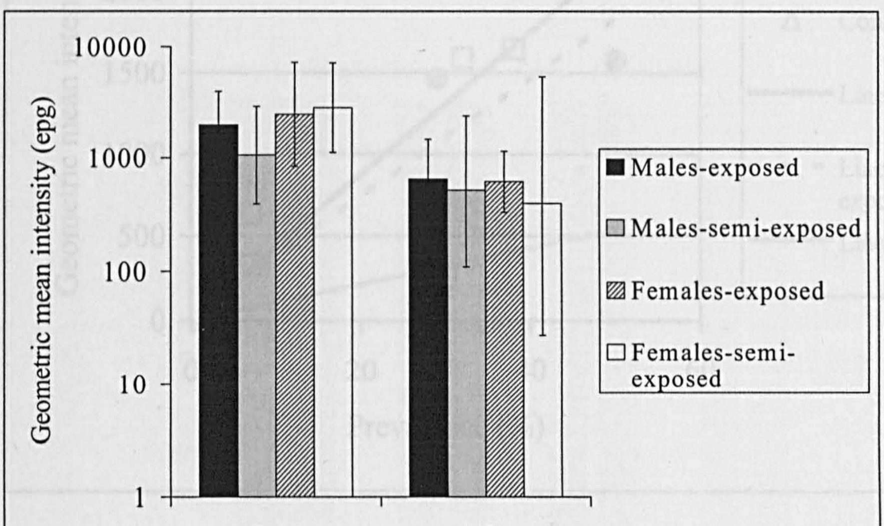
There were no significant differences in geometric mean intensity of *Ascaris* infection between the exposed and semi-exposed groups, whether comparing males, females or a specific age group (Figure 7-10). In the exposed group, 5-14 year old boys had a higher GMA than men (p=0.018) and 5-14 year old girls had a higher GMA than women (p=0.01). In the semi-exposed, 5-14 year old girls also had a higher GMA than women (p=0.024).

[42] Geometric means and 95% CI were not calculated in the control group because samples size <5

Table 7-17 Reinfection geometric mean abundance of *Ascaris* infection - effect of gender

Gender	Age group (years)	Total	Geometric mean (epg)	95% CI
Males	2-4	12	1525	(593-3919)
	5-9	24	2129	(928-4885)
	10-14	24	1059	(462-2428)
	15-34	18	704	(282-1756)
	35+	8	426	(150-1212)
Females	2-4	12	559	(124-2523)
	5-9	18	2151	(870-5319)
	10-14	9	4101	(1540-10923)
	15-34	23	650	(300-1410)
	35+	9	401	(150-1068)

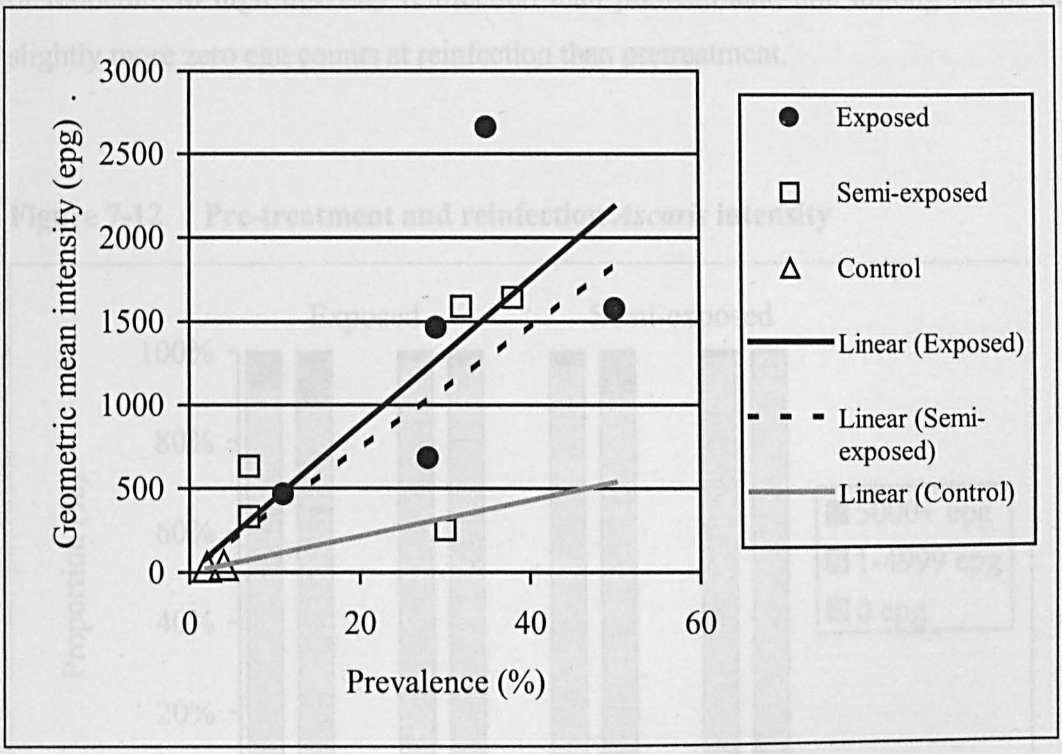
Figure 7-10 Age, gender and exposure intensity profile of reinfection *Ascaris* infection



7.2.2.3 Relationship between prevalence and abundance of Ascaris *infection*

The relationship between the age-specific prevalence and abundance of *Ascaris* infection reinfection for all age groups in the exposed, semi-exposed and control groups is displayed in Figure 7-11. There was no significant difference between the exposed and semi-exposed groups in the expected intensity for a given prevalence of *Ascaris* infection (Regression p-value=0.85). The correlation coefficients were low, exposed r=0.58, semi-exposed r=0.63 and control r=0.66.

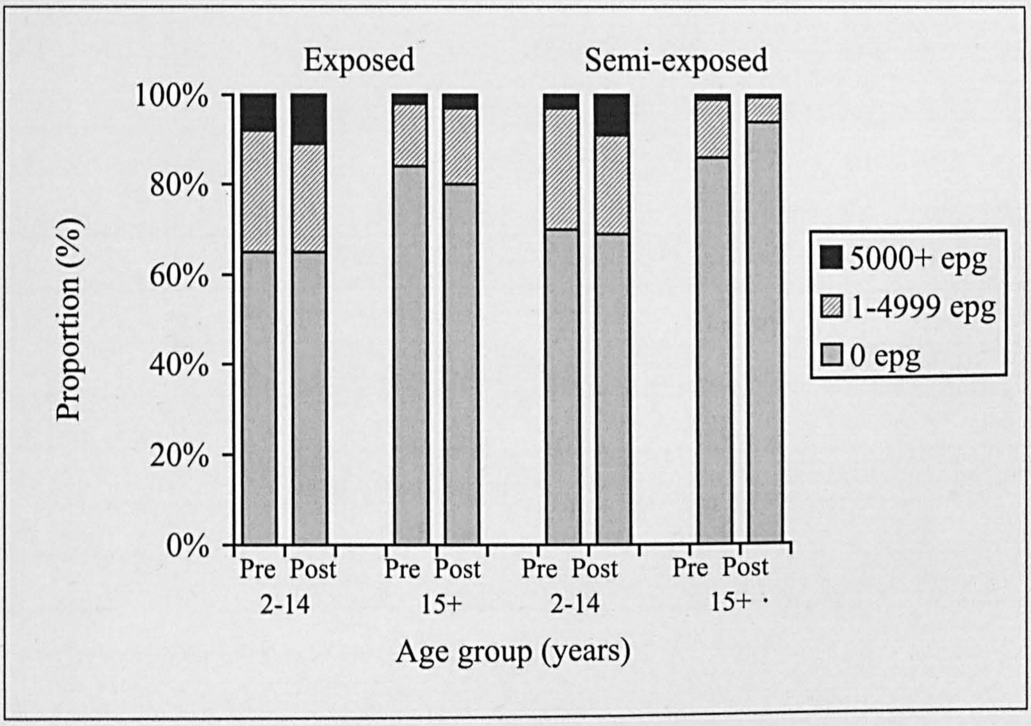
Figure 7-11 Relationship between prevalence and abundance of reinfection *Ascaris* infection



7.2.3 Comparison of pre-treatment and reinfection *Ascaris* intensity levels

Pre-treatment and reinfection intensity levels were compared by examining the proportions of individuals with 0 epg, 1-4999 epg and ≥5000 epg. These thresholds for classes of intensity were proposed by WHO (1987) and were termed none, low and, moderate to high intensity infections. Overall in the exposed and semi-exposed groups, there was a return to pre-treatment infection levels at 9-10 months after treatment. No significant differences in the proportions of individuals with low and moderate to high intensity infections were found when pre-treatment and reinfection intensity levels were compared ($p=0.095$) (Figure 7-12). In the semi-exposed, there were some small differences between pretreatment infection and reinfection levels. Among children in the semi-exposed group, there was a greater proportion of moderate to high intensity reinfection than pretreatment, and among adults there were slightly more zero egg counts at reinfection than pretreatment.

Figure 7-12 Pre-treatment and reinfection *Ascaris* intensity



7.3 OTHER ISSUES

7.3.1 Household clustering of *Ascaris* infection

In Table 7-18 household clustering of pre-treatment *Ascaris* infection is assessed (all individuals for whom an initial stool sample was collected are included). The expected values were calculated using the binomial distribution, where the probability of being infected was equal to the total number of infected divided by the total number of individuals. There is clearly some degree of household clustering of *Ascaris* infected individuals. An overall chi-squared test was performed by comparing all observed and expected values. The overall chi-squared test gave $p<0.001$ regardless of whether families with 10 or more members were included in the test. This household clustering of *Ascaris* infection could be caused by familial infections or by families having a common source of contamination. Household clustering was significant in both the exposed and semi-exposed groups.

Table 7-18 Household clustering of *Ascaris* infected individuals (observed and expected distributions)[43]

HH size (Indiv/HH)	No. HH		No. Individuals in HH infected											χ^2 p-value
			0	1	2	3	4	5	6	7	8	9	10	
1	35	O	29	6										0.56
		E	27.6	7.4										
2	42	O	32	9	1									0.17
		E	26.1	14.0	1.9									
3	46	O	27	12	7	0								0.23
		E	22.5	18.2	4.9	0.4								
4	59	O	30	15	8	2	4							0.00
		E	22.7	24.5	9.9	1.8	0.1							
5	56	O	26	14	10	5	1	0						0.07
		E	17.0	22.9	12.3	3.3	0.4	0.0						
6	52	O	16	18	8	5	4	0	1					0.00
		E	12.4	20.1	13.5	4.9	1.0	0.1	0.0					
7	40	O	16	12	6	5	1	0	0	0				0.08
		E	7.5	14.2	11.5	1.4	0.2	0.0	0.0	0.0				
8	20	O	5	4	3	4	0	2	1	1	0			0.00
		E	3.0	6.4	3.2	1.1	0.2	0.3	0.0	0.0	0.0			
9	10	O	2	3	1	0	2	1	0	0	0	1		0.00
		E	1.7	2.8	3.1	1.9	0.8	0.2	0.0	0.0	0.0	0.0		
10	2	O	0	1	1	0	0	0	0	0	0	0	0	1.00
		E	0.2	0.5	0.6	0.4	0.2	0.1	0.0	0.0	0.0	0.0	0.0	
11	1	O	0	0	0	1	0	0	0	0	0	0	0	0.99
12	0	O	0	0	0	0	0	0	0	0	0	0	0	-
13	0	O	0	0	0	0	0	0	0	0	0	0	0	-
14	0	O	0	0	0	0	0	0	0	0	0	0	0	-
15	2	O	0	0	1	0	1	0	0	0	0	0	0	1.00
16	2	O	0	0	0	0	0	0	0	1	0	0	1	0.00
17	1	O	0	0	1	0	0	0	0	0	0	0	0	1.00

43 O=observed, E=expected, expected values not shown for HH size > 10, because numbers were small

7.3.2 Predisposition to *Ascaris* infection

Individuals predisposed to *Ascaris* infection, are defined as those that for one or more reasons, are more likely than the remainder of the population to become re-infected with *Ascaris* infection and to have high (or low) intensity *Ascaris* infections. The debate surrounding predisposition was discussed in Chapter 2 (section 2.3.5). In the study population as a whole, individuals initially infected with *Ascaris* were predisposed to reinfection ($p<0.001$) (Table 7-19). In the exposed group, children aged 5-14 years and women, were predisposed to reinfection by *Ascaris* ($p<0.001$), but men were not predisposed to infection. No such relationship was observed in the semi-exposed group, irrespective of the gender or age group considered.

Overall in the study population, there was predisposition to high (or low) intensity infections ($p<0.001$) (Table 7-20). In the exposed group, there was predisposition to high (or low) intensity of *Ascaris* infection, among both children and adults, and among males and females. No age-gender comparisons could be made due to small numbers. There was no evidence of such predisposition in the semi-exposed group as a whole ($p=0.12$), nor among any specific gender or age group in the semi-exposed group. In the control group, the numbers of *Ascaris* infected individuals were too small to make comparisons. Exclusions of uninfected individuals from the "low" intensity group resulted in a very low sample size. Overall in the study population, with this revised definition of intensity, there was a slight predisposition to high (or low) intensity infections although this was not significant ($p=0.072$).

Table 7-19 Predisposition to *Ascaris* infection

Study Group	Pre-treatment infection	Reinfection prevalence No/total	(%)	Odds Ratio (95% CI)	LRT p-value
All	Uninfected	94/767	(12)	3.25 (2.21-4.78)	<0.001
	Infected	54/173	(31)		
Exposed	Uninfected	53/278	(19)	3.87 (2.31-6.48)	<0.001
	Infected	41/86	(48)		
Semi-exposed	Uninfected	33/221	(15)	1.68 (0.83-3.43)	0.16
	Infected	13/57	(23)		
Control	Uninfected	8/268	(3)	-	-
	Infected	0/30	(0)		

Table 7-20 Predisposition to high (or low) intensity *Ascaris* infection[44]

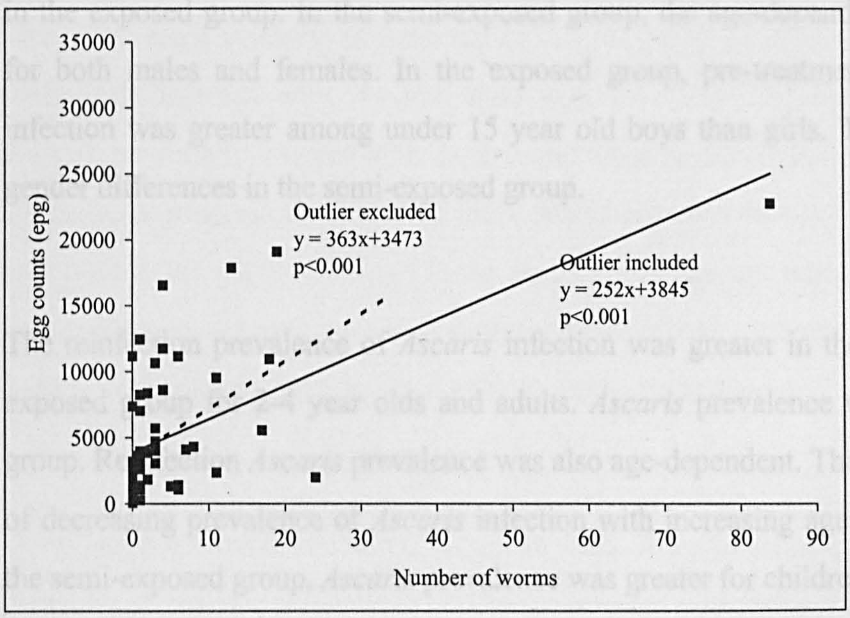
Study Group	Pre-treatment intensity of infection	Proportion with moderate to high intensity reinfection No/Total	(%)	Odds Ratio (95% CI)	LRT p-value
All	Uninfected or low	28/919	(3)	15.91 (5.96-42.49)	<0.001
	Moderate to high	7/21	(33)		
Exposed	Uninfected or low	15/348	(4)	13.32 (4.27-41.51)	<0.001
	Moderate to high	6/16	(38)		
Semi-exposed	Uninfected or low	11/273	(4)	5.95 (0.61-57.80)	0.124
	Moderate to high	1/5	(20)		
Control	-	-	-	-	-

[44] A threshold of 5000 epg to separate low and moderate intensity infections was defined by WHO (1987)

7.3.3 *Ascaris* Worm Burden

Comparison of egg counts and worm burden for a subsample of individuals (n=54) is displayed in Figure 7-13. A simple regression analysis reveals that the correlation is significant (continuous line), however there is considerable scatter in the data (R^2= 0.43). After exclusion of a possible outlier, the one individual from whom 84 worms were recovered, the correlation remains significant (dotted line), however as before there is considerable scatter in the data (R^2= 0.41).

Figure 7-13 *Ascaris* eggs counts and worm burden



7.4 SUMMARY

Throughout this chapter it has been demonstrated that differences in prevalence and intensity of *Ascaris* infection were associated with age, gender and exposure group.

The pre-treatment prevalence of *Ascaris* infection was greater in the exposed than the semi-exposed group for children but not for adults. *Ascaris* prevalence in the control group was lower than in both the exposed and the semi-exposed groups for all age groups. There was a significant age-related trend of decreasing prevalence of *Ascaris* infection prior to anthelmintic treatment. This age-related trend was significant for males but not for females in the exposed group. In the semi-exposed group, the age-dependent trend was significant for both males and females. In the exposed group, pre-treatment prevalence of *Ascaris* infection was greater among under 15 year old boys than girls. There were no significant gender differences in the semi-exposed group.

The reinfection prevalence of *Ascaris* infection was greater in the exposed than the semi-exposed group for 2-4 year olds and adults. *Ascaris* prevalence was lowest in the control group. Reinfection *Ascaris* prevalence was also age-dependent. There was a significant trend of decreasing prevalence of *Ascaris* infection with increasing age in the exposed group. In the semi-exposed group, *Ascaris* prevalence was greater for children than adults, although no gradual trend was observed. *Ascaris* prevalence was greater for 10-14 year old boys than girls in the exposed and semi-exposed groups, although this difference was only significant in the exposed group. No age nor gender effects were detected in the control group.

The reinfection prevalence of *Ascaris* infection returned in 9-10 months to pre-treatment levels for all ages in the exposed group, but not in the semi-exposed group. *Ascaris* infection levels in the control group were significantly lower than pre-treatment levels for all ages.

Prior to treatment, the geometric mean abundance of *Ascaris* infection (GMA) using egg counts was greater in the exposed than the semi-exposed and lowest in the control group. There was an age-related trend of decreasing GMA with increasing age for males in the exposed group. In the semi-exposed group there were no clear trends. There were no significant differences in the GMA of *Ascaris* reinfection between the exposed and semi-exposed groups. The GMA reinfection decreased with increasing age in the exposed but not in the semi-exposed group.

There was evidence of an association between exposure group and infection intensity, and the degree of overdispersion of pretreatment and reinfection *Ascaris* intensity. The association found with age caused by an interaction with *Ascaris* intensity.

There was some evidence of household clustering, with more families having few or many individuals infected with *Ascaris*, than would be expected by chance. Comparison of individuals initially infected with *Ascaris* and those re-infected after treatment demonstrated a significant proportion of infected individuals became re-infected (i.e. were predisposed to infection) in the exposed but not in the semi-exposed group. Individuals were also found to be predisposed to reinfection with high (or low) intensity infections, as determined by egg counts, in the exposed group, but not in the semi-exposed group.

There has been much discussion in recent years of the explanations for predisposition of a particular group of individuals to become re-infected with *Ascaris* infection and with similar intensity infections (Chapter 2 - section 2.3.5). Patterns of *Ascaris* infection and intensity, similar to those described here, have been reported in several studies (Chapter 2 - section 3.2-3.4). Much of the debate centres on the role of environment and behaviour in the acquisition of *Ascaris* infection and in the determination of *Ascaris* infection intensity. In Chapter 8, the relative roles of environment and of behaviour in the determination of *Ascaris* infection levels are analysed

CHAPTER 8. RELATIONSHIP BETWEEN PREVALENCE AND INTENSITY OF *ASCARIS* REINFECTION AND EXPOSURE AND REPORTED MORBIDITY

8.1 INTRODUCTION

The relationship between exposure to wastewater and the prevalence, abundance and intensity of *Ascaris* reinfection 9-10 months after treatment is described in this chapter. The relationship between prevalence and abundance of *Ascaris* reinfection and morbidity in 5-16 year olds is then described.

In the univariate analysis, odds ratios were calculated for all variables using logistic regression to examine the crude effect of exposure to wastewater and possible confounding variables on *Ascaris* reinfection. Multivariate analyses, also using logistic regression, then determined the effect of exposure to wastewater on *Ascaris* reinfection after adjusting for all possible confounding factors as determined in the univariate analyses.

To determine the crude effects of exposure to wastewater and possible confounding variables on the intensity of *Ascaris* reinfection two approaches were adopted. Firstly, means and coefficients for each variable were calculated using linear regression of the logarithm of the egg counts for infected individuals. Multivariate analyses, also using linear regression, then assessed the relationship between exposure to wastewater and the intensity of *Ascaris* reinfection among infected individuals (i.e. abundance of reinfection) after allowing for all significant confounding factors as determined in the univariate analyses. Secondly, coefficients for each variable were calculated using negative binomial regression of the raw egg counts for all individuals (including both uninfected and infected). To solve the problem of zero egg counts, 1 was added to the value of each egg count prior to performing the negative binomial regression. Multivariate analyses, also using negative binomial regression, then assessed the effect of exposure to wastewater on the intensity of *Ascaris* reinfection after allowing for all significant confounding factors as determined in the univariate analyses.

