Intestinal helminthiasis in children of early primary school going age in North-eastern peninsular Malaysia:

The effects of the infection on intestinal permeability, occult colonic blood loss, growth and school attendance rates.

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Summary

Ascaris lumbricoides and *Trichuris trichiura* are the two predominant intestinal helminth infections in North-eastern peninsular Malaysia. This thesis is a contribution towards evaluating the effects of the infections on children of early primary school going age in the area. The effect on small intestinal mucosal integrity as measured by the lactulose/mannitol differential permeability test was determined in a study of 246 children from two schools, 106 of whom were infected with intestinal helminths (Chapter 3). Intestinal permeability was assessed on all children at baseline and infected children were treated with albendazole. Permeability studies were then repeated on the infected children and on 68 uninfected subjects, 6-8 weeks after intervention. Infected and uninfected groups were compared with respect to baseline permeability, change in permeability after treatment, the correlation between baseline worm intensity and baseline permeability as well as the correlation between the fall in worm intensity and change in permeability. Analysis of the data suggested that helminthiasis has a marginal effect on intestinal permeability, the impact of which on children of lower socioeconomic groups was negligible in comparison with the cumulative effects of other potentially noxious factors.

The availability of accurate school attendance registers was exploited to simultaneously study the effect of helminthiasis on school attendance in the above group (Chapter 6). Ascariasis was associated with absenteeism at one of the schools but treatment did not improve the attendance rates in comparison with a control group of *Ascaris* negative children. The study did not provide any evidence that helminthiasis independently affected school attendance rates.

Overt rectal bleeding is part of the *Trichuris* Dysentery syndrome; a rare complication of trichuriasis. The possibility that trichuriasis causes occult colonic bleeding in the absence of the overt *Trichuris* Dysentery syndrome was explored in a study conducted on another cohort of 104 schoolchildren; 61 of whom harboured *Trichuris* worms (Chapter 4). A commercially available guaiac test kit was used to detect occult blood in the faeces of all 104 children. To

increase the chances of detecting occult bleeding, additional serial testing was conducted on the 11 children who had heavy trichuriasis and 8 uninfected controls. Although the initial design allowed for repeat stool occult blood testing after treatment, the baseline results obviated the need for this. There was no evidence that trichuriasis predisposes to occult colonic bleeding in the absence of the overt dysenteric syndrome.

The children who took part in the occult blood loss study were also the subjects of a simultaneous investigation on the effect of intestinal helminthiasis on growth (Chapter 5). The overall helminth positive rate in this cohort was 70%. Height and weight measurements were taken at baseline and 14 weeks after antihelminthic treatment. The increases in height, weight, weight for age, height for age and weight for height were greater among infected children who were treated than uninfected controls despite the fact that infected children were on average drawn from lower socioeconomic backgrounds. The gains were modest but statistically significant and occurred predominantly in girls. Although the independent effects of treating *Ascaris* and *Trichuris* infections on growth were difficult to determine with certainty because of the high prevalence of mixed infections, the trends did suggest that successful treatment of trichuriasis may be the critical factor in promoting growth.

The suggestion that helminthiasis in North-eastern Peninsular Malaysia adversely affects growth among early primary schoolchildren requires validation by further interventional studies utilising regimes with greater efficacy particularly against trichuriasis. Investigation of the effect of *Trichuris* infection on colonic mucosal integrity may shed further light on the question of whether modest burdens of *Trichuris* infection are indeed detrimental to health.

In conclusion, the results suggest that intestinal helminthiasis among young primary school children in North-eastern Peninsular Malaysia, has a modest independent effect on growth and a marginal effect on intestinal permeability (the impact of which on children from lower socioeconomic backgrounds is negligible); but does not independently cause school absenteeism nor occult colonic bleeding.

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Abbreviations used in the text.