In the logistic regression (for *Ascaris* prevalence), the linear regression (for abundance of *Ascaris* reinfection) and the negative binomial regression (for intensity of *Ascaris* reinfection), the significance of each variable was tested using the likelihood ratio test. Variables with a likelihood ratio test $p<0.10$ were included in the initial multivariate model. For the final model, variables were included if the likelihood ratio test $p<0.05$, or if there was a strong plausible rationale for including them. Age, sex and area were included in the final model for prevalence, abundance and intensity of *Ascaris* reinfection regardless of their p-value. All analysis was performed on individuals (not families) although some of the variables in the final models were calculated at the family level. In order to allow for the correlation of individuals within families, the confidence intervals of variables in the final model were also calculated to adjust for household clustering. No variables in the final models lost their significance as a result of adjustment for clustering by household. The 95% confidence intervals (95% CI) shown in the final models are not adjusted for clustering.

Sections 8.2, 8.3 and 8.4 examine the effect of exposure to wastewater on the prevalence of *Ascaris* reinfection in children, men and women respectively. Analyses were performed separately for children (2-14 years), men (15+ years) and women (15+ years) because of the differences in wastewater contact activities between these three groups (see Chapter 6). Section 8.5 determines the effect of exposure to wastewater on the abundance of *Ascaris* reinfection in children and adults, that is the intensity of *Ascaris* reinfection among infected children and adults. Section 8.6 determines the effect of exposure to wastewater on the intensity of *Ascaris* reinfection in all children and adults, regardless of reinfection status. Section 8.7 describes morbidity in 5-16 year olds related to *Ascaris* reinfection. The control group was excluded from analyses unless stated otherwise.

8.2 *ASCARIS* REINFECTION IN CHILDREN UNDER 15 YEARS OLD

The relationship between exposure to wastewater, socioeconomic status, hygiene and other variables and the prevalence of *Ascaris* reinfection for under 15 year olds in the exposed and semi-exposed groups are displayed in Table 8-1. For each variable, the prevalence of *Ascaris* reinfection, the crude odds ratio and confidence interval, and the adjusted odds ratio, confidence interval and p-value are shown. The odds ratios for all variables are adjusted for all other variables in the model.

8.2.1 Relationship between exposure through wastewater contact and prevalence of *Ascaris* reinfection

The prevalence of *Ascaris* reinfection was greater among 2-14 year olds reporting wastewater contact through crop irrigation than among those reporting no crop irrigation during the reinfection period (Table 8-1). Despite small numbers of children being involved in crop irrigation, there was a trend of increasing *Ascaris* prevalence from 33.1% among those reporting no crop irrigation, to 41.7% among those with sedimented wastewater contact, to 66.7% among those with raw wastewater contact during crop irrigation (trend p=0.076). The prevalence of *Ascaris* reinfection was also significantly greater among 2-14 year olds reporting play activities involving contact with wastewater (adjusted OR=2.03, 95% CI (1.06-3.86), p=0.031). There was no significant difference in the prevalence of *Ascaris* infection when children who played with raw and sedimented wastewater were compared.

Children under 15 years old were predisposed to *Ascaris* reinfection. 44% of those initially infected became reinfected compared with 28% of those initially not infected (crude OR=2.01, 95% CI (1.17-3.45), p=0.012). After adjustment for wastewater contact and other risk factors in Table 8-1, predisposition was no longer significant (adjusted OR=1.73, 95% CI (0.91-3.29), p=0.093).

8.2.2 Relationship between other variables and prevalence of *Ascaris* reinfection

As well as direct contact with wastewater, the effect of consuming products irrigated with wastewater was considered (Table 8-1). There was an increased risk of *Ascaris* reinfection associated with consumption of crops from local fields. The prevalence of *Ascaris* reinfection was greater among those consuming crops from local fields (41.1%) than among those who obtained crops from other sources (16.4%).

There was no excess risk of *Ascaris* reinfection among those living in the untreated wastewater area than in the sedimented wastewater area. Girls had a much lower risk of *Ascaris* reinfection than boys (OR=0.54, p=0.039), with boys more than twice as likely to be infected with *Ascaris*. There was no age effect having adjusted for other variables. *Ascaris* reinfection was less prevalent among individuals whose head of household had at least completed primary school. The prevalence of *Ascaris* reinfection was greater if the head of the household's main occupation (rather than his secondary occupation) was farming. *Ascaris* prevalence was greater among 2-14 year olds in households without domestic fowl, even after allowing for the lack of domestic fowl in households in the exposed than the semi-exposed group (see Chapter 5, Table 2-1).

Table 8-1 Relationship between exposure to wastewater and other variables and *Ascaris* prevalence in under 15 year olds in the exposed and semi-exposed groups

Variable		No / Total	Prev	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)	LRT p-value (trend p-value)
Wastewater contact						
Irrigation with wastewater	None	84/254	33.1	1.00	1.00	0.19
	Sedimented	5/12	41.7	1.45 (0.45-4.69)	1.17 (0.31-4.40)	(0.081)
	Untreated	10/15	66.7	4.05 (1.34-12.22)	3.43 (0.91-12.89)	
Playing with wastewater	None	57/190	30.0	1.00	1.00	0.070
	Sedimented	26/59	44.1	1.84 (1.01-3.35)	2.61 (1.10-6.15)	
	Untreated	16/32	50.0	2.33 (1.09-4.99)	1.50 (0.59-3.79)	
Other effects related to living in ww irrigated area						
Eat crops from local field	No	11/67	16.4	1.00	1.00	0.034
	Yes	88/214	41.1	3.56 (1.76-7.17)	2.41 (1.07-5.44)	
Demographic factors						
Exposure area	Untreated	55/151	36.4	1.00	1.00	0.39
	Sedimented	44/135	33.9	0.89 (0.55,1.46)	0.71 (0.33-1.54)	
Gender	Male	60/136	44.1	1.00	1.00	0.039
	Female	39/145	26.9	0.47 (0.28,0.77)	0.54 (0.30-0.97)	
Age group (years)	2-4	24/57	42.1	1.00	1.00	0.18
	5-9	42/115	36.5	0.79 (0.42-1.51)	0.53 (0.24-1.13)	
	10-14	33/109	30.3	0.60 (0.31-1.16)	0.51 (0.24-1.11)	
Socioeconomic factors						
Schooling of head of HH	None	10/21	47.6	1.00	1.00	0.042
	Primary incomplete	69/176	39.2	0.71 (0.29-1.76)	0.64 (0.23-1.74)	
	At least primary	20/84	23.8	0.34 (0.13-0.93)	0.31 (0.23-1.74)	
Main job of head of HH	Farmer	97/256	37.9	1.00	1.00	0.062
	Other	2/25	8.0	0.14 (0.03-0.62)	0.23 (0.05-1.07)	
Fowl owned by HH	Yes	57/201	28.4	1.00	1.00	0.008
	No	42/80	52.5	2.79 (1.63-4.77)	2.39 (1.26-4.54)	

Note : HH=household, ww=wastewater

The following variables were significantly associated with a **higher** *Ascaris* prevalence in the univariate analysis (likelihood ratio test $p<0.10$), but were not significant when included in the multivariate model :-

Wastewater contact

- Any agricultural work relating to maize production with wastewater contact (p=0.087)

Other effects related to living in a wastewater irrigated area

- Live increasingly near to wastewater irrigation channel (exposed group only) (trend p=0.021). Greatest risk if ≤5 min walk from channel.

Socioeconomic variables

- Head of household could not write a short note (p=0.018)
- Wife of head of household had not completed primary school (p=0.098)
- Increasing number of individuals living in the house (p=0.002)
- Increasing number of individuals per room (trend p=0.028)
- Use of both wood and gas for cooking, rather than just one fuel (p=0.053)
- Household has a horse (p=0.017)
- House does not have a fridge (p=0.04)
- Decreasing weekly egg consumption (trend p=0.033)

Hygiene variables

- Drink water direct from tap (p=0.03)

- At least one member of household defecates in open (effect increases when defecation near to house reported) (p=0.052)

8.3 *ASCARIS* REINFECTION IN MEN (AGED ≥15 YEARS)

The relationship between exposure to wastewater and other variables and the prevalence of *Ascaris* reinfection among men aged ≥15 years in the exposed and semi-exposed groups are displayed in Table 8-2. As in section 8.2, the prevalence of *Ascaris* reinfection, the crude odds ratio and confidence interval, and the adjusted odds ratio, confidence interval and p-value are displayed for all variables in the model. The odds ratio for all variables are adjusted for all variables in the model.

8.3.1 Relationship between exposure through wastewater contact and prevalence of *Ascaris* reinfection

The prevalence of *Ascaris* reinfection decreased from 43.5% (10/23) for men irrigating chilli to 9.5% (16/169) among those not involved in chilli irrigation during the reinfection period (adjusted OR[45]=7.09 (2.17-23.15), LRT p=0.001). Planting chillies was associated with an increased risk of *Ascaris* infection. (adjusted OR[46]=5.20 (1.37-19.66), LRT p=0.016). Men planting chillies had a higher *Ascaris* prevalence than men who did not plant chillies [7/13 (53.9%) and 10.6% (19/179) respectively]). The number of men involved in chilli related activities was small and most individuals who irrigated chillies also planted chillies, which meant it was not possible to separate out the effects of irrigation and planting. Consequently, it was decided to display the risks related to any agricultural activity related to chilli production which involved wastewater contact. Chilli production involving contact with raw

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[45] Odds ratio was adjusted for all variables in model in Table 8.2

[46] Odds ratio was adjusted for all variables in model in Table 8.2

wastewater was associated with a significantly higher prevalence of *Ascaris* reinfection (39.3%) when compared with men not producing chillies (9%) (Table 8-2). Separate analyses of men in the semi-exposed group examined the effect of chilli irrigation and planting with sedimented wastewater; however there was no significant risk of *Ascaris* reinfection associated with either irrigation or planting. No other wastewater contact variables were significantly associated with an increased risk of *Ascaris* reinfection.

8.3.2 Relationship between other variables and prevalence of *Ascaris* reinfection

Consumption of crops from local fields irrigated with untreated wastewater was associated with a greater risk of *Ascaris* reinfection. The prevalence of *Ascaris* reinfection was higher among those consuming local crops (22.4%) were compared with those that did not consume (8.8%). The only other factor associated with *Ascaris* reinfection in men, was the time the household had been in the community (Table 8-2). Households present for at least 6 years, had a lower risk of *Ascaris* reinfection than households resident for less than 6 years (trend OR=0.35 (0.18-0.67), LRT p=0.002). There was lower *Ascaris* prevalence in men aged ≥35 years than in younger men; however this was not significant. There was no significant exposure area effect.

Men in the exposed and semi-exposed groups were not predisposed to *Ascaris* reinfection (crude OR=1.58, 95% CI (0.42-5.95), p=0.50). However after adjustment for wastewater contact and other risk factors in Table 8-2, older boys (10-14 year olds) and adult men who reported contact with wastewater were predisposed to reinfection (adjusted OR=3.09, 95%CI (1.11-8.58), p=0.031) whereas those with no such contact were not predisposed to reinfection (adjusted OR=0.91, 95% CI (0.23-3.63), p=0.59).

The following variables were significantly associated with a **higher** *Ascaris* prevalence in the univariate analysis (likelihood ratio test p<0.10), but were not significant when included in the multivariate model :-

Other effects related to living in a wastewater irrigated area

- Live increasingly near to wastewater irrigation channel (exposed group only) (trend p=0.075)

Socioeconomic variables

- Head of HH ≤30 years old (p=0.004)
- Decreasing age of wife of head of HH (trend p=0.031)
- Do not own home (p=0.006)
- Kitchen in a communal room as opposed to separate room (p=0.063)
- Do not have dog (p=0.03) or donkey (p=0.096)

Hygiene variables

- Open defecation by at least one member of HH near to house (p=0.036)

Table 8-2 Relationship between exposure to wastewater and other variables and *Ascaris* prevalence in men ≥15 years in the exposed and semi-exposed groups[47]

Variable		No/Total	Prev	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)	LRT p-value (trend p-value)
Wastewater contact						
Work relating to chilli production	None	14/156	9.0	1.00	1.00	0.011
	Sedimented	1/8	12.5	1.45 (0.17-12.64)	1.56 (0.13-18.59)	(0.003)
	Untreated	11/28	39.3	6.56 (2.57-16.74)	5.37 (1.79-16.10)	
Other effects related to living in ww irrigated area						
Crops irrigated with raw ww eaten in HH	No	11/125	8.8	1.00	1.00	0.048
	Yes	15/67	22.4	2.99 (1.29-6.95)	3.93 (1.01-15.24)	
Demographic factors						
Exposure area	Untreated	19/109	17.4	1.00	1.00	0.70
	Sedimented	7/83	8.4	0.44 (0.17-1.09)	1.34 (0.30-6.03)	
Age group (years)	15-34	18/97	18.6	1.00	1.00	0.19
	35+	8/95	8.4	0.40 (0.17-0.98)	0.52 (0.19-1.38)	
Socioeconomic factors						
Time HH has lived in village (years)	1-5	7/16	43.8	1.00	1.00	0.004
	6-10	5/29	17.2	0.27 (0.07-1.06)	0.17 (0.04-0.86)	
	≥11	14/147	9.5	0.14 (0.04-0.42)	0.10 (0.03-0.39)	

[47] HH=household, ww=wastewater

8.4 *ASCARIS* REINFECTION IN WOMEN (AGED ≥15 YEARS)

The relationship between exposure to wastewater and other factors and the prevalence of *Ascaris* reinfection for women aged ≥15 years in the exposed and semi-exposed groups are displayed in Table 8-3. For each variable, as in section 8.2 and 8.3, the prevalence of *Ascaris* reinfection, the crude odds ratio and confidence interval, and the adjusted odds ratio, confidence interval and p-value are displayed. The odds ratio for all variables are adjusted for all other variables in the model.

8.4.1 Relationship between exposure through wastewater contact and prevalence of *Ascaris* reinfection

Women who reported wastewater contact while tending to livestock in fields that received untreated wastewater for crop irrigation had a greater risk of *Ascaris* reinfection than those reporting no wastewater contact or who did not take animals to pasture (adjusted OR=3.26, p=0.065) (Table 8-3). There was no such excess risk when the fields were irrigated with sedimented wastewater, compared with those reporting no wastewater contact or who did not take animals to pasture.

8.4.2 Relationship between other variables and prevalence of *Ascaris* reinfection

The use of wastewater to clean the yard around the house was associated with a greater risk of *Ascaris* reinfection (adjusted OR=4.28, 95% CI (0.93-19.72), p=0.068) (Table 8.3). Though the number of individuals was small, there was a greater *Ascaris* prevalence associated with this practice in both the exposed and semi-exposed groups. In the exposed group, the prevalence of *Ascaris* reinfection was 60% (3/5) among those reporting wastewater contact compared with 21.1% (20/95) among those who reported no wastewater contact while cleaning the yard. In the semi-exposed group, *Ascaris* prevalence was 18.2% (2/11) among those with wastewater contact and 4.6% (3/66) among those with no wastewater contact while cleaning the yard.

Women were predisposed to *Ascaris* reinfection and remained significantly predisposed to reinfection after adjustment for wastewater contact and other risk factors (adjusted OR=4.37, 95% CI (1.51-12.66), p=0.007).

After adjustment, living in the sedimented wastewater area was associated with a significantly lower risk of *Ascaris* reinfection as compared with living in the untreated wastewater area (p=0.008). No significant age effect was observed in women, though a trend was discernible. The time the household had lived in the community was associated with a lower *Ascaris* prevalence; households present for at least 6 years had a lower risk of *Ascaris* reinfection than those present for less than 6 years (adjusted OR=0.17, 95%CI (0.04-0.67), p=0.01). There was a greater *Ascaris* prevalence amongst those who ate eggs the day prior to the interview, from 5.0% to 21.4%.

The following variables were significantly associated with a **higher** *Ascaris* prevalence in the univariate analysis (likelihood ratio test p<0.10), but were not significant when included in the multivariate model :-

Wastewater contact

- Any contact with untreated wastewater versus none or sedimented wastewater contact (p=0.002)

Other effects related to living in a wastewater irrigated area

- Live increasingly near to wastewater irrigation channel (exposed group only) (p=0.083)

Socioeconomic variables

Increasing number of individuals per bedroom (p=0.096)

Kitchen in a communal room as opposed to separate room (p=0.048)

Hygiene variables

Do not boil water before drinking (p=0.044)

Table 8-3 Effect of exposure to wastewater and other variables on *Ascaris* prevalence in women ≥15 years in the exposed and semi-exposed groups[48]

Variable		No / Total	Prev	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)	LRT p-value (trend p-value)
Wastewater contact						
Tending to livestock in wastewater irrigated fields	No	21/135	15.6	1.00	1.00	0.11
	Sedimented	1/26	3.9	0.22 (0.03-1.72)	0.70 (0.06-8.33)	(0.065)
	Untreated	6/16	37.5	4.33 (1.63-11.49)	4.39 (1.08-17.81)	
Other effects related to living in ww irrigated area						
Use ww to clean yard	No	23/161	14.3	1.00	1.00	0.063
	Yes	5/16	31.3	2.73 (0.87-8.58)	4.28 (0.93-19.72)	
Demographic factors						
Exposure area	Untreated	23/100	23.7	1.00	1.00	0.008
	Sedimented	5/77	6.0	0.23 (0.08-0.64)	0.14 (0.03-0.59)	
Age group (years)	15-34	20/96	20.9	1.00	1.00	0.26
	35+	8/81	10.3	0.42 (0.18-1.00)	0.52 (0.16-1.63)	
Socioeconomic factors						
Time HH has lived in village (years)	1-5	7/16	43.8	1.00	1.00	0.005
	6-10	7/31	22.6	0.38 (0.10-1.37)	0.43 (0.09-2.07)	(<0.001)
	≥11	14/130	10.8	0.16 (0.05-0.48)	0.09 (0.02-0.44)	
Ate eggs yesterday	No	3/60	5.0	1.00	1.00	0.021
	Yes	25/117	21.4	5.16 (1.49-17.88)	4.89 (1.27-18.86)	

[48] HH=household, ww=wastewater; 20 women lost because of missing data for variable 'clean yard with ww'

8.5 INTENSITY OF *ASCARIS* REINFECTION AMONG INFECTED INDIVIDUALS (ABUNDANCE)

8.5.1 Children under 15 years old

Among children, there was no significant difference in geometric mean abundance (GMA) of *Ascaris* reinfection when comparing those with and without wastewater contact in the exposed and semi-exposed groups. There was no difference detected in children with wastewater contact during irrigation, chilli production, play or while tending animals, when compared with those children who did not report the activity during the reinfection period.

There was no relationship between gender or age and *Ascaris* intensity (Table 8-4). The exposure area was not significant and was excluded because of problems of collinearity with other variables (no significant interactions were found). The GMA of *Ascaris* reinfection was significantly higher among the grandchildren, nieces and nephews of the head of the household than among those whose father was the head of the household. GMA of *Ascaris* reinfections was greater among under 15 year olds in households where the yard was swept at least 3 times each week. Crowding was associated with the geometric mean abundance of *Ascaris* reinfection. Intensity was greater among those with 3 or more individuals per bedroom. Consumption of eggs and of pasta broth was associated with a significant reduction in the GMA of *Ascaris* reinfection.

The following variables were significantly associated with a **greater** geometric mean abundance of *Ascaris* reinfection in the univariate analysis (likelihood ratio test $p<0.10$), but were not significant when included in the multivariate model :-

Wastewater contact

Activities relating to chilli production (p=0.094)

Play with raw wastewater (p=0.04)

Socioeconomic variables

Head of household had not completed primary school (p=0.027)

Hygiene variables

Water for drinking boiled for over 5 minutes (p=0.014)

Table 8-4 Relationship between exposure to wastewater and other variables and abundance of *Ascaris* reinfection in children under 15 years old in the exposed and semi-exposed groups[49]

Variable		Total (GM)	Crude regression coefficient	Adjusted regression coefficient (95% CI)	LRT p-value (trend p-value)
Demographic factors					
Gender	Male	59 (1563)	Baseline	Baseline	0.95
	Female	39 (1649)	0.05	-0.03 (-0.77,0.71)	
Age group (years)	2-4	24 (923)	Baseline	Baseline	0.13
	5-9	42 (2138)	0.84	0.93 (-0.05, 1.92)	
	10-14	32 (1640)	0.57	1.07 (-0.05, 2.19)	
Relation with head of HH	Son/daughter	85 (1435)	Baseline	Baseline	0.001
	Other family member	13 (3209)	0.80	2.26 (0.94,3.58)	
Socioeconomic factors					
Sweep yard (times / week)	1-2	8 (284)	Baseline	Baseline	0.078
	3+	90 (1862)	1.88	1.27 (-0.14,2.67)	
Crowding (no. per bedroom)	<2	16 (880)	Baseline	Baseline	0.006
	2-2.99	26 (973)	0.04	-0.43 (-1.58,0.71)	
	3+	56 (2382)	1.00	1.13 (0.10,2.15)	
Eat pasta broth (days per week)	0-2	63 (2135)	Baseline	Baseline	0.064
	3-4	17 (900)	-0.86	-0.91 (-1.91,0.09)	(0.026)
	5+	18 (992)	-0.77	-1.03 (-2.10,0.03)	
Eat eggs (days per week)	0	11 (2974)	Baseline	Baseline	0.23
	1-2	43 (1854)	-0.47	-0.40 (-1.60,0.80)	(0.043)
	3-4	24 (1383)	-0.77	-0.59 (-1.86,0.68)	
	5+	20 (976)	-1.11	-1.23 (-2.53,0.06)	

[49] GM=geometric mean, HH=household

8.5.2 Adult men (≥15 years old)

There was a significantly higher geometric mean abundance (GMA) of *Ascaris* reinfection among men planting chillies in the untreated wastewater area, when compared with those who planted chillies in the sedimented wastewater area or, who did not plant chillies (Table 8-5. There was also a trend of increasing intensity of *Ascaris* reinfection associated with an increased number of days planting chillies during the reinfection period. Living in the untreated wastewater irrigated area was not associated with any increase in *Ascaris* intensity when compared with those living in the sedimented wastewater irrigated area.

Age had no significant association with the intensity of *Ascaris* reinfection in men (Table 8-5). A combination of storing water and not boiling it prior to drinking was associated with an increase in *Ascaris* intensity of reinfection compared with those who did not store, or who stored and boiled water. Individuals in households that did not own a horse had a greater GMA of *Ascaris* reinfection than those who did own a horse.