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
^{14}C	Carbon 14 radioisotope
CDC	Centers for Disease Control
95% CI	95% confidence interval
^{51}Cr	Chromium 51 radioisotope
EDTA	Ethylene diamine tetra-acetic acid
EEG	Electroencephalography
epg	Eggs per gram of faeces.
ERCP	Endoscopic cholangio-pancreatography.
fig.	Figure.
IgA	Immunoglobulin A
IgE	Immunoglobulin E
HAZ	Height for age Z-score
ΔHAZ	Change in height for age Z-score from baseline to follow-up.
L/M1	Urinary recovery ratio of lactulose to mannitol at baseline.
L/M2	Urinary recovery ratio of lactulose to mannitol ratio at follow-up.
ΔL/M	Change in urinary lactulose to mannitol recovery ratios
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide (reduced form)
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide diphosphate (reduced form)
NCHS	National Center for Health Statistics
NSAID	Non-steroidal antiinflammatory drugs.
QD	Quartile deviation
r	Correlation coefficient
RM	Ringgit Malaysia (Malaysian currency)
SD	Standard deviation
TNFα	Tumour necrosis factor α
USA	United States of America
WAZ	Weight for age Z-score.
WHO	World Health Organization
WHZ	Weight for height Z-score.
ΔWAZ	Change in weight for age Z-score from baseline to follow-up.
ΔWHZ	Change in weight for height Z-score from baseline to follow-up.

Declaration

I declare that the work has been done and the thesis composed by myself, and the books and papers cited were all consulted by me personally unless it is otherwise stated.

Signed,

Sundramoorthy Mahendra Raj

7th January 1999

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This thesis is dedicated to the memory of my late father, Ponnampalam Sundramoorthy who taught me the art of optimism.

Full papers published or accepted for publication in peer reviewed journals.

1 S. Mahendra Raj, K.T. Sein, A. Khairul Anuar, B.E. Mustaffa. Effect of intestinal helminthiasis on intestinal permeability of early primary schoolchildren. *Trans. R. Soc. Trop. Med. Hyg.* 1996; 90: 666-9.

2 S. Mahendra Raj. Fecal occult blood testing of *Trichuris*-infected primary school children in Northeastern Peninsular Malaysia. *Am. J. Trop. Med. Hyg.* 1999 (accepted; in press).

3 S. Mahendra Raj, N.N. Naing. Ascariasis, trichuriasis and growth of schoolchildren in Northeastern Peninsular Malaysia. *Southeast Asian J. Trop. Med. Public Health.* 1998 (accepted, in press).

4 S. Mahendra Raj, K.T. Sein, A. Khairul Anuar, B.E. Mustaffa. Effect of intestinal helminthiasis on school attendance by early primary schoolchildren. *Trans. R. Soc. Trop. Med. Hyg.* 1997; 90: 131-2.

5 S. Mahendra Raj, K.T. Sein, A. Khairul Anuar, B.E. Mustaffa. Intestinal helminthiasis in relation to height and weight of early primary school children in Northeastern Peninsular Malaysia. *Southeast Asian J. Trop. Med. Public Health* 1997; 28: 314-20.

6 S. Mahendra Raj. Intestinal geohelminthiasis and growth in pre-adolescent primary school children in Northeastern Peninsular Malaysia. *Southeast Asian J. Trop. Med. Public Health* 1998; 29: 112-117.

NB. Papers # 1, 2, 3 and 4 correspond to the main studies decribed in chapters 3, 4, 5 and 6 respectively. Papers # 5 and 6 represent the preliminary data alluded to in section 5.3 of chapter 5 which served as the foundation for the main study described in that chapter.

Chapter 1. Literature review on the prevalence, biology and morbidity of intestinal geohelminthiasis

Literature review on the prevalence, biology and morbidity of intestinal geohelminthiasis

1.1 General introduction

The nematodes *Ascaris lumbricoides* and *Trichuris trichiura* infect a quarter of the world's population.[1-3] Despite the scale of the global burden of infection and the fact that worm infection of the human gastrointestinal tract has been recognised at least since the time of Hippocrates,[4] there is a lack of empirical data on the extent of human morbidity attributable to these infections.[2,3] The term intestinal helminthiasis refers to an infection with any worm species whose life cycle includes an obligatory phase of inhabitation in the lumen of the gastrointestinal tract. This thesis focuses on the effects of ascariasis and trichuriasis, the two most prevalent nematode infections among primary schoolchildren in North-eastern Peninsular Malaysia. Elucidating the effects of helminthiasis on schoolchildren is pertinent not only because the health of schoolchildren is an important determinant of the future of a community but also because schoolchildren represent a readily accessible group for targetting preventive health measures.[5] As the vast majority of infected individuals reside in the third world, accurate assessment of helminth induced morbidity has a bearing on the allocation of scarce health care resources.