The following variables were significantly associated with a **greater** geometric mean abundance of *Ascaris* reinfection in the univariate analysis (likelihood ratio test $p<0.10$), but were not significant when included in the multivariate model :-

Wastewater contact
Tending to livestock in raw wastewater irrigated fields ($p=0.06$)

Other effects related to living in wastewater irrigated area
Live increasingly near to wastewater irrigation channel ($p=0.056$)

Socioeconomic variables
Head of household had not completed primary school ($p=0.002$)

Increasing number of individuals returning to house at weekends (p=0.071)

Kitchen in communal room as opposed to a separate room (p=0.066)

Table 8-5 Relationship between exposure to wastewater and other variables and abundance of *Ascaris* reinfection in men (≥15 years old) in the exposed and semi-exposed groups[50]

Variable		Total (GM)	Crude regression coefficient	Adjusted regression coefficient (95% CI)	LRT p-value (trend p-value)
Wastewater contact					
No. days planting chillies in untreated ww field	None	19 (431)	Baseline	Baseline	0.11
	1-5	4 (847)	0.67	0.96 (-0.71, 2.63)	(0.034)
	6+	3 (3209)	2.01	1.66 (-0.06,3.39)	
Demographic factors					
Exposure area	Untreated	19 (641)	Baseline	Baseline	0.51
	Sedimented	7 (511)	-0.23	0.44 (-0.94,1.82)	
Age group (years)	15-34	18 (704)	Baseline	Baseline	0.36
	35+	8 (426)	-0.50	-0.60 (-1.94,0.74)	
Socioeconomic factors					
Horse owned by HH	Yes	6 (194)	Baseline	Baseline	0.049
	No	20 (848)	1.48	1.34 (0.01,2.66)	
Hygiene factors					
Drinking water	Direct from tap & not boiled	13 (357)	Baseline	Baseline	0.015 (0.012)
	Stored & boiled	4 (211)	-0.53	-0.32 (-1.99,1.36)	
	Store & not boiled	9 (2051)	1.75	1.72 (0.48,2.96)	

[50] GM=geometric mean, HH=household, ww=wastewater

8.5.3 Adult women (≥15 years old)

There was no significant difference in geometric mean abundance (GMA) of *Ascaris* reinfection between women with and without wastewater contact. In particular, no difference was detected in women with wastewater contact during any agricultural activities or while tending animals, when compared with those women who did not report that activity involving wastewater contact during the reinfection period.

There was no association between age and the intensity of *Ascaris* reinfection (Table 8-6). *Ascaris* intensity was slightly higher among those living in the untreated wastewater area when compared with those living in the sedimented wastewater area. There was a significant reduction in GMA of *Ascaris* reinfection for women who had lived in the community at least 6 years when compared with those that had lived in the community for less than 6 years. Consumption of beans and vegetables was associated with a lower GMA of *Ascaris* reinfection. There was a slight (but not significant) trend of decreasing geometric mean abundance of *Ascaris* reinfection with increasing consumption of vegetables.

The following variables were significant associated with a **greater** geometric mean abundance of *Ascaris* reinfection in the univariate analysis (likelihood ratio test p<0.10), but were not significant when included in the multivariate model :-

Wastewater contact

- Tending livestock in wastewater irrigated fields (p=0.071)

  (untreated ww > sedimented ww > none)

Socioeconomic variables

- Do not own home (p=0.054)
- Increasing number of bedrooms (p=0.074)
- Sweep yard 3+ times each week (p=0.013)

Table 8-6 Relationship between exposure to wastewater and other variables and abundance of *Ascaris* reinfection in women (≥15 years old) in the exposed and semi-exposed groups[51]

Variable		Total (GM)	Crude regression coefficient	Adjusted regression coefficient (95% CI)	LRT p-value (trend p-value)
Demographic factors					
Exposure area	Untreated	27 (609)	Baseline	Baseline	0.062
	Sedimented	5 (389)	-0.45	-1.73 (-3.55,0.09)	
Age group (years)	15-34	23 (650)	Baseline	Baseline	0.92
	35+	9 (401)	-0.48	0.92 (-1.30,1.44)	
Socioeconomic factors					
No. years in community	1-5	7 (2450)	Baseline	Baseline	0.024
	6+	25 (377)	-1.87	-1.50 (-2.79,-0.21)	
Ate beans yesterday	Yes	7 (1544)	Baseline	Baseline	0.006
	No	25 (429)	-1.28	-2.31 (-3.90,-0.73)	
No. days ate vegetables in last week	0	3 (1603)	Baseline	Baseline	0.36
	1-2	15 (638)	-0.92	-0.83 (-2.80,1.13)	(0.071)
	3-4	10 (462)	-1.24	-1.42 (-3.44,0.59)	
	5-7	4 (281)	-1.74	-1.92 (-4.24,0.41)	

[51] GM=geometric mean, HH=household, ww=wastewater

8.6 INTENSITY OF *ASCARIS* REINFECTION

8.6.1 Children under 15 years old

There was significantly higher intensity of *Ascaris* reinfection among children reporting contact with sedimented wastewater during play than among children with no wastewater contact during play (Table 8-7). There was no significant difference in intensity of *Ascaris* infection between children with no wastewater contact during play and children with contact with untreated wastewater during play. There was higher intensity *Ascaris* reinfection among children eating crops from local fields than among children who did not consume crops from local fields.

Ascaris intensity was greater among younger children than among older children (Table 8-7). There was no relationship between gender and *Ascaris* intensity. There was however an association between exposure area and *Ascaris* intensity, with higher *Ascaris* intensity among children in the exposed than the semi-exposed area.

There was lower *Ascaris* intensity among children in households where the head of household main job was not farming and also among households where the head of the household had completed at least primary school (Table 8-7). However, among children in households where the wife of the head of the household had attended school, even if primary school was not completed, there was higher intensity *Ascaris* reinfection than among children in households where the wife had not been to school. Consumption of eggs and of beans was associated with significantly lower intensity of *Ascaris* reinfection. Household ownership of a car was also associated with significantly lower intensity of *Ascaris* reinfection, than children in households which did not own a car. Children in households which did not own a dog had significantly lower intensity of *Ascaris* reinfection than children in households which did own dogs.

Table 8-7 Relationship between exposure to wastewater and other variables and *Ascaris* intensity in children under 15 years old in the exposed and semi-exposed groups[52]

Variable	Category	Prevalence (%)	GM (infected only)	Crude regression coefficient	Adjusted regression coefficient	95% Confidence interval	LRT p-value
Wastewater contact							
Playing with wastewater	None	57/190 (30)	1325	Baseline	Baseline		<0.001
	Sedimented ww	26/59 (44)	1245	0.74	2.10	1.04, 3.16	
	Untreated ww	16/32 (50)	4044	1.33	1.45	0.36, 2.54	
Other effects related to living in ww irrigated area							
Vegetable from local fields	No	54/200 (27)	1072	Baseline	Baseline		<0.001
	Yes	45/81 (56)	2450	1.35	2.14	1.35, 2.93	
Demographic factors							
Area	Exposed	55/151 (36)	1923	Baseline	Baseline		0.004
	Semi-exposed	44/130 (34)	1203	0.12	-1.38	-2.27, -0.49	
Age group	2-4	24/57 (42)	923	Baseline	Baseline		0.001
	5-9	42/115 (37)	2138	0.50	-1.95	-3.04, -0.86	
	10-14	33/109 (30)	1533	0.19	-0.95	-2.02, 0.12	
Gender	Male	60/136 (44)	1507	Baseline	Baseline		0.86
	Female	39/145 (27)	1649	-0.37	-0.06	-0.72, 0.60	

(this table is continued on the next page)

[52] GM=geometric mean, HH=household, ww=wastewater

Table 8-7 continued.

Variable	Category	Prevalence (%)	GM (infected only)	Crude regression coefficient	Adjusted regression coefficient	95% Confidence interval	LRT p-value
Socioeconomic factors							
Head of HH main occupation	Farmer	97/256 (38)	1577	Baseline	Baseline		<0.001
	Not farmer	2/25 (8)	967	-1.59	-3.16	-4.41, -1.92	
Head of HH schooling	None	10/21 (48)	1440	Baseline	Baseline		<0.001
	Primary incomplete	69/176 (39)	2224	-0.57	0.52	-1.00, 2.03	
	At least primary	20/84 (24)	479	-1.84	-2.28	-3.86, -0.70	
Wife of head of HH schooling	None	13/33 (39)	660	Baseline	Baseline		0.004
	Primary incomplete	71/185 (38)	1943	1.09	1.84	0.64, 3.04	
	At least primary	15/63 (24)	1169	0.46	2.64	1.19, 4.09	
Eat eggs (days / week)	0	11/25 (44)	2974	Baseline	Baseline		<0.001
	1-2	43/111 (39)	1854	-0.37	-0.31	-1.83, 1.22	
	3-4	25/64 (39)	1273	-0.78	0.64	-0.78, 2.06	
	5+	61/81 (25)	976	-1.65	-2.01	-3.42, -0.59	
Eat beans (days / week)	0-2	29/53 (55)	2837	Baseline	Baseline		<0.001
	3-4	14/37 (38)	1118	-1.18	-0.43	-1.79, 0.94	
	5+	56/191 (29)	1245	-1.00	-2.07	-3.04, -1.09	
HH own car	Yes	4/21 (19)	978	Baseline	Baseline		<0.001
	No	95/260 (37)	1592	1.98	2.70	1.47, 3.92	
HH own dog	Yes	87/241 (36)	1637	Baseline	Baseline		0.021
	No	12/40 (30)	1109	-0.65	-1.33	-2.41, -0.26	

8.6.2 Men (≥15 years old)

Men involved in maize production in the sedimented wastewater area had higher intensity *Ascaris* reinfection than those who did not produce maize, or else produced maize but did not have any wastewater contact as a result (Table 8-8). There was also significantly higher intensity of *Ascaris* reinfection among men producing chillies in the untreated wastewater area, when compared with those who did not produce chillies. There was no significant difference in intensity of *Ascaris* reinfection between those who did not produce chillies and those producing chillies in the sedimented wastewater area.

Age had a significant association with the intensity of *Ascaris* reinfection in men, with higher intensity reinfection in younger men than older men (Table 8-8). Living in the untreated wastewater irrigated area was not associated with greater *Ascaris* intensity when compared with those living in the sedimented wastewater irrigated area.

Men living in houses constructed with modern building materials had higher intensity of *Ascaris* reinfection than men living in houses constructed with traditional mud bricks (Table 8-8). There was also higher intensity reinfection among households using just gas for cooking, rather than wood, or gas and wood. Consumption of chicken on the day prior to the interview was associated with lower intensity of *Ascaris* reinfection, compared with men that had not eaten chicken the previous day.

Table 8-8 Relationship between exposure to wastewater and other variables and *Ascaris* intensity in men (≥15 years old) in the exposed and semi-exposed groups[53]

Variable	Category	Prevalence (%)	GM (infected only)	Crude regression coefficient	Adjusted regression coefficient	95% Confidence interval	LRT p-value
Wastewater contact							
Work relating to maize production	None	10/91 (11)	451	Baseline	Baseline		<0.001
	Sedimented ww	6/44 (14)	581	0.60	2.14	0.94, 3.33	
	Untreated ww	10/57 (18)	824	1.34	0.97	0.08, 1.86	
Work relating to chilli production	None	14/156 (9)	639	Baseline	Baseline		<0.001
	Sedimented ww	1/8 (13)	85	-2.96	-1.07	-2.74, 0.59	
	Untreated ww	11/28 (39)	669	0.93	2.45	1.43, 3.47	
Demographic factors							
Area	Exposed	19/109 (17)	641	Baseline	Baseline		0.62
	Semi-exposed	7/83 (8)	551	-1.03	-0.27	-1.34, 0.80	
Age group	15-34	18/97 (19)	704	Baseline	Baseline		<0.001
	35+	8/95 (8)	426	-1.98	-3.05	-3.90, -2.21	
Socioeconomic factors							
Wall material	Mud bricks	5/28 (18)	228	Baseline	Baseline		<0.001
	Stone, clay brick or block	21/164 (13)	760	1.82	2.24	1.28, 3.20	
Fuel for cooking	Wood	2/27 (7)	1218	Baseline	Baseline		<0.001
	Gas	12/88 (14)	1099	0.57	2.61	1.50, 3.71	
	Wood and gas	12/77 (16)	294	-0.96	1.11	-0.47, 2.27	
Ate chicken yesterday	No	23/154 (15)	999	Baseline	Baseline		<0.001
	Yes	3/38 (8)	565	-0.97	-2.08	-3.04, -1.13	

[53] GM=geometric mean, HH=household, ww=wastewater

8.6.3 Women (≥15 years old)

There was no significant association between wastewater contact during any agricultural activities or while tending animals and intensity of *Ascaris* reinfection, when compared with those women who did not report that activity involving wastewater contact during the reinfection period. There was however higher intensity of *Ascaris* reinfection among women using wastewater to clean the yard than among those that did not use wastewater (Table 8-9).

There was no relationship between age and *Ascaris* intensity (Table 8-9). There was however an association between exposure area and *Ascaris* intensity, with higher *Ascaris* intensity of reinfection among women in the exposed than the semi-exposed area.

There was lower *Ascaris* intensity of reinfection among women in households where the head of the household had completed at least primary school than where the head of the household had not attended school (Table 8-9). There was also lower intensity of reinfection among women in households where the wife of the head of the household was under 30 year olds, compared to where the wife was over 40 years old. The time the household had lived in the community was associated with *Ascaris* intensity, with lower *Ascaris* intensity of reinfection among women in households present for at least 6 years in the community than among women in households present for less than 6 years. Use of wood, rather than gas for cooking was associated with higher *Ascaris* intensity of reinfection. Consumption of eggs on the day prior to the interview was also associated with higher intensity of *Ascaris* reinfection, compared with women that had not eaten eggs the previous day. While consumption of vegetables on the day prior to the interview was also associated with lower intensity of *Ascaris* reinfection, compared with women that had not eaten vegetables on the previous day.

Table 8-9 Relationship between exposure to wastewater and other variables and *Ascaris* intensity in women (≥15 years old) in the exposed and semi-exposed groups[54]

Variable	Category	Prevalence (%)	GM (infected only)	Crude regression coefficient	Adjusted regression coefficient	95% Confidence interval	LRT p-value
Other effects related to living in ww irrigated area							
Use ww to clean yard	No	5/16 (31)	566	Baseline	Baseline		<0.001
	Yes	23/161 (14)	557	0.04	2.07	0.80, 3.35	
Demographic factors							
Area	Exposed	27/114 (24)	609	Baseline	Baseline		<0.001
	Semi-exposed	5/83 (6)	389	-2.39	-3.25	-4.16, -2.35	
Age group	15-34	23/110 (21)	650	Baseline	Baseline		0.17
	35+	9/87 (10)	401	-2.04	-0.52	-1.26, 0.22	
Socioeconomic factors							
Schooling of head of HH	None	1/16 (6)	2700	Baseline	Baseline		0.006
	Primary incomp.	22/140 (16)	462	-0.08	1.24	-0.02, 2.51	
	At least primary	9/41 (22)	788	1.99	2.44	1.03, 3.86	
Age of wife of head of HH (years)	>40	12/97 (12)	322	Baseline	Baseline		0.006
	30-40	12/60 (20)	740	0.97	0.20	-0.79, 1.19	
	<30	8/40 (20)	892	2.52	-2.24	-3.63, -0.85	
Time HH has lived in village (years)	1-5	7/17 (41)	2450	Baseline	Baseline		<0.001
	6-10	8/34 (24)	314	-2.43	-3.36	-4.91, -1.82	
	11+	17/146 (12)	410	-3.38	-5.75	-7.34, -4.15	
Fuel for cooking	Wood	3/32 (9)	427	Baseline	Baseline		0.034
	Gas	16/92 (17)	643	2.08	1.46	0.21, 2.71	
	Wood and gas	13/73 (18)	519	1.37	1.66	0.50, 2.82	
Ate eggs yesterday	No	28/129 (22)	540	Baseline	Baseline		<0.001
	Yes	4/68 (6)	808	-1.13	1.87	1.12, 2.63	
Ate vegetables yesterday	No	19/111 (17)	524	Baseline	Baseline		0.012
	Yes	13/86 (15)	637	0.61	-0.90	-1.60, -0.20	

[54] GM=geometric mean, HH=household, ww=wastewater

8.7 *ASCARIS* REINFECTION AND REPORTED MORBIDITY IN 5-16 YEAR OLDS

Information concerning reported illness over the past week was collected for 5-16 year olds in the study population. In order to maximise the sample size, all individuals aged 5-16 years for whom morbidity data and a reinfection result was available were included in the analyses below, whether or not wastewater contact and socioeconomic data were obtained.

An analysis of the relationship between *Ascaris* reinfection and the different measures of morbidity (as described in Chapter 4, section 4.4.2) is displayed in Table 8-10. After adjusting for the exposure area, gender and age, three morbidity variables were significantly associated with *Ascaris* reinfection. Earache was more common among children with *Ascaris* reinfection than among uninfected children. Children with *Ascaris* reinfection were more likely to complain of wind or acidity than those not infected. Children infected with *Ascaris* reinfection also slept slightly more than others. *Ascaris* reinfection was not significantly associated with any other morbidity variables (see Chapter 4, section 4.4.2. for full details).

The effects of moderate to high intensity *Ascaris* reinfection (≥5000epg) on different measures of morbidity (as described in Chapter 4, section 4.4.2) are displayed Table 8-11. After adjusting for the quality of the wastewater near the house, gender and age, two morbidity variables were significantly associated with higher intensity *Ascaris* reinfection. Children with higher intensity *Ascaris* reinfection had more general illness and more stomach ache than children with lower intensity infections. Moderate to high intensity *Ascaris* reinfection was not significantly associated with any other morbidity variables.

Table 8-10 Morbidity variables associated with *Ascaris* reinfection among children aged 5-16 years old in the exposed and semi-exposed groups[55]

Variable	*Ascaris* reinfection	Prevalence of morbidity variable No./Total (%)	Crude odds ratio (95% CI)	Adjusted[56] odds ratio (95% CI)	LRT p-value (trend p-value)
Earache	No	11/171 (6)	1.00	1.00	0.032
	Yes	13/81 (16)	2.78 (1.19-6.52)	2.66 (1.09-6.48)	
Wind or acidity	No	29/171 (17)	1.00	1.00	0.050
	Yes	21/81 (26)	1.71 (0.91-3.24)	1.97 (1.00-3.86)	
Sleep more	No	157/171 (92)	1.00	1.00	0.079
	Yes	79/81 (98)	3.52 (0.78-15.88)	3.93 (0.85-18.14)	

Table 8-11 Morbidity variables associated with moderate to high intensity *Ascaris* reinfection in children aged 5-16 years in the exposed and semi-exposed groups[57]

Variable	Moderate to high intensity *Ascaris* reinfection	Prevalence of morbidity variable No./Total (%)	Crude odds ratio (95% CI)	Adjusted[58] odds ratio (95% CI)	LRT p-value (trend p-value)
Recent illness	No	17/62 (27)	1.00	1.00	0.024
	Yes	10/19 (53)	2.94 (1.02-8.48)	3.79 (1.19-12.06)	
Stomach ache	No	18/63 (29)	1.00	1.00	0.065
	Yes	9/18 (50)	2.50 (0.85-7.31)	2.88 (0.94-8.83)	

[55] A separate model was produced for each morbidity variable

[56] Adjusted for exposure area (exposed, semi-exposed), gender & age (5-9,10-16 years)

[57] A separate model was produced for each morbidity variable

[58] Adjusted for exposure area (exposed, semi-exposed), gender & age (5-9,10-16 years)

8.8 SUMMARY

There was an increased risk of *Ascaris* reinfection for under 15 year olds associated with contact with untreated wastewater. Children under 15 years old who reported contact with untreated wastewater whilst irrigating crops had a greater *Ascaris* prevalence than those that did not irrigate crops during the 9-10 month reinfection period. The prevalence of *Ascaris* reinfection was also greater among children who had contact with untreated wastewater whilst playing, than among those who had no wastewater contact while playing. There was a trend of increasing *Ascaris* prevalence for irrigation. Prevalence was lowest among those children with not irrigating, was slightly higher among children with sedimented wastewater contact and was greatest in children with untreated wastewater contact while irrigating. Contact with sedimented wastewater was associated with a significant increase in *Ascaris* prevalence for play activities but not for irrigation.

The odds of *Ascaris* reinfection were greater among men who had contact with untreated wastewater while irrigating or planting chillies than among those not involved in chilli production. *Ascaris* prevalence was lowest among those not producing chillies, slightly higher among those producing chillies in the sedimented wastewater area and was greatest among those producing chillies in the untreated wastewater area.

Among women, tending to livestock in untreated wastewater irrigated fields was associated with a higher risk of *Ascaris* reinfection. Shepherding in fields irrigated with sedimented wastewater was not associated with a greater risk of *Ascaris* reinfection.

There was no significant association between geometric mean abundance (GMA) of *Ascaris* reinfection and wastewater contact in general or during any particular activity for children under 15 year olds or for women. There was however higher intensity *Ascaris* reinfection among children in contact with sedimented wastewater during play activities. Among men, there was a significant trend of increasing GMA associated with increasing frequency of chilli planting in the untreated wastewater area. There was also higher intensity of *Ascaris* reinfection associated with chilli production in untreated wastewater irrigated area and with maize production in the sedimented wastewater irrigated area.

There were several significant effects of living in a wastewater irrigated area that were not related to direct wastewater contact. Eating crops from local fields irrigated with untreated or sedimented wastewater was associated with a greater prevalence and higher intensity of *Ascaris* reinfection among children under 15 years old. This association was highly significant in the untreated wastewater irrigated area. This practice was also associated with a higher *Ascaris* reinfection among men.

Using wastewater to clean the yard around the house was associated with a greater prevalence and a higher intensity of *Ascaris* among women when compared with women not using wastewater to clean the yard. For men, women and children, living increasingly close to a wastewater channel was significant in the univariate but not in the multivariate analysis. There was a greater prevalence, a higher geometric mean abundance and a higher intensity of *Ascaris* reinfection among women living near untreated wastewater as opposed to sedimented wastewater. In the exposed group, there was higher intensity of *Ascaris* reinfection among children than in the semi-exposed group. This association was not observed for men.

Several socioeconomic variables were associated with a higher *Ascaris* prevalence. Prevalence was greater among children under 15 years old in households where the head of the household had not been to school, and also where there were no chickens. For men and women, the longer the family had lived in the community the lower the prevalence of *Ascaris*.

There was a higher geometric mean abundance (GMA) of *Ascaris* reinfection among children who were related to the head of the household, but were not his children. Frequent sweeping of the yard around the house and bedroom crowding was also associated with a higher GMA among children. The GMA of *Ascaris* reinfection was lower among children eating more pasta broth or more eggs, and among women eating beans or more vegetables. An increase in the number of years living in the community was also associated with a lower GMA among women. Men who owned a horse had a lower GMA of *Ascaris* reinfection than those who had no horses. Consumption of drinking water that had been stored and not boiled was associated with a higher GMA among men, when compared with men consuming drinking water that had either not been stored or had been stored and boiled.

Completion of primary school by the head of the household was associated with lower intensity of *Ascaris* reinfection among children and women. The intensity of *Ascaris* reinfection was lower among children eating more beans or more eggs, and among women eating vegetables. An increase in the number of years living in the community was also associated with lower intensity of reinfection among women. Use of gas, rather than wood, or wood and gas was associated with higher intensity of *Ascaris* reinfection. Ownership of a car was associated with lower intensity of *Ascaris* reinfection.

Morbidity associated with *Ascaris* reinfection was difficult to assess due to small sample sizes. Among those 5-16 year olds with *Ascaris* reinfection, significantly more had earache and wind or acidity when compared with children not infected with *Ascaris*. More 5-16 year olds with *Ascaris* reinfection slept more than those not infected. Children with moderate to high intensity *Ascaris* reinfection reported more general recent illness and more stomach aches than children with low intensity *Ascaris* reinfection.

CHAPTER 9. DISCUSSION

9.1 EPIDEMIOLOGY OF *ASCARIS* INFECTION

The initial and reinfection prevalence of *Ascaris* infection (initial 18% and reinfection 17% for all ages) were lower than previously reported by studies in Mexico. Stoopen and Beltran (1964) reported a prevalence of *Ascaris* of over 70% in school-age children in the Sierra Madre while Navarrete Espinosa *et al.* (1993) reported an *Ascaris* prevalence as 42% among 2-14 year olds. These differences between the study population and other studies reflect differences in exposure to *Ascaris* infection, related possibly to better living conditions in the study population when compared with other studies that were carried out in more marginalised communities. The dry climate in the Mezquital Valley may also favour desiccation of *Ascaris* ova and so reduce the risk of transmission.

The age-prevalence profile of *Ascaris* reinfection in the study population differed from that previously reported by other studies (Bundy *et al.*, 1988; Forrester *et al.*, 1988). In both the exposed and semi-exposed groups, *Ascaris* infection levels were highest in younger children and then showed evidence of a decrease with increasing age through pubescence and adulthood. Bundy *et al.* (1988) and Forrester *et al.* (1988) reported that *Ascaris* prevalence increased steadily from early to late childhood, and then either remained at that level or declined slightly in adulthood.

Despite suggestions that the 'usual' age-intensity profile of *Ascaris* infection shows an increase during childhood and then declines into adulthood (Bundy *et al.*, 1988), studies have reported a variety of age-intensity profiles of *Ascaris* infection (Croll *et al.*, 1982; Elkins *et al.*, 1986; Thein-Hlaing, 1984). In the present study population, *Ascaris* intensity levels (epg) rose from early childhood, peaked among 5-9 year olds, and then declined slightly among adults.

Reported differences in *Ascaris* prevalence between males and females have generally been either small (Arfaa & Ghadirian, 1977; Tedla & Ayele, 1986; Henry, 1988) or not significant (Stoopen & Beltran, 1964; Forrester *et al.,* 1988). Forrester *et al.* (1988) and Elkins *et al.* (1986) found higher intensity infection among females than males, while Arfaa & Ghadirian found no difference between the sexes. In this study, there were no overall differences in *Ascaris* prevalence or intensity between genders in the exposed or semi-exposed groups. Age-specific gender differences in *Ascaris* reinfection were however observed. In the exposed group, 10-14 year old boys had a greater prevalence and intensity of *Ascaris* reinfection than 10-14 year old girls.

Generalisations regarding the prevalence and intensity profile of *Ascaris* infection can not be made. The different age-dependent and gender-dependent effects observed in this study and in other studies suggest that there may be no intrinsic relationship between infection and age or gender. This could indicate that factors that are age- or gender-dependent and that vary between individuals in different communities and cultures, are determining the prevalence and intensity profiles of *Ascaris* infection. This would tend to suggest that environmental or behavioural rather than genetic factors may be influencing *Ascaris* prevalence and intensity distributions.

The impact of socioeconomic status, hygiene and sanitation on the prevalence and intensity of *Ascaris* infection has been widely studied (Arfaa & Ghadirian, 1977; Croll *et al.,* 1982; Henry, 1988; Holland *et al.,* 1988; Thein-Hlaing *et al.,* 1987, Kightlinger *et al.,* 1998; Curtale *et al.,* 1999), although the conclusions reported by these studies are often inconsistent. Despite this, Stoopen and Beltran (1964) found that poor housing associated with higher intensity infections, Holland *et al.* (1988) found poor housing and open defecation were associated with *Ascaris* infection, and Henry (1988) identified a lack of sanitation and of no piped water supply as risk factors. The differences between studies are due in part to inadequate adjustment for confounding factors and also probably to the complexity of peridomestic transmission. In this present study, the relative importance of different socioeconomic markers, and of hygiene and sanitation varied between children and adults (see Chapter 8, Tables 8.1-8.3).

The results of this study and other studies reported in the literature indicate that the importance of socioeconomic, hygiene and sanitation factors vary according to the population studied. Some factors are more common in a particular group of the population and their effect on *Ascaris* reinfection surpasses the effects of all other variables. In this study, the population was exposed to an additional transmission route by its exposure to wastewater during agricultural work and associated activities. This exposure is in addition to the other routes of transmission within the study community, and in particular within the peridomestic environment of households. This may partially explain the limited number and significance of socioeconomic, hygiene and sanitation risk factors identified in this study.

There has been a long debate regarding the factors which determine the distribution of *Ascaris* in the population. In this study, the intensity of *Ascaris* reinfection (as indicated by egg counts in stools) was overdispersed, with a few individuals having very high intensity infections. There was also a tendency for individuals to reacquire high (or low) intensity infections after treatment, as had been previously demonstrated by Anderson (1986), Bundy *et al.,* (1988) and Forrester *et al.,* (1988). There were groups of individuals that seem predisposed, not only to being infected, but also to a particular intensity of *Ascaris* infection. However, these studies, while demonstrating a predisposition to reinfection, have not examined the causal mechanisms.

The relative roles of exposure and susceptibility in determining whether an individual is infected, and the intensity of that infection have been discussed by several authors (Keymer & Pagel, 1988; Bundy, 1988). It has been suggested that behaviour, genetic susceptibility or resistance to helminth invasion and establishment or a combination of these processes may be implicated. The impact of changes in exposure due to behavioural changes has been addressed by several authors. Studies have suggested that contact with contaminated water, soil or food increases the risk of *Ascaris* infection and of higher intensity infections (Stephenson, 1985; Elkins *et al.,* 1986; Thein-Hlaing *et al.,* 1987; Bundy *et al.,* 1987) and measurement of silica levels in stool samples (as a proxy for geophagia) suggested differences in the patterns of geophagia, although the intensity measured in the study was not of *Ascaris*, but of *Trichuris*.

Age-dependency of overdispersion was reported to be an effect of co-variance with mean intensity (Guyatt *et al.,* 1990; Needham *et al.,* 1998). In this present study, it was also shown that age-dependency of overdispersion, was due mean intensity levels. It was also found that initial egg counts were more overdispersed in the exposed than the control group, and that reinfection egg counts were more overdispersed in the exposed than the semi-exposed group.

Reported morbidity related to *Ascaris* reinfection was associated with minor discomfort from wind or acidity and possibly altered sleep patterns. Moderate to high intensity *Ascaris* reinfection was associated with a slightly higher prevalence of stomach ache. It was difficult to be certain whether these were caused by other infections, since such symptoms are not *Ascaris*-specific. Abdominal discomfort and lethargy are symptoms reported to be associated with heavy infections (de Silva *et al.,* 1997). There is little evidence to indicate that there is any morbidity associated with low intensity reinfection. Global calculations suggest 4.2% of infected 5-16 year olds are at risk of some morbidity (de Silva *et al.,* 1997). Based on these calculations, in the present study (where the of number of 5-16 year olds infected was 81), four individuals would be expected to report some morbidity. In fact, more children appeared to report minor symptoms, however since the symptoms are not *Ascaris* specific, there may have been confounding with other illnesses that were not reported.

9.2 QUANTIFYING CONTACT WITH WASTEWATER

Exposure to water has been measured in several studies in relation to the associated risk of schistosomiasis (Dalton 1976; Dalton & Pole, 1978; Kloos & Lemma, 1980; Tiglao & Camacho, 1983; Kloos *et al.,* 1983; Butterworth *et al.,* 1984; Wilkins *et al.,* 1984; Blumenthal, 1985; Etard, J, Audibert, M & Dabo, A., 1995, Kloos *et al.,* 1997). For a particular community there are often a number of defined sites where the water is known to contain infectious cercariae. This has enabled investigators to observe individuals visiting those sites, rather than observe individuals in their homes, as is often required in studies of peridomestic transmission.

Studies have provided circumstantial evidence that reuse of untreated wastewater is associated with an increased risk of *Ascaris* infection (Khalil, 1931; Krishnamoorthi *et al.*, 1973; Shuval *et al.*, 1986) but no attempt has been made to measure the amount of contact with wastewater or to determine the components of contact and their relative roles in determining the prevalence of *Ascaris* and the intensity of infection. There have been a number of studies that have measured the levels of environmental contamination with *Ascaris* ova, but few have looked at the degree of contact with the environment by the exposed population.

This study measured contact with wastewater and, in the same Mexican population, determined the prevalence and estimated the intensity of *Ascaris* reinfection, as well as measuring the concentration of *Ascaris* ova in the wastewater.

The results demonstrate that activities involving contact with wastewater varied according to age and gender. Nearly all agricultural activities were done by males, and most activities involving wastewater contact and related to crop production were performed by men aged 15 years or older. In contrast, the traditional task in the region of taking the family's livestock to graze in local fields was done mainly by women, although men and children also helped. As well as economic activities involving wastewater contact, there were also domestic activities that lead to exposure to wastewater. Women reported using wastewater to sweep the yard around the home and to irrigate houseplants. Children under 15 years old, and particularly 5-9 year olds, had wastewater contact through a variety of play activities that were linked to the irrigation ditches that often ran past their homes and to the larger irrigation channels. These findings agree with the studies of water contact relating to the transmission of schistosomiasis which found activities involving water contact to be both age- and gender-dependent (Dalton, 1976; Fulford *et al.*, 1996).

The observation studies of farmers in the fields and the in-depth reports from individuals of past wastewater contact demonstrated that different agricultural activities resulted in different amounts of wastewater contact. The crop also affected the degree of contact with the wastewater. There was wastewater contact in over 80% of irrigation sessions. The amount of contact varied according to whether the crop irrigated was maize, alfalfa or chilli. There was more hand contact with wastewater during alfalfa irrigation. At the same time farmers had to wait around more during alfalfa irrigation than during maize irrigation.

No previous studies have shown the full complexities of wastewater contact. However Dalton (1976) in studies of schistosomiasis transmission had demonstrated that there were variations in the level of body contact and the duration of contact according to the activity performed. Water contact was found to be high during bathing, but the duration was lower than during clothes washing.

Age and gender, as well as influencing the activity performed, also affected the amount of contact during a particular activity. The amount of water contact during irrigation was lower in younger individuals. Children were more likely than women to have wastewater contact while tending livestock. These results agree with similar findings in water contact studies related to schistosomiasis transmission (Dalton, 1976; Kloos & Lemma, 1980; Fulford *et al.*, 1996).

Activities involving wastewater contact varied slightly between the exposed and semi-exposed groups, despite the socioeconomic and cultural similarities between these two groups. There was a large chilli-growing region in the exposed area, whereas chilli was grown on a smaller scale in the semi-exposed group.

Dalton (1976) found that proposals to introduce a domestic water supply to reduce contact with river water were flawed because the river had a social value as a meeting place for the community, and that crossing the river and various economic activities involving water contact would continue.

The situation in the present study area is equally complicated. Contact with wastewater occurs in the study population, not only as a result of direct contact with the wastewater during irrigation or because of irrigation prior to transplanting chillies, but also through the presence of the wastewater ditches and channels in the communities studied. The wastewater is used by the housewife and occasionally played with by the children. The irrigation ditches around fields are open and there is often no way to cross but jump (unpublished data). As a result, many reported contacts with wastewater occur accidentally, and consequently are less common among adults than children, who reported more contact through slipping into irrigation ditches than did adults. Despite the wastewater being grey or black, and having a strong odour, there would appear no intense aversion to wastewater contact. A farmer preparing to irrigate will often remove his shoes and roll up his shirt sleeves to protect his clothing, although many farmers said that contact with untreated wastewater often caused skin irritations.

9.3 IMPACT OF EXPOSURE TO WASTEWATER AND OTHER VARIABLES ON *ASCARIS* REINFECTION

9.3.1 Exposure to wastewater

Once patterns of wastewater contact are established, the effect of exposure to wastewater on *Ascaris* reinfection can be assessed. This study has shown that exposure to *Ascaris* eggs through contact with wastewater can, in certain circumstances, be associated with an increase in the prevalence and intensity of *Ascaris* reinfection.

The pretreatment prevalence and intensity of *Ascaris* infection was low in the control group and reinfection levels lower still. This meant that the relationship between wastewater exposure and *Ascaris* infection was investigated by comparisons made between individuals with varying degrees of wastewater contact in the exposed and semi-exposed groups. It was not possible to assess the excess infection due to living in a wastewater irrigated area. There was concern, that choosing a non-wastewater irrigated area may have been an inappropriate control group for this study. It was possible that *Ascaris* infection levels in the control group were low because of the dry environment causing desiccation of the *Ascaris* eggs and that a white water irrigated area should have been chosen as the control group. After the conclusion of the study described in this thesis, and while the author was working on a subsequent study, an area where farmers used well water to irrigate the fields was located in the municipality of Tecozautla, Hidalgo State. A small substudy of *Ascaris* prevalence was carried out in 3 communities in this area (El Paso, Banzha and Bothe). A description of the area and the population can be found in Blumenthal *et al* (1999) (in preparation). *Ascaris* prevalence levels in Tecozautla were low (8/626=1.3%), and found not to be not dissimilar to those obtained from the control group studied for this thesis (8/310=2.6%) (p=0.15) (Table 9-1). It may be argued retrospectively that a more suitable control group could have been found. However, this comparison of the control group and a well water irrigated region suggests that the rain-fed group was indeed a reasonable control population.

Table 9-1 Comparison of prevalence of *Ascaris* infection in control group and a well water irrigated area[59]

Age group (years)	Control group in present study (rainfed) No/Total (%)	Tecozautla group (well-water irrigated) No/Total (%)
2-4	0/22 (0.0)	2/80 (2.5)
5-9	1/52 (1.9)	3/118 (2.5)
10-14	2/53 (3.8)	2/80 (2.5)
15+	5/183 (2.7)	1/348 (0.3)

In the study population as a whole, there was no increased risk of *Ascaris* reinfection related to the total number of occurrences of wastewater contact. This is maybe not surprising, since it has been shown that the amount of wastewater contact and the degree of body contact vary according to the activity and to the age and gender of the individual. Activities such as irrigation were shown to result in frequent contact of feet and periodic contact of hands with wastewater, while other reports of contact were short in duration but involved a greater degree of wastewater contact. For example, one individual had spent the day in the fields tending livestock and at one point during the day went into a deep irrigation ditch (wastewater can be 1m deep), to push out a cow, and had to stand up to his waist in the wastewater.

Specific activities were identified as being associated with *Ascaris* reinfection for different sections of the population. Children reporting to have irrigated during the reinfection period had four times the risk of *Ascaris* reinfection than children not irrigating. Play involving wastewater contact was also associated with an increased risk of *Ascaris* reinfection. Compared with children not having contact with wastewater during play, the risk of *Ascaris* reinfection was 2.6 times greater where children had contact with sedimented wastewater. Men who grew chillies in the untreated wastewater area had an higher risk of *Ascaris*

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[59] Data from Tecozautla is unpublished.

reinfection than those no cultivating chillies. It was not possible to assess the risk associated with chilli production in the sedimented area because of the small number of men involved in this practice. Women that took livestock to pasture in fields irrigated with untreated wastewater had more than a three-fold increase in risk of *Ascaris* reinfection compared with those who did not have wastewater contact or who were not involved in this activity. There was no excess risk associated with contact with sedimented wastewater while tending livestock in fields previously irrigated with sedimented wastewater.

Studies of schistosomiasis and water contact have also shown that an increase in exposure to infectious cerceriae is associated with an increased risk of infection. Blumenthal (1985) found a relationship between exposure and reinfection, with higher exposure among children who were reinfected than among those that were not.

In this study, chilli planting by men was associated with higher intensity reinfection. There was an increased risk of *Ascaris* reinfection associated with increased frequency of chilli planting during the re-infection period. Similar associations have been reported for schistosomiasis, where studies have found increasing exposure associated with increasing intensity of infection (Butterworth *et al.,* 1984; Wilkins *et al.,* 1987).

The results from this study demonstrate that environmental contamination and the amount of exposure to infection are important factors in determining whether an individual is infected and the intensity of that infection.

9.3.2 Other Variables

After adjustment for significant wastewater contact and confounding variables, although the association with age was no longer significant, the risk of *Ascaris* reinfection was three times greater among boys than girls under 15 years old. Frequency of contact with the wastewater during the reinfection period was greater for boys than girls. Among 5-14 year olds, boys accounted for 92% of all reported wastewater contact during the reinfection period. This suggests that there was an unexplained residual effect of wastewater contact among boys.

Crowding, defined as number of individuals per bedroom, was associated with more intense reinfection among children, an indication of intra-familial transmission of *Ascaris* infection in the domestic environment. Household clustering of *Ascaris* reinfection was significant in the study population as a whole. A study of *Ascaris* in Dar es Salaam (Killewo *et al.*, 1991) found a significant degree of household clustering and reported that this as an indication that exposure in the home was an important route of transmission.

Storing and not boiling water before consumption was related to an increased risk of *Ascaris* reinfection among men. This practice was also significant in the univariate analysis for women, but not in the multivariate analyses. It is unclear why this association was only among adults, unless only adults drank stored water and it was kept for long periods and contaminated prior to use. Alternatively this may have been a general indicator of "hygiene awareness".

Among under 15 year olds there was a more than two-fold increase in the risk of *Ascaris* reinfection from living in households that did not have domestic fowl, and a trend of decreasing intensity of *Ascaris* reinfection associated with increased consumption of eggs. It is difficult to explain these findings, but since it has appeared for under 15 year olds in both the prevalence and intensity analyses (only positive counts) suggests the association may be real. A conflicting result was obtained for women, for whom "eating eggs yesterday" was associated with a greater prevalence and a higher intensity of *Ascaris* reinfection. However, numbers were small and this may have been an anomalous result; also studies of consumption in the region (unpublished data) indicate that consumption patterns vary enormously depending on the day of the week, so there may have been confounding, since households were not visited in random order, but households in each community were visited in turn for logistical reasons.

Consumption of pasta broth, beans and eggs was associated with lower intensity of *Ascaris* reinfection in children. In women, consumption of vegetables was associated with a similar decrease in intensity of *Ascaris* reinfection. As it seems unlikely that there are direct benefits from consumption of pasta broth, beans, vegetables or eggs (mentioned previously), it is more likely that increased consumption of these products is a proxy for generally improved living standards.

Living in the village for over 5 years was strongly associated with a reduced risk of *Ascaris* reinfection in adults and a lower risk of high intensity *Ascaris* reinfection in women. Individuals who had been there for less than 6 years may have had a slightly different cultural background. There was a close correlation between duration of residence and home ownership. This would suggest that families that had been in the village less time may have been less well established and that such families are more exposed to infection.

No area effect was associated with prevalence of *Ascaris* reinfection for children or men. There was however a significantly lower risk of *Ascaris* reinfection and lower egg counts in women in the semi-exposed group as compared with the exposed group. There was also lower intensity of *Ascaris* reinfection among children in the semi-exposed than the exposed group. There was no significant difference in reported wastewater contact between women in the exposed and semi-exposed groups. This suggests that there may have been other activities involving wastewater contact that were not reported or other sources of infection common to women.

As well as an increased risk of *Ascaris* reinfection related to direct contact with wastewater, there were several other risks associated with living in wastewater irrigated areas. For children, consumption of crops from any local field, whether irrigated with untreated or sedimented wastewater, was associated with an increased risk of *Ascaris* and higher intensity of *Ascaris* reinfection. Among men, there was only such a risk with crops from fields irrigated with untreated wastewater. This factor was also significant for women in the univariate analyses.

Living close to an irrigation channel (defined as the time to walk to the nearest irrigation channel) was associated with a significantly greater risk of *Ascaris* reinfection in children and adults in the exposed group (trend $p<0.001$, when adjusting for age and gender) but not in the semi-exposed group. This effect was lost in the multivariate analyses, because of the interaction with exposure group. Among women, the practice of sweeping the yard with wastewater was associated with over a two-fold increased risk of *Ascaris* reinfection. Increased sweeping of the yard (without noting whether wastewater was used) was associated with a slightly greater risk of intense infections among children. This may be reflecting the use of wastewater to sweep the yard. No association was found with the variable "sweep yard with wastewater" for children. However this was an individual variable; therefore it is quite likely that children would not have been recorded as sweeping the yard, since this is a job normally carried out by the adult females. Sweeping the yard with wastewater would have resulted in a general increase in contamination of the yard and would have been an additional exposure for members of the household frequenting the yard. This agrees with reports in the literature (Moraes, 1996; Smith, 1993). Moraes (1996) found that communities with drains (which prevented flooding of sewage) had lower levels of *Ascaris* infection, compared with communities with no drains. This was also associated with a decrease in *Ascaris* intensity. Smith (1993) found that for children, living next to a street with faecal contaminated sand (due to flooding of sewage) had a greater risk of *Ascaris* reinfection as compared to living beside a street with no contaminated sand.