1.2 Global prevalence and population biology of *Ascaris* lumbricoides, *Trichuris trichiura* and hookworm infections.

Half a century ago, Stoll, in an often quoted paper estimated from the available survey data of the time that 29%, 16% and 21% of the world's population were infected with *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm respectively.[6] Altough the global population has since more than doubled, more recent estimates of the prevalence rates of ascariasis and trichuriasis have changed by no more than a few percentage points. Based on an extensive and critical review of parasitological surveys reported after 1975, Crompton in 1989 estimated the global frequency of human *Ascaris* infection to be 1008 million.[1] Warren *et al.* in a 1993 publication for the World Bank placed the worldwide *Ascaris*, *Trichuris* and hookworm infection frequencies at 1,129 million, 770 million and 877 million respectively.[2] In an even more recent paper based on an extensive database of prevalence data accumulated over the preceding 20 years, Chan *et al.* estimated the prevalences of the three infections to be somewhat higher at 1471 million, 1048 million and 1297 million respectively.[3] In arriving at their estimation Chan *et al.* were selective in only considering data derived from large community based surveys. There are obvious limitations in such estimates. Prevalence data may not be available in many areas. Sample selection of even community based studies may not be truly random and representative of the population. Furthermore, stool examination which is the commonest mode of detecting infection has its diagnostic limitations. Nonetheless the estimates give an indication of the scale of infection.

In arguably the most comprehensive national survey undertaken in recent times, a randomly selected sample of 1.5 million subjects from almost 3,000 sites in China were examined for intestinal helminth infection.[7] Based on the survey data, the frequencies of human infections with *Ascaris*, *Trichuris* and hookworm in China were estimated to be 531, 212 and 194 million respectively. The *Ascaris* figures derived from this study[7] are comparable to the previous estimates for China by Warren *et al.*[2] and Chan *et al.*[3] but the *Trichuris* and hookworm figures are a little lower than previously estimated. Although separate prevalence figures have been quoted for *Ascaris*, *Trichuris* and hookworm infections, it should be recognised that the three infections often coexist in the same host not only with each other but with other intestinal parasites.[2, 8-11] The bulk of the global burden of infection is found in the tropical and subtropical world[1-2] where climatic conditions condusive for transmission act in synergy with inadequate provisions for sanitation, clean water supply and housing to establish the endemicity of intestinal helminthiasis. A warm and moist climate favours high prevalence rates. This was clearly illustrated in the data of the Chinese national survey where prevalence rates were observed to fall progressively northwards with decreasing temperatures and annual rainfall.[7] *Trichuris* and hookworm transmission seem to be particularly sensitive to low temperatures and lack of moisture; the prevalence rates in the cold and arid areas of China often falling to below 1%.[7] Crompton and Tully had earlier observed from a review of the published data that *Ascaris* prevalence rates were generally lower in the drier countries of Africa.[12] It is notable however that marked variations in prevalence are observed within nations, not only between provinces but between communities within a province.[7,13]

The age prevalence profile of intestinal helminthiasis is generally convex or *asymptotic* (Fig 1.1). *Ascaris* and *Trichuris* prevalence rates usually peak in childhood or less commonly in adolescence[1,3,7,14] while hookworm prevalence tends to peak later in adolescence or adulthood.[3,7] A consistent observation is that the prevalence of infection is low in the first year of life. Irrespective of whether the age prevalence profile is convex or *asymptotic*, children of primary school going age constitute a major pool of infection. Indeed Chan *et al.* estimated a global prevalence of 194 million *Ascaris*, 141 million *Trichuris* and 128 million hookworm infections among 5-10 year old children.[3]

Figure 1.1 Typical age-prevalence profiles of *Ascaris* and *Trichuris* infections showing: a) an asymptotic and b) a convex pattern.