9.3.3 Predisposition to infection

Previous studies of predisposition to *Ascaris* infection (Hall *et al.*, 1992; Chan *et al.*, 1992; Chan *et al.*, 1994) have shown that certain individuals are predisposed to high (or low) intensity infections. The relative roles of environment, behaviour, genetic factors and susceptibility have been discussed by several authors (Bundy *et al.*, 1988; Forrester *et al.*, 1988). The identification of a co-dominant gene that appears to control susceptibility to *Schistosoma mansoni* suggested that about 5% of the sample population were genetically predisposed to intense infections (Abel *et al.,* 1991). On the other hand, a recent study of genetic relatedness and *Ascaris* infection (Chan *et al.,* 1994b) found that while familial predisposition to infection had previously been demonstrated (Forrester *et al.,* 1990; Chan *et al.,* 1994a), predisposition was no more closely correlated between parents and their children than between parents. In fact, there was a closer association of predisposition between parents than between parents and their children (Chan *et al.,* 1994). This suggested that environmental and behavioural factors and not genetic relatedness were the predominant determinants of infection status in the population.

This present study showed that there was predisposition to reinfection in the exposed group, but none in the semi-exposed or control groups. In the exposed group, it initially seemed that predisposition only occurred among women and children. However further analysis of reinfection indicated that there was predisposition to reinfection among older boys and men who had reported contact with untreated wastewater, but not among men with sedimented wastewater contact, nor among those with no wastewater contact. The study also found that individuals in the exposed group were predisposed to high (or low) intensity infections.

This confirms the finding of Moraes (1996) that the degree of predisposition was affected by environmental interventions such as sanitation. He found that predisposition was increased by reducing "random" transmission in the public domain, which allowed domestic transmission to assume greater importance. In the present study by contrast it appears that, among those consistently exposed to wastewater in the public domain (in this case, in wastewater-irrigated fields), the tendency to predisposition is greater. The range and frequency of wastewater contact activities was similar in the exposed and semi-exposed groups. However the concentration of *Ascaris* eggs in the wastewater was higher in the exposed area and therefore the risk of *Ascaris* infection greater. Consequently, overdispersion of egg counts was greater in the exposed than the semi-exposed group.

The explanation lies in the fact that not all individuals living in the wastewater irrigated areas are equally exposed to infection. Predisposition to infection will be apparent whenever a certain group of people is highly exposed to specific risk factors, which do not change from the period of first infection to reinfection. As noted above, men cultivating chillies for example, are particularly at risk and the same individuals cultivate chillies year after year. Repeated exposure to a highly contaminated environment (e.g. contact with untreated wastewater) by a particular group of the population can be expected to result in that group appearing to be more predisposed to reinfection than individuals with no single overriding source of contamination, e.g. domestic transmission but no wastewater contact.

This explanation is supported by the results of multivariate analyses. Children under 15 years old were predisposed to *Ascaris* reinfection (OR=2.01, p=0.012). However, after adjustment for activities involving contact with wastewater and other risk factors for infection, the significance of such predisposition among children was reduced substantially (OR=1.73. p=0.093). Older boys (10-14 year olds) and adult men who had contact with untreated wastewater were predisposed to reinfection (OR=3.09, p=0.031) but those with no such contact were not predisposed to reinfection (OR=0.91, p=0.59).

The range and frequency of wastewater contact activities was similar in the exposed and semi-exposed groups. However the concentration of *Ascaris* eggs in the wastewater was higher in the exposed area and therefore the risk of *Ascaris* infection greater, as was demonstrated in the multivariate analyses. Consequently, overdispersion of egg counts was greater in the exposed than the semi-exposed group, because there was a greater range of exposures in the exposed group than the semi-exposed group.

Results from this present study, together with other studies, show that exposure to infection is important in the acquisition of *Ascaris* infection and in determining the distribution of intensity of infection in the population. This study has shown that exposure through contact with untreated wastewater can increase the risk of *Ascaris* reinfection, although transmission of *Ascaris* infection in the domestic domain was also shown to be important for certain groups of the population.

Only specific activities involving wastewater contact were associated with an increased risk of *Ascaris* reinfection in this population. It was shown that all children had at least one instance of contact with the wastewater during irrigation. Irrigation made up a significant proportion of all wastewater contact for under 15 year old boys. The multivariate analyses indicated that irrigating with untreated wastewater was associated with a four-fold increase in the risk of *Ascaris* reinfection.

The other activity involving wastewater contact that was significantly associated with an increased risk of *Ascaris* reinfection was play. Multivariate analyses indicated that play with sedimented wastewater contact was associated with more than a two-fold increase in the risk of *Ascaris* reinfection compared with children who had no wastewater contact during play activities. Playing with untreated wastewater was not associated with a significant increased risk of reinfection. Hand contact was included in over 80% of play involving contact with wastewater. Play was a significant component of all wastewater contact for under 15 year olds in the semi-exposed, and a much smaller component in the exposed group. This may explain why playing with untreated wastewater was not associated with an increased risk of *Ascaris* reinfection.

Shepherding was not identified as a risk factor for *Ascaris* infection in under 15 year olds, despite it being a major component of wastewater contact for this age group. However it was shown that the extent of wastewater contact during shepherding was lower than during play or irrigation. Therefore it seems likely that the combined effects of wastewater contact during irrigation and play masked any effect that may have resulted from wastewater contact during shepherding.

Similar comparisons of wastewater contact and infection can be made for adults. Men reported a range of activities related to farming that involved wastewater contact; however only planting chillies was found to be significant after adjustment for confounders. Chilli planting was a very small component of all wastewater contact by men. Observation studies and reports from farmers indicated that it was an intense activity involving prolonged hand contact with the wastewater, whereas irrigation did not involve continuous contact. Among women, over 80% of wastewater contact occurred while shepherding, so it was not surprising to find that shepherding in fields irrigated with untreated wastewater was associated with a four-fold increase in the risk of *Ascaris* infection when compared with women that did not shepherd or had reported no wastewater contact while shepherding.

Contact with wastewater played a lesser role in determining the intensity of infection. An association was only found for men, among whom increased planting of chillies was associated with higher intensity of *Ascaris* reinfection. Among both children and women, transmission in the domestic domain was more important. Increased *Ascaris* intensity in children was associated with crowding and a poorer diet, possibly a proxy for general conditions in the household.

Therefore it can be concluded that there is evidence to suggest that exposure, whether outside the home, in the public domain, or in the peridomestic environment, is a major determinant of the prevalence of *Ascaris* infection. Predisposition to reinfection in children in this study was not age or gender dependent but was shown to be largely dependent on environmental and behavioural factors. Long-term consistency of exposure in the public domain by a particular sector of the population resulted in predisposition to infection. In contrast, more random exposure in the domestic domain in the absence of wastewater contact reduced predisposition.

9.4 STRENGTHS AND WEAKNESSES OF THE STUDY

The strengths and weaknesses of this study are listed below,

Strengths

a) The exposure and reinfection data were collected on the same population over the same period of time.

b) The study estimated both prevalence and intensity of *Ascaris* infection and reinfection by measuring egg counts per gram of faeces

c) The setting made it easier to measure exposure to *Ascaris* infection than is possible in most other settings, where most exposure occurs in the peridomestic environment.

d) Structured observation studies of farmers performing agricultural activities enabled detailed measurement of the many aspects of wastewater contact according to activity and crop.

e) Two in-depth wastewater contact interviews with individuals established exposure patterns over the reinfection period. Information on the date of the wastewater contact, the irrigation channel, the activity, the crop and the amount of wastewater was collected.

f) Control for potential confounding factors was ensured through the application of a questionnaire to the wife of the head of the household concerning socioeconomic, hygiene and sanitation factors. Those factors found to be significant in the univariate analyses were then adjusted for in multivariate analyses for each outcome.

g) Multivariate analysis was also used to test the degree to which predisposition to reinfection could be explained by differential exposure to environmental and behavioural risk factors.

h) Random selection of households eligible for inclusion in the study, proportionate to the number of households in each community, minimised selection bias.

i) Sources of information bias were minimised by ensuring that all individuals, with the exception of young children, responded for themselves in the wastewater contact interview. Mothers responded for children under 10 years old, who were also present during the interview.

Weaknesses

a) The observation studies were not carried out exclusively with individuals from the selected study population. Logistical difficulties were caused by farmers often not knowing exactly when they would irrigate or plant; this meant that other farmers were also observed, so as to ensure a sufficient number of observations to allow data comparisons to be made.

b) A larger sample size in the control group would have enabled more detailed data comparisons between the exposed and semi-exposed groups and the control group, because of the very low reinfection prevalence of *Ascaris* infection.

c) It could be argued that it is better to study individuals who are all living in the same area (to reduce residual unexplainable effects often caused by cultural differences) but this was not possible. The wastewater quality in a particular area was uniform, therefore it would not have been possible to assess the effect of reduced contamination on infection, had all individuals come from one area. It would only have been possible to assess the effect of less frequent exposure on infection levels. The objectives relating to assessing the relationship between exposure to sedimented rather than untreated wastewater and the risk of *Ascaris* infection could **not** have been addressed. Adjustment for any area effect was however made in all multivariate analyses.

d) There was no irrigation in the control area. It may have been better to choose a control group from an area irrigating with surface water. The environment in a rain-fed area may be drier and may affect the survival of *Ascaris* ova. Unfortunately such an area was not identified until after the completion of this study. A subsequent substudy of *Ascaris* prevalence, in an area irrigating with borehole water, showed similar prevalence to the control group, suggesting that the rainfed group was indeed an appropriate control group.

e) The sensitivity of the laboratory methodology adopted to monitor the concentration of *Ascaris* eggs in raw wastewater was low, and consequently recovery of ova from raw wastewater was only 25% (Ayres 1989). As a result, the concentration of *Ascaris* ova in raw wastewater may have been underestimated.

f) Worm intensity was not measured directly by worm expulsion, but by inference from egg counts in stools.

9.5 CONCLUSIONS

9.5.1 *Ascaris* epidemiology

1) Exposure, defined as "any reported contact with untreated or sedimented wastewater", is **not** associated with an increased risk of *Ascaris* infection, nor with higher intensity infections.

2) On the other hand, the specific components of an individual's total exposure over a defined period that result in exposure to a highly contaminated environment or high exposure to a less contaminated environment **are** associated with an increased risk of *Ascaris* infection and with higher intensity infections.

3) Predisposition to *Ascaris* infection in children is largely a consequence of behavioural and environmental factors. Predisposition is a function of exposure to a contaminated environment. Changes in behaviour that reduce or eliminate exposure to this environment result in a significant reduction in predisposition.

4) Predisposition to *Ascaris* infection in adult men is related to exposure in the fields. Removal of that source of environmental contamination eliminates that predisposition.

5) Overdispersion of *Ascaris* intensity was greater in the exposed group than the semi-exposed group. Overdispersion was neither age- nor gender-dependent.

9.5.2 Wastewater reuse

1) Contact with untreated wastewater during crop irrigation is associated with a three-fold increase in *Ascaris* infection among children under 15 years old.

2) Contact with untreated wastewater while carrying out agricultural activities relating to the production of chillies is associated with a five-fold increase in *Ascaris* infection and in higher intensity infections among men.

3) Contact with untreated wastewater whilst tending livestock in wastewater irrigated fields is associated with more than a four-fold increase in *Ascaris* infection among women.

4) Consumption of crops from fields irrigated with untreated wastewater is associated with more than a two-fold increase in the risk of *Ascaris* infection in men and children, and higher intensity infections among children.

5) Use of untreated wastewater to sweep the yard around the home is associated with a 5-fold increase in *Ascaris* infection among women, higher intensity infections among women and is possibly associated also with higher intensity infections in children

6) Contact with sedimented wastewater during play is associated with more than a two-fold increase in *Ascaris* infection among children under 15 years old

7) Contact with sedimented wastewater during maize production is associated with higher intensity infections in men.

8) Contact with sedimented wastewater is not associated with any increase in the prevalence or intensity of *Ascaris* infection in women

9.5.3 Wastewater quality

1) The untreated wastewater in the exposed area has a mean concentration 96 *Ascaris* ova per litre over a 12-month period

2) The sedimented wastewater in the semi-exposed area has a mean concentration of <1 *Ascaris* ova per litre over a 12-month period

9.6 IMPLICATIONS OF THE FINDINGS AND FURTHER AREAS OF RESEARCH

This study has demonstrated that predisposition to *Ascaris* infection can be a function of behaviour and environment, whether exposure is in the public or domestic domain. It has shown that exposure to untreated wastewater is associated with the prevalence and intensity of *Ascaris* infection. If particular activities, whether economic or domestic, are associated with an increase in *Ascaris* infection, then changes in these activities would reduce infection. There is a need for more awareness by the population of the risks associated with particular activities. Simple solutions to reducing exposure to environmental contamination need to be identified. Some activities, such as irrigation, are essential and could obviously not be stopped. However a greater awareness of the risks associated with contact with untreated wastewater, and the benefits of reducing this may help to minimise unnecessary contact.

Recent advances in thinking regarding effective and cost-efficient helminth control through chemotherapy suggest a strategy of targeting three particularly vulnerable groups; pre-school children, schoolchildren and women of reproductive age (Hall *et al.*, 1997). Establishing that certain types of exposures are associated with *Ascaris* infection means that at-risk sectors of the population could also be identified and targeted for treatment. Determination of whether this exposure and therefore transmission is in the domestic or public domain has implications for helminth control (Cairncross *et al.,* 1996).

Further research is now required to investigate the role of environment and behaviour in determining the prevalence and intensity of other geohelminth infections. Establishing that exposure is a major determinant of infection for the common geohelminth infections affecting man will have important consequences for helminth control.

Living in an area where parasitic infections are frequent and where wastewater channels are commonplace seems to create a degree of misconception among the population. The economic benefits of wastewater are enormous and the associated health risks often not well understood. There is a need to promote a better understanding of these risks. Authorities in charge of wastewater irrigation schemes need to address the risks to farmers from using untreated wastewater. Health authorities must recognise the public health risks associated with wastewater contact not directly related to crop irrigation. As well as periodic chemotherapy campaigns, promotion of hygiene awareness is essential if such risks are to be reduced.

References

Abel, L., Demenais, F., Parata, A., Souza, A.E. & Dessein, A. 1991. Evidence for the segregation of a major gene in human susceptibility / resistance to infection by *Schistosoma mansoni. American Journal of Human Genetics* **48**, 959-970.

Anderson, R.M. 1982. Population dynamics and control of hookworm and roundworm infections. In Population Dynamics of Infectious Diseases: Theory and Applications (ed. R.M. Anderson), pp 67-108. London: Chapman and Hall.

Anderson, R.M. 1986. The population dynamics and epidemiology of intestinal nematode infections. *Transactions of the Royal Society Tropical Medicine and Hygiene* **80**, 686-696.

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

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

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

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

Arfaa, F. & Ghadirian, E. 1977. Epidemiology and mass-treatment of ascariasis in six rural communities in Central Iran. *American Journal of Tropical Medicine and Hygiene* **26(5)**, 866-871.

Augustine, D.L. 1926. Studies and observations of soil infestation with hookworm in southern Alabama from October 1923 - September 1924. *American Journal of Hygiene* **6**, 63-79. Cited in Anderson, R.M. 1986. The population dynamics and epidemiology of intestinal nematode infections. *Transactions of the Royal Society Tropical Medicine and Hygiene* **80**, 686-696

Ayres, R.M. 1989. A practical guide to the enumeration of intestinal helminths in raw wastewater and effluent from waste stabilisation ponds. Tropical Public Health Engineering, Department of Civil Engineering, Leeds University.

Baumhogger, W. 1949. Ascariasis in Darmstadt and Hessen as seen by a Wastewater Engineer. *Zeitschrift fur Hygiene und Infektionskrankheiten* **129**, 448-506. Cited in Wastewater Irrigation in Developing Countries : Health Effects and Technical Solutions (eds. Shuval *et al.*). World Bank Technical Paper No. 51 (1986).Washington, DC : The World Bank.

Bell, S. 1965. The Amru people of Kenya. A medical and sociological study. Part IX. Ascariasis, taeniasis and ancylostomiasis; conclusion. *Journal of Tropical Medicine and Hygiene* **59**, 121-133.

Ben-Ari, J. 1962. The incidence of *Ascaris lumbricoides* and *Trichuris trichiura* in Jerusalem during the period 1934-1960. *American Journal of Tropical Medicine and Hygiene* **11**, 336-368. Cited in Wastewater Irrigation in Developing Countries : Health Effects and Technical Solutions (eds. Shuval *et al.*). World Bank Technical Paper No. 51 (1986).Washington, DC : The World Bank.

Blumenthal, U.J. 1985. Transmission of *Schistosoma haematobium* in seasonal pools in The Gambia with particular reference to the role of human water contact. PhD. Thesis. University of Liverpool.

Blumenthal, U.J. 1988. Generalised Model of the Reduction in Health Risk Associated with Different Control Measures for the Use of Human Wastes. *IRCWD News* **24/25**, 13-17.

Blumenthal, U.J., Peasey, A., Quigley, M. & Ruiz-Palacios, G. 1999. Risk of enteric infections through consumption of vegetables irrigated with contaminated river water. In preparation.

Blumenthal, U.J., Strauss, M., Mara, D.D. & Cairncross, S. 1989. Generalised model of the effect of different control measures in reducing health risks from waste reuse. *Water, Science and Technology* **21**, 567-577.

Bouhoum, K. & Schwartzbrod, J. 1998. Epidemiological study of intestinal helminthiasis in a Marrakech raw sewage spreading zone. *Zentralblat für Hygiene und Umweltmedizin* **200** (5-6), 553-561.

Bundy, D.A.P. 1986. Epidemiological aspects of *Trichuris* and trichuriasis in Caribbean communities. *Transactions of the Royal Society Tropical Medicine and Hygiene* **80**, 706-718.

Bundy, D.A.P. 1988a. This Wormy World. *Parasitology Today* 4(8), 234 (including reply from Crompton D.W.T.).

Bundy, D.A.P. 1988b. Population ecology of intestinal helminth infections in human communities. *Phil. Transactions of the Royal Society of London* B **321**, 405-420.

Bundy, D.A.P. 1997. This Wormy World - Then and Now. *Parasitology Today* **13** (11), 407-408.

Bundy D.A.P. & Blumenthal U.J. 1990. Human behaviour and the epidemiology of helminth infection: the role of behaviour in exposure to infection. In: Parasitism and host behaviour. Ed. CJ Barnard and JM Behnke. London: Taylor and Francis, 1990, pp 264-289.

Bundy, D.A.P. & Cooper, E.S. 1988. The evidence for predisposition to trichuriasis in humans: comparison of institutional and community studies. *Annals of Tropical Medicine and Parasitology* **82**(3), 251-256.

Bundy, D.A.P., Cooper, E.S., Thompson, D.E., Didier, J.M. & Simmons, I. 1987. Epidemiology and population dynamics of *Ascaris lumbricoides* and *Trichuris trichiura* infection in the same community. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **81**, 987-993.

Bundy, D.A.P., Kan, S.P. & Rose, R. 1988. Age-related prevalence, intensity and frequency distribution of gastrointestinal infection in urban slum children from Kuala Lumpa, Malaysia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **82**, 289-294.

Bundy, D.A.P., Thompson, D.E., Golden, M.H.N., Cooper, E.S., Anderson, R.M. & Harland, P.S.E. 1985. Population distribution of *Trichuris trichiura* in a community of Jamaican children. *Transactions of the Royal Society Tropical Medicine and Hygiene* **79**, 232-237..

Butterworth, A.E., Dalton, P.R., Dunne, D.W., Mugambi, M., Ouma, J.H., Richardson, B.A., Arap Siongok, T.K. & Sturrock, R.F. 1984. Immunity after treatment of human schistosomiasis mansoni. I. Study design, pretreatment observations and the results of treatment. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **78**, 108-123.

Castilho, V.L.P., Guizelini, E., Turri, E.S., Campos, R., Amato Neto, V., Moreira, A.A.B. & Pinto, P.L.S. 1984. Exame Parasitologico Quantitativo das Fezes: Estudo Comparativo entre Os Metodos de McMaster, Stoll-Hausheer e Kato-Katz. *Revista da Sociedade brasileira de Medicina Tropical* **17**, 209-212.

Castaneda, I.G., Cerda, J.H., Lozano, A.G. & Trujillo, J.A. 1983. Study of the reuse of wastewater and the health risks for farm workers. Master's thesis, School of Public health, Mexico City, Mexico.

CEPIS 1995. Uso de Aguas residuales. Repindex No.53, Introduccion. Mar. 1995.

CEPIS 1996. Curso de tratamiento y uso de aguas residuales. OPS/CEPIS/PUB96.20.

Chai, J.Y., Kim, K.S., Hong, S.T., Lee, S.H. & Seo, B.S. 1985. Prevalence, Worm Burden and Other Epidemiological Parameters of *Ascaris lumbricoides* Infection in Rural Communities in Korea. *Korean Journal of Parasitology* **23**, 241-246.

Chan Chac-Tai 1989. Prevalence of intestinal helminth infections among Chinese schoolchildren in Macao City. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **83**, 238.

Chan, M-S. 1997. The global burden of intestinal nematode infections - fifty years on. *Parasitology Today* **13** (11), 436-443.

Chan, L., Bundy, D.A.P. & Kan, S.P. 1994a. Aggregation and predisposition to *Ascaris lumbricoides* and *Trichuris trichiura* at the familial level. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 46-48.

Chan, L., Bundy, D.A.P. & Kan, S.P. 1994b. Genetic relatedness as a determinant of predisposition to *Ascaris lumbricoides* and *Trichuris trichiura* infection. *Parasitology* **108**, 77-80.

Chan, L., Kan, S.P. & Bundy, D.A.P. 1992. The effect of repeated chemotherapy on age-related predisposition to *Ascaris lumbricoides* and *Trichuris trichiura. Parasitology* **104**, 371-377.

Chandler, A.C. 1925. The rate of loss of hookworm in the absence of reinfections. *Indian Journal of medical Research* **13**, 625-634. Cited in Anderson, R.M. 1986. The population dynamics and epidemiology of intestinal nematode infections. *Transactions of the Royal Society Tropical Medicine and Hygiene* **80**, 686-696.

Chen, E.R. & Hsieh, H.C. 1969. Study of Ascariasis control in Taiwan. *J.F.M.A.* **68(8)**, 411-428.

Cifuentes, E. 1996. Impact of wastewater irrigation on intestinal infections in a farming community in Mexico. PhD thesis. London School of Hygiene and Tropical Medicine. University of London.

Comission Nacional de Agua, CNA (1998) El Uso de las Aguas Residuales en la Agricultura de Riego. Documento Interno. Subgerencia de Ingenieria de Riego y Drenaje. Mexico.

Cooper, E.S. & Bundy, D.A.P. 1988. Trichuriasis. In Intestinal helminthic infections (ed. Z. Pawlowski) (Balliere's Clinical Tropical Medicine and Communicable Diseases), pp. 629-643. London : Tindall.

Cort, W.W. 1922. A graphic analysis of certain factors in hookworm control. *American Journal of Tropical Medicine* **2**, 449-463. Cited in Anderson, R.M. 1986. The population dynamics and epidemiology of intestinal nematode infections. *Transactions of the Royal Society Tropical Medicine and Hygiene* **80**, 686-696.

Cort, W.W. 1931. Recent investigations on the epidemiology of human ascariasis. *Journal of Parasitology* **17**, 121-144.

Croll, N.A. & Ghadirian, E. 1981. Wormy persons: Contributions to the nature and patterns of overdispersion with *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Necator americanus* and *Trichuris trichiura*. *Tropical and Geographical Medicine* **33**, 241-248.

Croll, N.A., Anderson, R.M., Gyorkos, T.W. & Ghadirian, E. 1982. The population biology and control of *Ascaris lumbricoides* in a rural community in Iran. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **76**(2), 187-197.

Crompton, D.W.T. 1988. The Prevalence of Ascariasis. *Parasitology Today* **4**(6), 162-169.

Crompton, D.W.T. 1999. How much human helminthiasis is there in the world? *Journal of Parasitology* **83**(3), 397-403.

Crompton, D.W.T. & Tulley, J.J. 1987. How much Ascariasis is there in Africa? *Parasitology Today* **3**(4), 123-127.

Curtale, F., Pezzotti, P., Saad, Y.S. & Aloi, A. 1999. An analysis of individual, household, and environmental risk factors for intestinal helminth infection among children in Qena Governorate, Upper Egypt. *Journal of Tropical Pediatrics* **45**(1), 14-17.

de Silva, N.R., Chan, M.S. & Bundy, D.A.P. 1997. Morbidity and mortality due to ascariasis: re-estimation and sensitivity of global numbers at risk. *Tropical Medicine and International Health* **2** (6), 519-528.

Dalton, P.R. 1976. A socioecological approach to the control of *Schistosoma mansoni* in St Lucia. *Bulletin of the World Health Organisation* **54**, 587-595.

Dalton, P.R. & Pole, D. 1978. Water-contact patterns in relation to *Schistosoma mansoni* infection. *Bulletin of the World Health Organisation* **56**, 417-426.

Elkins, D.B., Haswell-Elkins, M. & Anderson, R.M. 1986. The epidemiology and control of intestinal helminths in the Pulicat Lake region of Southern India. I. Study design and pre- and post- treatment observations on *Ascaris lumbricoides* infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **80**, 774-792.

Engelberg Report 1985. Health Aspects of Wastewater and Excreta Use in Agriculture and Aquaculture. Report of a Review Meeting of Environmental Specialists and Epidemiologists, Engelberg, Switzerland, July 1-4 1985. Sponsored by the World Bank/WHO. Dubendorf, Switzerland : International Reference Centre for Wastes Disposal. Also published in *IRCWD News* **23**, 11-18, 1985.

Etard, J.F., Audibert, M & Dabo, A. 1995. Age-acquired resistance and predisposition to reinfection with *Schistosoma haematobium* after treatment with praziquantal in Mali. *American Journal of Tropical Medicine and Hygiene* **52**, 549-558.

Feachem, R., Bradley, D., Garelick, H. & Mara, D. 1983. Sanitation and Disease: health aspects of excreta and wastewater management. Chichester: John Wiley & Sons.

Forrester, J.E., Scott, M.E., Bundy, D.A.P. & Golden, M.H.N. 1988. Clustering of *Ascaris lumbricoides* and *Trichuris trichiura* infections within households. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **82**, 282-288.

Forrester, J.E., Scott, M.E., Bundy, D.A.P. & Golden, M.H.N. 1990. Predisposition of individuals and families in Mexico to heavy infection with *Ascaris lumbricoides* and *Trichuris trichiura. Transactions of the Royal Society of Tropical Medicine and Hygiene* **84**, 272-276.

Fulford, A.J.C., Ouma, J.H., Kariuki, H.C., Thiongo, F.W., Klumpp, R.K., Kloos, H., Sturrock, R.F. & Butterworth, A.E. 1996. Water contact observations in Kenyan communities endemic for schistosomiasis: methodology and patterns of behaviour. *Parasitology* **113**, 223-241.

Gonzales Richmond, J.A., Madrigal Fritsch, H., Naranjo Banda, A. & Moreno Terrazas, O. 1985. Consumo de alimentos, estados nutricional y parasitosis intestinal en una comunidad indigena. *Salud Publica de Mexico* **27**(4), 336-345.

Guyatt, H.L., Bundy, D.A.P., Medley, G.F. & Grenfell, B.T. 1990. The relationship between the frequency distribution of *Ascaris lumbricoides* and the prevalence and intensity of infection in human communities. *Parasitology* **101**, 139-143.

Guyatt, H.L., Brooker, S. & Donnelly, C.A. 1999. Can prevalence of infection in school-aged children be used as a measure for assessing community prevalence? *Parasitology* **118**, 257-268.

Halsted, J.A. 1968. Geophagia in Man: Its nature and Nutritional Effects. *The American Journal of Clinical Nutrition* **21**(12), 1384-1393.

Hall, A. 1981. Quantitative variability of nematode egg counts in faeces: a study among rural Kenyans. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **75**, 682-687.

Hall, A. 1982. Intestinal helminths of man:the interpretation of egg counts. *Parasitology* **85**, 605-613.

Hall, A., Anwar, K.S. & Tomkins, A. 1992. Intensity of reinfection with *Ascaris lumbricoides* and its implications for parasite control. *Lancet* **339**, 1253-1257.

Hall, A., Orinda, V., Bundy, D.A.P. & Broun, D. 1997. Promoting Child Health through Helminth Control - A Way Forward?. *Parasitology Today* **13**(11), 411-413.

Henry, F.J. 1981. Environmental sanitation, infection and nutritional status of infants in rural St. Lucia, West Indies. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **75**, 507-513.

Henry, F.J. 1988. Reinfection with *Ascaris lumbricoides* after chemotherapy : a comparative study in three villages with varying sanitation. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **82**, 460-464.

Hill, R.B. 1926. Hookworm reinfestation for three years after treatment in a sanitated area in Puerto Rico, and its bearing on permanent hookworm control in the population studied. American Journal of Hygiene 6, 103-117. Cited in Anderson, R.M. 1986. The population dynamics and epidemiology of intestinal nematode infections. *Transactions of the Royal Society Tropical Medicine and Hygiene* **80**, 686-696.

Holland, C.V., Taren, D.L., Crompton, D.W.T., Nesheim, M.C., Sanjur, D., Barbeau. I., Tucker, K., Tiffany, J. & Rivera, G. 1988. Intestinal Helminthiases in relation to the Socioeconomic Environment of Panamian Children. *Social Science and Medicine* **26**(2), 209-213.

Hpay, El-Zawahry, M.M., Lay-Maung, Ohn-Kyi, Sein-Dwe, R., Than Sein, Tong, L., Khin-Mar-Mar, Mala-Maung, Mya-Mya-Lin & Kwin-Win 1970. Epidemiological features of intestinal parasitoses in Dayabo village, Burma. *Union of Burma Journal of Sciences* **3**, 289-307. Cited in Thein-Hlaing, Than Saw, Htay-Htay-Aye, Myint-Lwin & Thein-Maung-Myint 1984. Epidemiology and transmission dynamics of *Ascaris lumbricoides* in Okpo village, rural Burma. *Transactions of the Royal Society Tropical Medicine and Hygiene* **78**, 497-504.

INEGI 1990. Hidalgo. Perfil Sociodemografico. XI Censo General de Poblacion y Vivienda, 1990. Instituto Nacional de Estadistica, Geografia e Informatica. Mexico.

Kagei, N. 1982. Techniques for the Measurement of environmental Pollution by Infective Stage of Soil-Transmitted Helminths. In "Collected Papers on the Control of Soil-transmitted Helminthiases". Volume II. APCO Research Group. Asian Parasite Control Organisation. Tokyo, Japan.

Kan. S.P., Guyatt, H.L. & Bundy, D.A.P. 1989. Geohelminth infection of children from rural plantations and urban slums in Malaysia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **83**, 817-820.

Keymer, A. 1982. Density-dependent mechanisms in the regulation of intestinal helminth populations. *Parasitology* **84**, 573-587.

Keymer, A. & Pagel, M. 1988. Predisposition to Helminth Infection. Presented at Rockefeller Foundation conference on "Hookworm Disease: Current Status and New Directions" at Bellagio, September 1988. Unpublished document (to be published by Taylor & Francis).

Khalil, M. 1931. The Pail Closet as an efficient means of controlling human helminth infections as observed in Tura Prison, Egypt, with discussion on the source of *Ascaris* infection. *Annals of Tropical Medicine and Parasitology* **25**, 35-62. Abstracted in : Wastewater Irrigation in Developing Countries : Health Effects and Technical Solutions (eds. Shuval *et al.*) World Bank Technical Paper Number 51 (1986). Washington, DC : The World Bank.

Kightlinger, L.K., Seed, J.S. & Kightlinger, B. 1998. *Ascaris lumbricoides* in relation to environmental, socioeconomic, and behavioural determinants of exposure to infection in children from Southeast Madagascar. *Journal of Parasitology* **84**(3), 480-484.

Killewo, J.Z.J., Cairncross, S., Smet, J.E.M., Maikwano, L.F. & van Asten, H. 1991. Patterns of hookworm and *Ascaris* infection in Dar es Salaam. *Acta Tropica* **48**, 247-249.

Kloos, H. & Lemma, A. 1980. The epidemiology of *Schistosoma haematobium* infection in Tensae Behran: human water contact patterns. *Ethiopian Medical Journal* **18**, 91-98.

Kloos, H., Higashi, I., Cattani, J.A., Schlinski, V.D., Mansour, N.S. & Murrell, K.D. 1983. Water contact behaviour and schistosomiasis in an Upper Egyptian village. *Social Science and Medicine* **17**, 545-562.

Kloos, H., Fulford, A.J., Butterworth, A.E., Sturrock, R.F., Ouma, J.H., Kariuki, H.C., Thiongo, F.W., Dalton, P.R. & Klumpp, R.K. 1997. Spatial patterns of human water contact and *Schistosoma mansoni* transmission and infection in four rural areas in Machakos District, Kenya. *Social Science and Medicine* **44**(7), 949-968.

Krey, W. 1949. The Darmstadt Ascariasis Epidemic and its Control. *Zeitschrift fur Hygiene und Infektions Krankheiten* **129**, 507-518. Abstracted in : Wastewater Irrigation in Developing Countries : Health Effects and Technical Solutions (eds. Shuval *et al.*) World Bank Technical Paper Number 51 (1986). Washington, DC : The World Bank.

Krishnamoorthi, K.P., Abdulappa, M.K. & Aniwikar, A.K. 1973. Intestinal parasitic infections associated with sewage farm workers with special reference to helminths and protozoa. In : Proceedings of Symposium on Environmental Pollution. Central Public Health Engineering Research Institute, Nagpur, India. Abstracted in : Wastewater Irrigation in Developing Countries : Health Effects and Technical Solutions (eds. Shuval *et al.*) World Bank Technical Paper Number 51 (1986). Washington, DC : The World Bank.

Lara Aguilera, R. 1984. Las geohelminthiasis en mexico y perspectivas de su control. *Salud Publica de Mexico* **26**(6), 573-578.

Mara, D.D. & Cairncross, S. 1989. Guidelines for the Safe Use of Wastewater and Excreta in Agriculture and Aquaculture : Methods for Public Health Protection. *IRCWD News* **24/25**, 4-12.

Mara, D.D. & Silva, S.A. 1986. Removal of intestinal nematode eggs in tropical waste stabilisation ponds. *Journal of Tropical Medicine and Hygiene* **89**, 71-74.

McCullough, F. 1974. Observations on *Ascaris lumbricoides* infection in Mwanza, Tanzania. Cited in Tedla, S, & Ayele, T. 1986. Ascariasis Distribution in Ethiopia. *Ethiopian Medical Journal* **24**, 79-86.

Mello, D.A. 1974. A Note on Egg Production of *Ascaris lumbricoides*. *Journal of Parasitology* **60**(2), 380-381.

Moraes, L. 1996. Health impact of drainage and sewerage in poor urban areas in Salvador, Brazil. PhD thesis. London School of Hygiene and Tropical Medicine. University of London.

Navarrete Espinosa, J., Navarrete Cadena, E., Escandon Romero, C. & Escobedo de la Pena, J. 1993. Prevalencia de parasitosis intestinal en la poblacion infantil de Santiago Jamiltepec, Oaxaca. *Revista Medica del Instituto Mexicano del Seguro Social* **319**(2), 157-161.

Needham, C., Kim H.T., Hoa, V., Cong, L.D., Michael, E. Drake, L., Hall, A. & Bundy, D.A.P. 1998. Epidemiology of soil-transmitted nematode infection in Ha Nam Province, Vietnam. *Tropical Medicine and International Health* 3(11), 904-912.

Norma Técnica Ecológica NTE-CCA-033/91 Que establece las condiciones, para el uso de aguas residuales de origen urbano o municipal o de la mezcla de éstas con la de los cuerpos de agua, en el riego agrícola. Diario Oficial de la Federation Octubre 24 de 1991. pp 6-9. Mexico.

Peasey, A. & Blumenthal, U.J. 1992. Use of Time-Point Direct Observation in the Measurement of Hygiene Behaviour Associated with Irrigation with Wastewater. In: Studying hygiene behaviour methods, issues, and experiences. Ed. Cairncross, S & Kochar, V. New Delhi Thousand Oaks [Calif.] Sage 1994

Peng, W., Zhou, X., Cui, D., Crompton, D.W.T., Whitehead, R.R., Xing, H., Wu, H., Peng, J., Yang, Y., Wu, W., Xu, K. & Yan, Y. 1996. *Ascaris*, people and pigs in a rural community of Jiangxi Province, China. *Parasitology* **113**, 545-557.

Pescod, M.B. & Arar, A. 1988. Treatment and use of sewage effluent for irrigation. Proceedings of the FAO Regional Seminar on The Treatment and Use of Sewage Effluent for Irrigation, held in Nicosia, Cyprus in October 1985.

Peters, W. 1978. Medical aspects - comments and discussion II, The Relevance of Parasitology to Human Welfare Today (Symposia of the British Society of Parasitology. Vol 16). Edited by E.R.A. Taylor & R.Muller. Oxford, Blackwell Scientific Publications, 1978, pp25-41.

Ridley, D.S. & Hawgood, B.C. 1956. The Value of Formol-Ether Concentration of Faecal Cysts and Ova. *Journal of Clinical Pathology* **9**, 74-76.

Rivera Arguero, F., Acevedo-Cruz, N.C. & Alcocer-Duran, J. 1985. Capacidad de eliminacion de helmintos parasitos de una laguna de estabilizacion en Santo Tomas Atzingo, Estado de Mexico. *Revista Latinoamericana de Microbiologia* **27**, 335-340.

Rivera Ramirez, L. 1980. Effects on Public Health of the Use of Wastewater in Irrigation District 03 of the Western Area of Tula, State of Hidalgo. Master's thesis, School of Public Health, Mexico City, Mexico.

Rose, J.B. 1985. Microbial aspects of wastewater reuse for irrigation. *CRC Critical Reviews in Environmental Control* **16**, 230-256.

Sanchez Leyva, R. 1976. Use of Wastewater for Irrigation in District 03 and 88 and its Impact on Human Health. Master's thesis, School of Public Health, Mexico City, Mexico.

Sawyer, W.A. 1925. Factors that influence the rate of increase in hookworm infection. *American Journal of Hygiene* **5**, 790-817. Cited in Anderson, R.M. 1986. The population dynamics and epidemiology of intestinal nematode infections. *Transactions of the Royal Society Tropical Medicine and Hygiene* **80**, 686-696.

Schad, G.A., Nawalinski, T.A. & Kochar, V. 1983. Human Ecology and the Distribution and Abundance of Hookworm. In Human Ecology and Infectious Diseases (ed. Croll, N.A. and Cross, J.H..), pp188-225. Academic Press, London.

Schaller, K.F. & Kuis, W. 1972. *Geomedical Monograph Series*, Volume **3**, pp72-159. Springer Verlag, Heidelberg, West Germany. Cited in Tedla, S, & Ayele, T. 1986. Ascariasis Distribution in Ethiopia. *Ethiopian Medical Journal* **24**, 79-86.

Schleiper, C. & Kalies, W. 1949. Quantitative Investigations on the Infection by Roundworm of the Inhabitants of the Darmstadt Rural District. *Zbl. Bakt.* **154**, 78-86. Cited in Wastewater Irrigation in Developing Countries : Health Effects and Technical Solutions (eds. Shuval *et al.*). World Bank Technical Paper No. 51 (1986).Washington, DC : The World Bank.

Schultz, S. & Kroeger, A. 1992. Soil contamination with *Ascaris lumbricoides* eggs as an indicator of environmental hygiene in urban areas of north-east Brazil. *Journal of Tropical Medicine and Hygiene* **95**, 95-103.

Scott, M.E., Santizo, M., Zepeda, E., Ramirez, I. & Forrester, J.E. 1989. Age-related differences in prevalence and intensity of *Trichuris trichiura* in sensory impaired children from two schools in Guatemala. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **83**, 542-544.

Seo, B.S., Cho, S.Y. & Chai, J.Y. 1979. Egg discharging patterns of *Ascaris lumbricoides* in low worm burden cases. *Korean Journal of Parasitology* **17**(2), 98-104.

Shield, J.M., Smith, P. & Heywood, P. 1981. The prevalence of alimentary helminthiasis and its association with nutritional status in children under five years old in the highlands of Papua New Guinea. *Papua New Guinea Medical Journal* **24**(1), 40-44.

Shuval, H.I. 1987. The Development of Water reuse in Israel. *AMBIO* **16**(4), 186-190.

Shuval, H.I. 1988. Rationale for Engelberg Guidelines. *IRCWD News* **24/25**, 18-19.

Shuval, H.I., Adin, A., Fattal, B., Rawitz, E. & Yekutiel, P. 1986. Wastewater Irrigation in Developing Countries : Health Effects and Technical Solutions. World Bank Technical Paper No. 51, Integrated Resource recovery Series, UNDP Project Management Report No. 6. Washington, DC : The World Bank.

Sinniah, B. 1982. Daily egg production of *Ascaris lumbricoides*: the distribution of eggs in the faeces and the variability of egg counts. *Parasitology* **84**, 167-175.

Smith, C. 1993. The Effect of the Introduction of Piped Sewerage on *Ascaris* infection and Environmental Contamination in a Gaza Strip Refugee Camp. PhD thesis. London School of Hygiene and Tropical Medicine. University of London.

Stephenson, L.S. 1985. In : Ascariasis and its Public Health Significance, edited by Crompton, D.W.T., Nesheim, M.C. & Pawlowski, Z.S. 1985 (London : Taylor and Francis).

Stoll, N.R. 1923. Investigation on the control of hookworm disease XVIII. On the relation between the number of eggs found in human faeces and the number of hookworms in the host. *American Journal of Hygiene* **2**, 103-117.

Stoll, N.R. 1947. This wormy world. *Journal of Parasitology* **33**, 1-18.

Stoopen, M.R. & Beltran, F.A. 1964. Caracteristicas Epidemiologicas de las Helmintiasis en Yancuictlalpan, Puebla (Mexico). *Revista Medicina* 28-33.

Strauss, M. 1985. Survival of Excreted Pathogens in Excreta and Faecal Sludges. In Health Aspects of Nightsoil and Sludge Use in Agriculture and Aquaculture. *IRCWD* **23**, 4-9.

Tedla, S, & Ayele, T. 1986. Ascariasis Distribution in Ethiopia. *Ethiopian Medical Journal* **24**, 79-86.

Thein-Hlaing, Than Saw, Htay-Htay-Aye, Myint-Lwin & Thein-Maung-Myint 1984. Epidemiology and transmission dynamics of *Ascaris lumbricoides* in Okpo village, rural Burma. *Transactions of the Royal Society Tropical Medicine and Hygiene* **78**, 497-504.

Thein-Hlaing, Than-Saw & Myint-Lwin 1987. Reinfection of people with *Ascaris lumbricoides* following single, 6-month and 12- month interval mass chemotherapy in Okpo village, rural Burma. *Transactions of the Royal Society Tropical Medicine and Hygiene* **81**, 140-146.

Tiglao, T.V. & Camacho, A.C. 1983. Water contact behaviour among humans in Leyte, Philippines. *Southeast Asian Journal of Tropical Medicine and Public Health* **14**, 18-24.

Tu, M., El-Zawahry, M.M., Ohn-Kyi, Kin-Hlaing, Hla-Kyaw, S., Kyaw-Myint, Sann-Myint, K., Kyi-Kyi-Khin, Aung-Thann & Lei-Lei-Win 1970. Epidemiological features of intestinal parasitoses in the inhabitants of Okpo village, Burma. *Union of Burma Journal of Life Science* **3**, 247-267. Cited in Thein-Hlaing, Than Saw, Htay-Htay-Aye, Myint-Lwin & Thein-Maung-Myint 1984. Epidemiology and transmission dynamics of *Ascaris lumbricoides* in Okpo village, rural Burma. *Transactions of the Royal Society Tropical Medicine and Hygiene* **78**, 497-504.

Walsh, J.A. & Warren, K.S. 1979. Selective Primary Health Care: An interim strategy for disease control in developing countries. *New England Journal of Medicine* **301**, 967-974.

WHO 1973. Reuse of Effluents : Methods of Wastewater Treatment and Health Safeguards. Technical Report Series 517. Geneva : World Health Organisation.

WHO 1984. Case Management of Acute Respiratory Infections in Children in Developing Countries. WHO/RSO/85.15.

WHO 1987. Prevention and Control of Parasitic Infections. Technical Report Series 749. Geneva. World Health Organisation.

WHO 1989. Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture. Technical Report Series 778. Geneva: World Health Organisation.

WHO/UNEP 1987. Global Pollution and Health - Results of Health-related Environmental Monitoring, GEMS: Global Environmental Monitoring System, World Health Organisation, Div. Of Env.Health. Geneva, Switzerland. Cited in Shuval, H. 1988. Rationale for Engelberg Guidelines. *IRCWD News* **24/25**, 18-19.

Wilkins, H.A., Goll, P.H., Marshall, T.F. & Moore, P.J. 1984. Dynamics *of Schistosoma haematobium* infection in a Gambian community. I. The pattern of human infection in the study area. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **78**, 216-221.

Wilkins HA; Blumenthal UJ; Hagan P; Hayes RJ; Tulloch S. 1987. Resistance to reinfection after treatment of urinary schistosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **81**(1), 29-35.

Wong, M.S. 1988. The role of environmental and host behavioural factors in determining exposure to infection with *Ascaris lumbricoides* and *Trichuris trichiura*. PhD Thesis - University of the West Indies.

Wong, M.S., Bundy, D.A.P. & Golden, M.H.N. 1988. Quantitative assessment of geophagous behaviour as a potential source of exposure to geohelminth infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **82**, 621-625.

Young, K.H., Bullock, S.L., Melvin, D.M. & Spruill, C.L. 1979. Ethyl Acetate as a Substitute for Diethyl Ether in the Formalin-Ether Sedimentation Technique. *Journal of Clinical Microbiology* **10**(6), 852-853.

Zapponi, G.A. 1989. Health risks connected with water reuse. Notes from a conference on wastewater reuse held in Sicily in 1989. Unpublished.

Appendix A Letter explaining the study

ESTUDIO DE LAS INFECCIONES Y EL ESTADO NUTRICIONAL DE CAMPESINOS Y SUS FAMILIAS

El mes pasado se realizó un Censo de Salud para los campesinos y sus familias en esta área. A partir de este censo, se está realizando un estudio sobre las infecciones por lombrices y el estado nutricional de los campesinos y sus familias. Usted y su familia han sido seleccionados para participar en este estudio. Específicamente, usted, todos los adultos y los niños mayores de 2 años.

En caso de que su familia pueda participar en el estudio, pasaremos a recojer una muestra de excremento, y a pesar y medir a cada miembro de su familia que esté incluido en el estudio.

Posteriormente, en noviembre o diciembre se darán medicinas a cada persona del estudio que lo necesite. Este tratamiento se dará para eliminar cualquier infección por lombrices. Habrá un médico disponible para revisar los casos graves.

Es importante que los adultos de la familia estén de acuerdo en participar en el estudio.

Muchas gracias a usted y a su familia por su cooperación.

INSTITUTO NACIONAL DE LA NUTRICION
"SALVADOR ZUBIRAN, CIUDAD DE MEXICO

Valle del Mezquital, Hidalgo, Octubre 1989.

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Valle del Mezquital, Hidalgo, Octubre 1989.

Appendix B Form for wastewater contact interview

CUESTIONARIO DE CONTACTO CON EL AGUA EL RIEGO

1. FECHA [| | | | |]1 dia mes ano 2. ENCUESTADOR [|]2

3. COMUNIDAD [|]3 4. FAMILIA [| | |]4

6. JEFE DE FAMILIA ______ apellido paterno ______ apellido materno ______ nombre(s) 5

ANOTAR HORARIO PARA VISITAR CADA MIEMBRO DE LA FAMILIA

ID	FECHA	HORA (AM/PM)	PROXIMA CITA (FECHA Y HORA)	

LLENAR POR CADA MIEMBRO DE LA FAMILIA (anotar los cuestionarios que ha aplicado)

OTRAS MANERAS DE CONTACTO CON EL AGUA DE RIEGO Y LA TIERRA (SI-1,NO-2)

ID	CUEST.APLIC		LAVAR ROPA	LIMPIAR PATIO	LAVARSE	TOMAR	PLANTAS	COME TIERRA		

Codigos de los cuestionarios : riego adultos (1), temporal adultos (3)
riego ninos (2), temporal ninos (4)

CUESTIONARIO DE CONTACTO CON EL AGUA EL RIEGO — RIEGO : PRIMERA PARTE

1. FECHA [02 | 19 | 90] 1 (mes, dia, ano) — 2. ENCUESTADOR [03] 2

3. COMUNIDAD [02] 3 — 4. FAMILIA [0011] 4 — 5. ID [06] 5

6. NOMBRE Cerón (apellido paterno) — Frías (apellido materno) — Pedro (nombre(s)) 6

FECHA DE TRATAMIENTO [03 | 21 | 90] (mes, dia, ano)

7. LISTA DE LOS LUGARES DONDE HA TRABAJADO DESDE QUE RECIBIO TRATAMIENTO - anota primero la ocupacion, la direccion de la parcela, los cultivos y si esta cerca de la casa p.ej. pequeno propietario, por la carreterra Progreso-C.Veracruz, 2 km de Progreso cerca la compuerta 102 de Canal X, alfalfa y esta cerca de la casa

	Ocupacion	Código	Direccion y canal	Códigos	Cultivo 1	Código	Cultivo 2	Código	Cerca de casa (si-1,no-2)
I	[illegible]	11	Ejido [illegible] Canal derecho	28, 08	Maíz	01	—		2 12
II	Pastorea	06	[illegible]	04, 99	—	10	—	99	1 18
III	Jornalero	26	en derecho	04, 07	—	99	—	99	2 24
IV									30
V									36
VI									42
VII									48

SI ES PEQUENO PROPIETARIO, EJIDATARIO O MEDIERO LLENAR LA SEGUNDA HOJA; SI ES JORNALERO LLENAR CUADRO SOLO PARA JORNALEROS

riego

SOLO PARA PEQUENO PROPIETARIO / EJIDATARIOS

CUESTIONARIO DE CONTACTO CON EL AGUA DE RIEGO

RIEGO : SEGUNDA PARTE

FECHA [illegible] (mes dia ano) COMUNIDAD [illegible] FAMILIA [illegible] ID [illegible]

12. Cultivo Maiz — 1
13. Lugar ejido [illegible] Canal [illegible] — 2
14. ¿ En que fecha siembra/planta su cultivo (anotar fecha) ? 0 [illegible] / 0 6 / 9 0 (mes dia ano) — 3
15. ¿ Cuantos dias antes de sembrar el cultivo riega la parcela con el agua de riego (mismo dia = 0) ? — 4
16. ¿ Cuantos dias despues de sembrar el cultivo es el primer riego con el agua de riego (mismo dia = 0) ? — 5
17. ¿ Desde que siembra hasta que cosecha cada cuando riega el cultivo ? codigo : dia (1), semana (2), mes (3) — Cada — 6 no. — 7 tiempo
18. ¿ Desde que siembra hasta que cosecha ¿ Como se da cuenta que el cultivo necesita agua ? [illegible] — 8 — 9

¿ La lluvia influye en los riegos (si-1,no-2) ? — 10

¿ Porque ? si llueve no se le riega. —

¿ La crecimiento del cultivo influye en los riegos (si-1,no-2) ? — 11

¿ Porque ? necesita [illegible] —

¿ La temperatura influye en los riegos (si-1,no-2) ? — 12

¿ Porque ? por el calor necesita [illegible] —

19. Llenar estos cuadros de lo hecho desde Enero hasta el momento (anotar todas las visitas a la parcela y el no. de veces que tiene contacto con el agua de riego y la tierra)

Mes	actividad	contacto agua	contacto tierra
Ene (13-24)			
Feb (25-36)			
Mar (37-48)	[illegible]	1	1
	Riego - [illegible]	1	1
Abr (49-60)	[illegible]	0	1
May (61-72)	Riego	1	1
	desenyerbe	0	2
Jun (73-84)			
Jul (85-96)	desenyerbe	0	2
Aug (97-108)			
Sep (109-120)			

20. ¿ Cuando comienza a cosechar el cultivo ? — no. — tiempo — 122
21. ¿ Que tiempo dura la cosecha ? — 124

8 7 6 5 4 3 2 1 0 1 2 3 4 5 6 7 8

riego1c

CUESTIONARIO DE CONTACTO CON EL AGUA DE RIEGO — RIEGO : TERCERA PARTE

FECHA [07.090] (mes dia ano) COMUNIDAD [04] FAMILIA [31] ID [01]

POR CADA VEZ QUE LA PERSONA TIENE CONTACTO CON EL AGUA DE RIEGO LLENAR UN CUADRO CON LOS DETALLES DEL CONTACTO COMENZANDO CON LA MAS RECIENTE p.ej. regando la parcela, sembrando

PEQUENO PROPIETARIO, EJIDATARIO - llenar por cultivo. JORNALERO - llenar por frecuencia Y MEDIERO

22. Cultivo Maiz [01] 61
23. Lugar/Fecha de siembra Ejido Atitalaquia 01 62
24. Fecha de contacto con el agua negra [05 06 90] (mes dia ano) 63
25. Nombre del trabajo Riego [01] 64
26. Descripcion de su trabajo pone Trabas en la zanja Abre Chiflones y Cierra Chiflones. [ ] 65
27. Hora de comienzo 3 pm Hora de terminacion 7 pm [04] horas [00] minutos 67
28. Numero de adultos y ninos que estan trabajando en la parcela (incluyendo al entrevistado) [01] 68
29. Cuando agarra o se moja el agua de riego ¿ que parte de su cuerpo se ensucia (1) y/o moja (2) ? (nada = 3)
Pies [1 2] Pantorrilla [1 2] Muslo [3 3] Brazos [1 2] Manos [1 2] Todo [3 3] 74
Otra 75
30. ¿ Usted toma alguna precaucion cuando se moja con el agua de riego o agarra la tierra (si-1,no-2) ? [2] 76
Usa guantes ? [2] Usa botas ? [2] Otra descalso [2] 79
31. ¿ Durante este trabajo comio ? [2] (si-1,no-2) ═══> Si comio ¿ se lavo las manos ? (si-1,no-2) [9] 81
═══> Si lavo las manos ¿ Con que agua (no aplicable-9) ? codigos : agua de riego (1), agua de llave (2), agua de rio (3) Otra [9] 82

22. Cultivo Maiz [01] 61
23. Lugar/Fecha de siembra Ejido Atitalaquia 01 62
24. Fecha de contacto con el agua negra [03 14 90] (mes dia ano) 63
25. Nombre del trabajo Riego - Remojo [01] 64
26. Descripcion de su trabajo Pone Trabas en la zanja Abre y Cierra chiflones [ ] 65
27. Hora de comienzo 9 AM Hora de terminacion 11 AM [02] horas [00] minutos 67
28. Numero de adultos y ninos que estan trabajando en la parcela (incluyendo al entrevistado) [01] 68
29. Cuando agarra o se moja el agua de riego ¿ que parte de su cuerpo se ensucia (1) y/o moja (2) ? (nada = 3)
Pies [1 2] Pantorrilla [1 2] Muslo [3 3] Brazos [1 2] Manos [1 2] Todo [3 3] 74
Otra 75
30. ¿ Usted toma alguna precaucion cuando se moja con el agua de riego o agarra la tierra (si-1,no-2) ? [2] 76
Usa guantes ? [2] Usa botas ? [2] Otra descalso [2] 79
31. ¿ Duranta este trabajo comio ? [2] (si-1,no-2) ═══> Si comio ¿ se lavo las manos ? (si-1,no-2) [9] 81
═══> Si lavo las manos ¿ Con que agua (no aplicable-9) ? codigos : agua de riego (1), agua de llave (2), agua de rio (3) Otra [9] 82

ASEGURARSE QUE TIENE TODA LA INFORMACION PARA LLENAR LAS PREGUNTAS 8-11. riego1d

CUESTIONARIO DE CONTACTO CON EL AGUA DE RIEGO

RIEGO : ULTIMA PARTE

PREGUNTAR ESTA PARTE AL FINAL (v=veces, c=codigo)

	JUEGA v	JUEGA c	PASTOREO v	PASTOREO c	TRABAJO v	TRABAJO c	
8. ¿ En los ultimos ... desde que ha recibido tratmiento, cuantas veces ha agarrado o se ha mojado con el agua de riego ?			9	6	9	7	51
9. ¿ En las ultimas cuatro semanas, cuantas veces ha agarrado o se ha mojado con el agua de riego ?	7	6	9	6	9	6	54
10. ¿ En los ultimos ... desde que ha recibido tratamiento, cuantas veces ha trabajado en el campo y ha agarrado la tierra pero no se ha mojado con el agua de riego ?	9	1	9	4	3	9	57
11. ¿ En las ultimas cuatro semanas, cuantas veces ha trabajado en el campo y ha agarrado la tierra pero no se ha mojado con el agua de riego ?	9	1	9	1	2	9	60

SOLO PARA JORNALEROS

1. ______
2. ______
3. ______
4. ______
5. ______
6. ______
7. ______

Llenar estos cuadros de todos los trabajos hechos desde Enero hasta el momento (anota todas las visitas a la parcela y EL NO. DE VECES QUE TIENE CONTACTO CON LA TIERRA Y CON EL AGUA DE RIEGO)

Preguntar por orden lo hecho en cada una de las parcelas de la primera hoja

Mes	actividad	contacto agua	contacto tierra
Ene			
Feb			
Mar			
Abr	[illegible]	0	1
May			
Jun			
Jul	[illegible]	0	2
Aug			
Sep			

CUANDO SE TENGA LA INFORMACION DE ESTE CUADRO, PASAR A LA TERCERA HOJA, PREGUNTAR PRIMERO POR LA ULTIMA VEZ DE CONTACTO CON EL AGUA DE RIEGO QUE APARECE EN ESTE CUADRO.

riego

CUESTIONARIO DE CONTACTO CON EL AGUA EL RIEGO — NINOS (2-12anos) : RIEGO : PRIMERA PARTE

1. FECHA 07 19 90 (mes dia ano) 1 2. ENCUESTADOR 05 2 FECHA DE TRATAMIENTO 03-22-90

3. COMUNIDAD 04 3 4. FAMILIA 0831 4 5. ID 02

6. NOMBRE Cerón (apellido paterno) Facio (apellido materno) Gaudencio (nombre(s)) 6

ESTA SECCION ES EN RELACION DE LO QUE HA HECHO DESDE QUE LO VISITAMOS PARA DARLE TRATAMIENTO

7. ¿ Usted ayuda / trabaja en el campo (si-1,no-2) ? 1 7

==> ¿ Cuales son los trabajos de los que usted hace en el campo y agarra o se moja con el agua de riego (por cada cosa preguntar donde se hace, el cultivo, si esta cerca de la casa y el contacto. ANOTAR ABAJO) ?

8. ¿ Cuando usted juega se moja con el agua de riego (si-1,no-2) ? 2 8

==> Jugar cerca casa .... 2 Jugar/Seguir padre que esta trabajando en el campo 2 11
Jugar/Pastoreo 2

PREGUNTAR SI HAY OTRA MANERA JUGANDO EN QUE SE MOJA CON EL AGUA DE RIEGO

==> Otra descripciones para jugando 1___
2___ 3___
4___ 5___ 16

(ANOTAR ABAJO los detalles donde se juega, como juega y si esta cerca de la casa)

9. ¿ De que otras maneras trabajando o jugando toca o AGARRA LA TIERRA pero no se moja con el agua de riego (ANOTAR ABAJO) ?

LISTA DE LOS LUGARES DONDE SE HA MOJADO CON EL AGUA DE RIEGO O HA AGARRADO LA TIERRA DESDE QUE RECIBIO TRATAMIENTO - anota la direccion del lugar, que hace, si esta cerca de la casa, el tipo de contacto (agua de riego-1,tierra-2) y el cultivo (N/A = 9) p.ej. carreterra de Progreso por Canal X, pastoreo/jugando, cerca de casa, contacto con el agua de riego.

1 Lugar y canal En el Ejido de Atotalaquia (Canal Vendho) | 37 | 04 | Cerca de casa 2
ultima fecha 07 19 90 | Contacto 2
Que hace Ayuda al Campo | 11 | Cultivo Maiz 1 22

2 Lugar y canal En Frente de su Casa Mohan Canal | 04 | 39 | Cerca de casa 1
ultima fecha 19 07 90 | Contacto 2
Que hace Pastorea | 06 | Cultivo — 9 28

3 Lugar y canal En su Casa en Dendhó | 04 | 99 | Cerca de casa 1
ultima fecha 19 07 90 | Contacto 2
Que hace Juega a la Pelota | 26 | Cultivo — 9 34

4 Lugar y canal ___ | Cerca de casa
ultima fecha | Contacto
Que hace ___ | Cultivo ___ 40

5 Lugar y canal ___ | Cerca de casa
ultima fecha | Contacto
Que hace ___ | Cultivo ___ 46

6 Lugar y canal ___ ultima fecha ___ | Cerca de casa Contacto
Que hace ___ | Cultivo ___ 52

7 Lugar y canal ___ ultima fecha ___ | Cerca de casa Contacto
Que hace ___ | Cultivo ___ 58

CUESTIONARIO DE CONTACTO CON EL AGUA DE RIEGO | NINOS (2-12 anos) : RIEGO : SEGUNDA PARTE

FECHA [0 7 | 1 9 | 9 0] mes dia ano COMUNIDAD [0 4] FAMILIA [| 3 1] ID [0 7]

POR CADA VEZ QUE LA PERSONA TIENE CONTACTO CON EL AGUA DE RIEGO O LA TIERRA LLENAR UN CUADRO CON LOS DETALLES DEL CONTACTO COMENZANDO CON LA MAS RECIENTE p.ej. jugando

15. Cultivo — [0 9] 71

16. Tipo de contacto (agua de riego-1, tierra-2) [2] 72

17. Lugar/Canal Ejido de Atitalaquia 01 73

18. Fecha(s) de contacto con el agua de riego o la tierra 1, 2 Dic 7 [ ] [ ] dias [0 6] mes [9 0] ano 75

19. Nombre del trabajo/juego Ay al Campo [1 1] 76

20. Descripcion de su trabajo/juego Le Ayuda a su Papá a sembrar semilla a tapar [ ] 77

21. Hora de comienzo — Hora de terminacion — [0 1] horas [0 0] minutos 78

22. Numero de adultos y ninos que estan trabajando/jugando en este lugar (incluyendo al entrevistado) [3] 79

23. Cuando agarra o se moja con el agua de riego ¿ que parte de su cuerpo se ensucia (1) y/o moja (2) ? (nada = 3)

Pies [1 3] Pantorrilla [3 3] Muslo [3 3] Brazos [3 3] Manos [1 3] Todo [3 3] 85
Otra — 86

24. ¿ Usted toma alguna precaucion cuando se moja con el agua de riego o agarra la tierra (si-1,no-2) ? [1] 87

Usa guantes ? [2] Usa botas ? [2] Otra Zapatos [1] 90

25. ¿ Durante este trabajo/juego comio ? [2] (si-1, no-2) => Si comio ¿ se lavo las manos ? [9] 92 (si-1, no-2)

=====> Si se lavo las manos ¿ Con que agua (si-1, no-2, no aplicable-9) ? [9] 93
codigos : agua de riego (1), agua de llave (2), agua de rio (3) Otra —

15. Cultivo — [0 9] 71

16. Tipo de contacto (agua de riego-1, tierra-2) [2] 72

17. Lugar/Canal En frente de su Casa [0 4] 73

18. Fecha(s) de contacto con el agua de riego o la tierra [1 9] [1 9] dias [0 7] mes [9 0] ano 75

19. Nombre del trabajo/juego Pastores [0 6] 76

20. Descripcion de su trabajo/juego Arreando y Cuidando los Animales [ ] 77

21. Hora de comienzo — Hora de terminacion — [0 2] horas [0 0] minutos 78

22. Numero de adultos y ninos que estan trabajando/jugando en este lugar (incluyendo al entrevistado) [0 2] 79

23. Cuando agarra o se moja con el agua de riego ¿ que parte de su cuerpo se ensucia (1) y/o moja (2) ? (nada = 3)

Pies [3 3] Pantorrilla [ ] Muslo [ ] Brazos [ ] Manos [ ] Todo [1 3] 85
Otra — 86

24. ¿ Usted toma alguna precaucion cuando se moja con el agua de riego o agarra la tierra (si-1,no-2) ? [1] 87

Usa guantes ? [2] Usa botas ? [2] Otra Tenis [6] 90

25. ¿ Durante este trabajo/juego comio ? [2] (si-1, no-2) => Si comio ¿ se lavo las manos ? [9] 92 (si-1, no-2)

=====> Si se lavo las manos ¿ Con que agua (si-1, no-2, no aplicable-9) ? [9] 93
codigos : agua de riego (1), agua de llave (2), agua de rio (3) Otra —

nrlc

PREGUNTAR ESTA PARTE AL FINAL (v=veces, c=codigo)

	JUEGA V	JUEGA C	PASTOREO V	PASTOREO C	TRABAJO V	TRABAJO C	
11. ¿ En los ultimos ... desde que ha recibido tratamiento, cuantas veces ha agarrado o se ha mojado con el agua de riego ?	9	6	9	6	9	6	61
12. ¿ En las ultimas cuatro semanas, cuantas veces ha agarrado o se ha mojado con el agua de riego ?	9	6	9	6	9	6	64
13. ¿ En los ultimos ... desde que ha recibido tratamiento, cuantas veces ha trabajado o ha jugado y ha agarrado la tierra pero no se ha mojado con el agua de riego ?	9	1	9	3	2	[illegible]	67
14. ¿ En las ultimas cuatro semanas, cuantas veces ha trabajado o ha jugado y ha agarrado la tierra pero no se ha mojado con el agua de riego ?	9	1	9	3	2	[illegible]	70

nr1b

CODIGOS

			POR SEMANA		POR MES
1	TODO EL TIEMPO				
2	MAYOR PARTE DEL TIEMPO	=	mas 4/semana	o	16/mes
3	MUCHAS VECES	=	3-4/semana	o	12-15/mes
4	REGULARMENTE	=	1-2/semana	o	4-8/mes
5	POCAS VECES	=	menos 1/semana	o	menos 4/mes
6	NUNCA	=	0/semana	o	0/mes

Appendix C Structured observation form

TIEMPO QUE COMENZO LAS OBSERVACIONES 15.15 TIEMPO TERMINO 18.15

ANTES DE SALIR AL CAMPO

1. FECHA 04/01/91 (m d a) 2. ENCUESTADOR Rocio !04!

3. COMUNIDAD !4!2! 4. FAMILIA !7!9!9!1! 5. ID !9!9!

6. POSICION EN LA FAMILIA Cabe de fam. 7. EDAD 47 8. SEXO M

9. NOMBRE Maqueda Aguilar Leopoldo

10. DIRECCION DE LA PARCELA Propiedades los Siete ... ! ! ! !

11. AREA (hectares.areas) 7 Ha. !7.00!

12. CANAL Manzanillo !0!2!

13. CULTIVO Maiz !0!1!

14. TRABAJO(S) OBSERVADO(S) Y ESTADO DE DESAROLLO DE CULTIVO !0!1! !9!9!
Riego de Maiz

AL TERMINAR LAS OBSERVACIONES

Hacer preguntas 11 y 12 a la persona

15. LA HORA QUE COMENZO SU TRABAJO (p.ej. 03.30, 12.45) !15.00! PM

16. LA HORA QUE VA A TERMINAR O TERMINO SU TRABAJO !18.00! PM del Proximo Jueves

Las preguntas 13 a 30 se hacen por observacion

17. LA TIERRA ES SECA (1), HUMEDA (4) O MOJADA (2) ? !2!

18. QUE TIENE EN SUS PIES ? !2!
Zapatos (1) Descalso (2) Botas (14)
SI LA PERSONA NO ESTA DESCALSA PASA A LA 15

19. HASTA QUE PARTE DE SU PIERNA LLEGAN LOS ... ? !9!
Tobillo (1) Rodilla (2) Entre tobillo y rodilla (3)

20. QUE TIENE EN SUS PIERNAS ? !2!
Pantalones hasta tobillo (1) Pantalones hasta rodilla (2)
OTRA

21. QUE TIENE EN SUS MANOS ? Otra Nada Guantes (12) !9!

22. QUE TIENE EN SUS BRAZOS ? Mangas entre codo y muneca (1) !2!
OTRA Mangas hasta codo o arriba (2)

Por cada parte del cuerpo anotar si en algun momento del trabajo estubo mojado (3). Si no estubo mojado anotar codigo = seco (1)

23. CUERPO !1! 26. MANOS !3! 29. PIERNAS !3!
24. BRAZOS - ARRIBA CODO !3! 27. CARA !1!
25. ENTRE CODO Y MUNECO !3! 28. PIES !3!

30. LA PERSONA COME (1) O VA COMER (2) EN LA PARCELA ? !9!

31. LAVO SUS MANOS ANTES DE COMER ? Si = 1, No = 2 !9!

32. CON QUE AGUA LAVO SUS MANOS ? !9!
Agua de riego (1) Agua de llave (2)

33. LA PERSONA FUE AL BANO(1) O VA IR AL BANO(2) EN LA PARCELA ? No fue = 3 !9!

34. SI FUE O VA A IR AL BANO, DONDE ? Dentro de su parcela (1), En sanja (2), En canal de riego (3), En otra parcela (4) !9!
Otra

FORMA DE REGISTRO DE LAS CONDUCTAS EN EL CAMPO   NOMBRE DE INDIVIDUAL *Magueda Aguilar Leopoldo*

ENCUESTADOR *Rocio*   FECHA (mm/dd/aa) *0 4 6 1 9 1*   COM *9 9*   FAM *9 9 9 9*   ID *9 9*

PARCELA *Venpiedades los siete pertenece a Tlahuelilpan*

	Mano a boca	Lavar manos [illegible]	Mano a pie	Mano a cara	Mano a [illegible]	Mano a tierra mojada	Mano a tierra	Mano a hierba mojada	Mano a [illegible]	[illegible]	Levanta terrón	Levanta piedras	Mano brazo agua	Pie tierra	Pie [illegible]	Pie a lodo	[illegible]	Uso [illegible]	Uso [illegible]	Mano a [illegible]	Planta a boca	Limpia boca	Come	Toma	Fuma	[illegible]	CHECADO					[illegible]
2-10	I									I				III	IIII	II			IIII	IIIII						IIIII	X X	X X	X X	X X	X X	
12-20	II													I	IIIII	IIII			IIII	IIIII II						III	X X	X X	X X	X X	X X	IIII
22-30	I													IIIII III	I	I			II	IIIII III						IIIII I	X X	X X	X X	X X	X X	II
30-40														IIIII IIIII					II	IIIII III						III	X X	X X	X X	X X	X X	IIIII
42-50				I										IIIII III		II			I	IIIII IIII						IIIII II	X X	X X	X X	X X	X X	II
52-60										I				IIIII IIIII					IIIII IIIII								X X	X X	X X	X X	X X	
2-10													II	IIIII III	II				III	IIIII I						II	X X	X X	X X	X X	X X	IIIII I
12-20	I													IIIII I	IIII	II			IIII	IIIII I						IIII	X X	X X	X X	X X	X X	II
22-30														IIIII	II	II				IIIII IIIII						IIIII	X X	X X	X X	X X	X X	IIIII
32-40	I													IIIII I	II	II			IIIII I	IIII						IIII	X X	X X	X X	X X	X X	
42-50														IIIII	II	III			IIIII	IIIII						II	X X	X X	X X	X X	X X	III
52-60														III	IIII	III			IIII	IIIII I						III	X X	X X	X X	X X	X X	III
2-10														IIIII IIIII					IIIII III	III						II	X X	X X	X X	X X	X X	
12-20	II													IIIII IIIII					II	IIII						II	X X	X X	X X	X X	X X	IIIII I
22-30		I								II				III	IIIII				II	IIIII I						IIIII I	X X	X X	X X	X X	X X	
32-40														IIIII	IIIII				IIIII III	II							X X	X X	X X	X X	X X	
42-50														IIIII I					IIIII III	II							X X	X X	X X	X X	X X	II
52-60														IIIII I		II			IIII	IIIII						IIII	X X	X X	X X	X X	X X	II
TOTAL	9	1		1						4			2	82	34	23			x59	96						59	36	36	36	36	36	42

Appendix D Summary of seeded water trials in UK

Evaluation of some Wastewater Analysis Techniques

The techniques were performing using samples of Thames water seeded with *Ascaris suum* ova. *Ascaris suum* worms were obtained from an abattoir in Yorkshire and the ova dissected out of the mature female worms. For each technique, the ova were counted out on a slide and transferred to a glass beaker containing a litre sample of water.

Three techniques were performed, the Doncaster dish technique (Ayres, 1989), the Raw wastewater technique (Ayres, 1989) and the Bailenger technique (Bailenger, 1979). The Doncaster dish technique is designed specifically for the measuring low concentrations of *Ascaris* eggs in the final effluent from waste stabilisation ponds. The Raw wastewater technique is as the name suggests designed to measure the concentration of *Ascaris* eggs in raw wastewater. Raw wastewater often has very high concentrations of *Ascaris* eggs. There is generally a problem with most techniques if used with raw wastewater, because of the high level of suspended solids, it can be very difficult to find the *Ascaris* eggs among the debris. This technique developed by Ayres (1989) is designed to cope with this problem. Although the technique has a low sensitivity, it has been reported to produce consistent results with seeded samples. The aim was to perform the techniques using the following concentrations of *Ascaris suum* eggs per litre of water (the chosen concentrations representing the range of possible values obtainable in the field),

Technique	No. eggs per litre
Doncaster	4, 10, 50, 100
Raw wastewater	50, 100, 200
Bailenger	10, 50, 100, 200

Logistical constraints however meant that the work carried out on the Doncaster technique was very limited, however the results gave a mean recovery of 86% and this agreed with the reported sensitivity of the technique. The results for the Raw wastewater technique and Bailenger technique are displayed in Table D-1.

Table D-1. Percentage recovery of *Ascaris suum* eggs from seeded water samples using three techniques for wastewater analysis

Technique	No. eggs / litre	Number of eggs recovered / litre		
		Test 1	Test 2	Test 3
Raw wastewater	50	30	32	34
	100	48	21	27
	200	41	44	50
Bailenger	10	0	0	1
	50	11	0	0
	100	72	40	37
	200	11	11	20

Appendix E Pictorial explanation of how to collect the stool sample

SURGERENCIAS PARA COLECCIONAR SU MUESTRA DE HECES

Cuando sea posible, es mejor defecar directamente en el vaso. Cuando no sea posible, entonces hacerlo en papel y hacer lo siguiente :-

1.

doblar el papel y vaciar la muestra dentro del vaso usando un pedazo de papel enrollado

---

2.

cuando la muestra esta en el vaso, cerrar perfectamente con el tapon y poner el vaso en un lugar con sombre

EN CASO de los ninos es necesario que la madre (u otra persona) ayude a coleccionar la muestra de heces.

Appendix F Questionnaire on socioeconomic, hygiene and sanitation factors

CUESTIONARIO DE ESTADO SOCIOECONOMICO

INSTITUTO NACIONAL DE LA NUTRICION
"SALVADOR ZUBIRAN"
MEXICO D.F.

CV V_A VA

DATOS GENERALES

1. FECHA (mes/dia/ano) 04/17/91

2. ENCUESTADOR ______________ 5

3. COMUNIDAD ______________ 22

4. FAMILIA 0020

5. NOMBRE DEL JEFE DE LA FAMILIA Juan Mendoza Cruz

OCTUBRE 1990

6. EDUCACION	PAGINA 2

Anotar el numero de identificacion y el nombre de cada miembro de la familia comenzando con el jefe de la familia. EN LA CASA checar que que no falta.

POR CADA MIEMBRO DE LA FAMILIA anotar,

1. sus ocupaciones (ESCRIBIRLOS)
2. la relacion con el jefe de la familia (ver CODIGOS abajo)
3. si sabe leer (escribir SI o NO)
4. hasta que nivel llego en la esuela (ver CODIGOS abajo)

ID	NOMBRE	OCUPACION 1		OCUPACION 2		RELACION (codigo)		LEER (si/no)		ESCRIBIR (si/no)		ESCUELA (codigo)	
1	Juan	El Campo	01	—	99	Jef. fami	01	Si	1	Si	1	Prim Inc.	02
2	Virginia	Hogar	02	[illegible]	03	Esposa	02	Si	1	Si	1	Prim Inc.	02
4	Rafael	Estudia	04	Pasto	03	Hijo	03	Si	1	Si	1	Prim Inc.	02
5	German	Estudia	04	Past.	03	Hijo	03	Si	1	Si	1	Prim Inc.	02
6	Alberto	Estudia	04	Past.	03	Nieto	14	Si	1	Si	1	Prim Inc	02
7	Yesenia	Estudia	04	—	99	Nieto	14	Si	1	Si	1	Prim Inc	02

OCUPACION 1 = OCUPACION QUE OCCUPA EL MAYOR PARTE DEL TIEMPO
LEER = SI, CUANDO LA PERSONA PUEDE LEER UN ESCRITO/UNA CARTA

CODIGOS - ESCUELA (si el nivel de escuela no esta incluido en estos codigos, anotar el nivel de escuela con flecha)

01 NO FUE A LA ESCUELA
02 PRIMARIA INCOMPLETA
03 PRIMARIA COMPLETA (termino 6o.prim y tiene certificado)
04 SECONDARIA INCOMPLETA
05 SECONDARIA COMPLETA (tiene certificado)
06 NIVEL TECNICO/COMERCIAL (DESPUES QUE TERMINO PRIMARIA)
07 NIVEL TECNICO/COMERCIAL (DESPUES QUE TERMINO SECONDARIA)
10 PREPARATORIA
11 ESCUELA NORMAL (para ser maestro en escuela)
12 UNIVERSIDAD
13 PREPARATORIA INCOMPLETA

88 NO SABE
99 NO APLICABLE

CODIGOS - RELACION (si la relacion no esta incluida en los codigos anotar la relacion)

01 JEFE DE FAMILIA
02 ESPOSA
03 HIJO/A
04 PADRE/MADRE
05 ABUELO/A
06 HERMANO/A
07 TIO/A
10 PRIMO/A
11 SOBRINO/A
12 SUEGRO/A
13 CUNADO/A
14 NIETO/A
15 YERNO/A

88 NO SABE
99 NO APLICABLE

OCTUBRE 1990

Encerrar la respuesta en un circulo. Si la respuesta no esta incluida en los opciones anotarla.

3 min | 3

7. ¿ Hace cuanto tiempo vive la familia en esta casa/pueblo ? 17 años. [17]

8. ¿ Esta casa donde vive la familia es de su propiedad ? [1]

1 SI 2 RENTADA 3 PRESTADA OTRO ____

9. ¿ De que material es la mayor parte del piso de esta vivienda ? [1 | 9]

1 TIERRA 2 CEMENTO 3 MOSAICO OTRO ____

¿ De que material es el piso de las recamaras de esta vivienda ? [1 | 9]

1 TIERRA 2 CEMENTO 3 MOSAICO OTRO ____

10. ¿ De que material es la mayor parte de las paredes o muros de esta vivienda ? [10 | 11]

1 LAMINA DE CARTON 2 LAMINA DE ASBESTO 3 LAMINA METALICA

4 MADERA 5 ADOBE 6 MAGUEY 7 TABIQUE 10 BLOCK 11 PIEDRA

OTRA ____

¿ De que material son las paredes o muros de las recamaras de esta vivienda ? [10 | 11]

1 LAMINA DE CARTON 2 LAMINA DE ASBESTO 3 LAMINA METALICA

4 MADERA 5 ADOBE 6 MAGUEY 7 TABIQUE 10 BLOCK 11 PIEDRA

OTRA ____

11. ¿ De que material es la mayor parte del techo de esta vivienda ? [01 | 02]

1 LAMINA DE CARTON 2 LAMINA DE ASBESTO 3 LAMINA METALICA

4 MADERA 5 PALMA 6 TEJA 7 LOSA DE CONCRETO 10 TABIQUE/LADRILLO

OTRO ____

¿ De que material es el techo de las recamaras de esta vivienda ? [01 | 02]

1 LAMINA DE CARTON 2 LAMINA DE ASBESTO 3 LAMINA METALICA

4 MADERA 5 PALMA 6 TEJA 7 LOSA DE CONCRETO 10 TABIQUE/LADRILLO

OTRO ____

12. ¿ Cuantos cuartos tiene la vivienda aparte del bano y la cocina (incl.cuartos no ocupados) ? [0 2]

13. ¿ Cuantos cuartos se usan para dormir en esta vivienda ? [0 2]

14. ¿ Cuantas personas viven normalmente en esta vivienda y cuantos vienen cada 8 o 15 dias ? [16] normal [0] a veces

15. ¿ Hay luz electrica en esta vivienda (si-1,no-2) ? [1]

16. ¿ Cada cuando barre el patio ? Diario [7] v/semana

17. ¿ Donde cocinan en esta vivienda ? [1]

1 COCINA (CUARTO ESPECIAL PARA COCINAR con paredes y techo)
2 EN LA SALA
3 EN LA RECAMARA
4 POR UN LADO DE LA CASA - TIENE TECHO
5 AIRE LIBRE

OTRO ______

18. ¿ Que combustible se usa para cocinar los alimentos en esta vivienda ? [4]

1 LENA y 2 GAS 3 PETROLEO OTRA ______

19. Que comieron ayer en esta familia (anotar SI o NO) y en los ultimos 7 dias CUANTOS DIAS comieron ....

ADULTOS

	ayer (si/no)		numero de dias en la ultima semana
carne	NO	2	0
pollo	NO	2	0
huevos	SI	1	3
frijoles	NO	2	7
verduras	SI	1	3
fruta	NO	2	2
tortillas	SI	1	7
sopa de pasta	NO	2	1

MENOS DE 12 ANOS

	ayer (si/no)		numero de dias en la ultima semana
carne	NO	2	0
pollo	NO	2	0
huevos	SI	1	5
frijoles	NO	2	7
verduras	SI	1	7
fruta	NO	2	2
tortillas	SI	1	7
sopa de pasta	NO	2	1

CODIGOS : FUENTES DE AGUA

01 RIO
02 CANAL DE AGUAS NEGRAS
03 POZO
04 AGUA POTABLE FUERA DE LA CASA/CALLE
05 AGUA POTABLE EN EL INTERIOR DE LA CASA
06 GARRAFON DE VIDRIO
07 JAGUEY O LAGUNA
10 MANANTIAL
11 LLUVIA
12 AGUA POTABLE EN EL PATIO

20. ¿ De donde toma el agua para beber ? A.P.P. [1 2]

¿ Cuanto tiempo hace caminando ? 1 min (minutos) [1]

21. ¿ Donde guardan el agua para beber ? olla/tambo [2] [1]

1 NO GUARDAN 3 OLLA/CUBETA SIN TAPAR 1 HERVIDA lugar hierve
2 OLLA/CUBETA TAPADA 4 CISTERNA 2 NO HERVIDA

22. ¿ Que hacen al agua antes de tomarla (HIERVE-1,NADA-3) ? Hierve. [1]

SI DICEN QUE HIERVE EL AGUA, ¿ Por cuantos minutos hierve el agua ?
1.hasta q'se forman burbujas abajo
2.hasta q'se forman burbujas arriba
3.menos de 1 minuto con burbujas arriba
4.1-2 minutos
5.2-5 minutos
6.mas de 5 minutos
[6]

VER CODIGOS : FUENTES DE AGUA

23. ¿ De donde toma el agua para cocinar ? A.P.P. [1 2]

24. ¿ De donde toma el agua para lavar los trastes ? A.P.P. [1 2]

25. ¿ De donde toma el agua para lavar la ropa ? A.P.P. [1 2]

26. ¿ De donde toman el agua para banarse ? A.P.P. [1 2]

27. ¿ De donde provienen las verduras que comen en la casa (DESDE TRATAMIENTO) ? Plaza Progreso + Campo [23 03]

28. ¿ Han consumido verduras de su jardin o de alguna parcela ? (DESDE TRATAMIENTO) [1]

¿ Si han consumido verduras de su jardin o de alguna parcela cuales son y con que agua se riegan ?

verdura = Quelites	agua (canal) = Norte.	01	13
verdura = Nopales.	agua (canal) = Hueco	07	23
verdura = —	agua (canal) = —	99	99
verdura = —	agua (canal) = —	99	99

29. ¿ De donde provienen las frutas que comen en la casa (DESDE TRATAMIENTO) ? Plaza Progreso - [23 03]

30. ¿ Si han consumido frutas del jardin o de alguna parcela cuales son y con que agua las riegan ?

verdura = —	agua (canal) = —	99	99
verdura = —	agua (canal) = —	99	99
verdura = —	agua (canal) = —	99	99
verdura = —	agua (canal) = —	99	99

47. Que animales hay en esta casa ?

Por ejemplo : ¿ tienen vacas en esta casa ? Si dicen que tienen, preguntar : ¿ son de su propiedad ?

ANIMAL	TIENE (SI/NO)	SON DE SU PROPIEDAD (SI/NO)		
vaca	Si	Si	vaca	1 1
borrego	Si	Si	borrego	1 1
cerdo	Si	Si	cerdo	1 1
gallina/aves	Si	Si	gallina	1 1
burro	Si	Si	burro	1 1
caballo	Si	Si	caballo	1 1
perro	Si	Si	perro	1 1
gato	Si	Si	gato	1 1

48. De estas cosas cuales tiene en su casa y son de su propiedad ?

POSSESIONES	TIENE (SI/NO)	SON DE SU PROPIEDAD		
radio	Si	Si	radio	1 1
estereo	NO	–	estereo	2 9
television	Si	Si	tv	1 1
video casetera	NO	–	video	2 9
refrigerador	NO	–	refrigerador	2 9
estufa de gas	Si	Si	estufa	1 1
bicicleta	Si	NO	bicicleta	1 2
motocicleta	NO	–	motocicleta	2 9
automovil	NO	–	automovil	2 9
camioneta	NO	–	camioneta	2 9
tractor	NO	–	tractor	2 9

GASTOS

49. Cuanto gasto la semana pasada en comida ? 30,000
Esto es lo que gasta generalmente a la semana ? Si $ 30000

SI DICEN QUE ESTO NO ES EL NORMAL
Cuanto gasta generalmente en comida a la semana ? ______

50. Quienes contribuyen con los gastos de esta familia ?

Nombre	ID	Origen del Ingreso	Cantidad con que contribuye	Cada cuanto tiempo
Juan	01	Del Campo 01	$ 60,000	8 dias 8

51. El ingreso de la casa es fijo (1) ¿ variable (2) ? Variable 2

52. Cual fue el INGRESO TOTAL de la familia la semana pasada ? $60,000 Generalmente este es el ingreso a la semana (si/no) ? Si [illegible]

SI DICEN QUE ESTO NO ES EL NORMAL
Cual es el ingreso generalmente a la semana ? ______