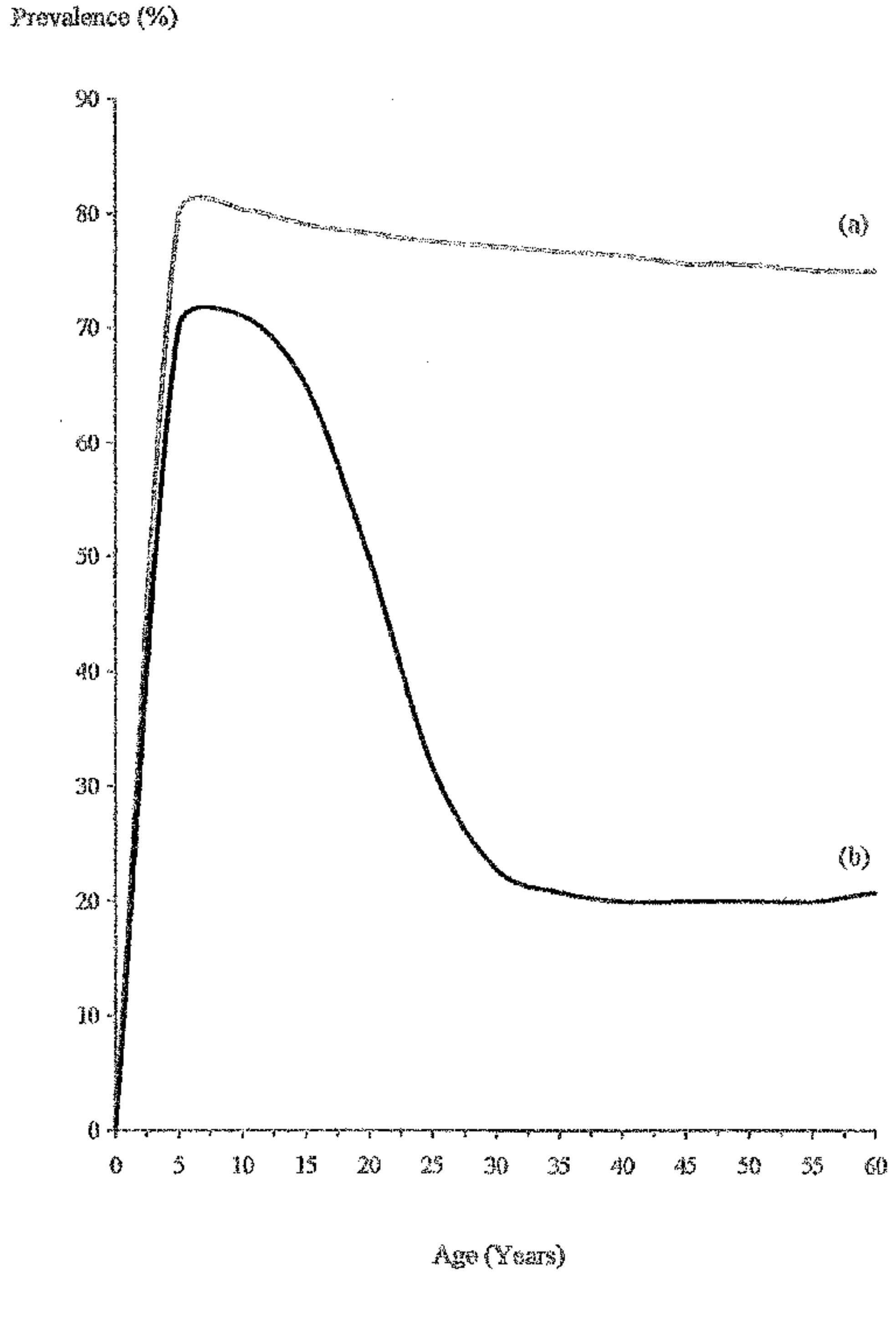
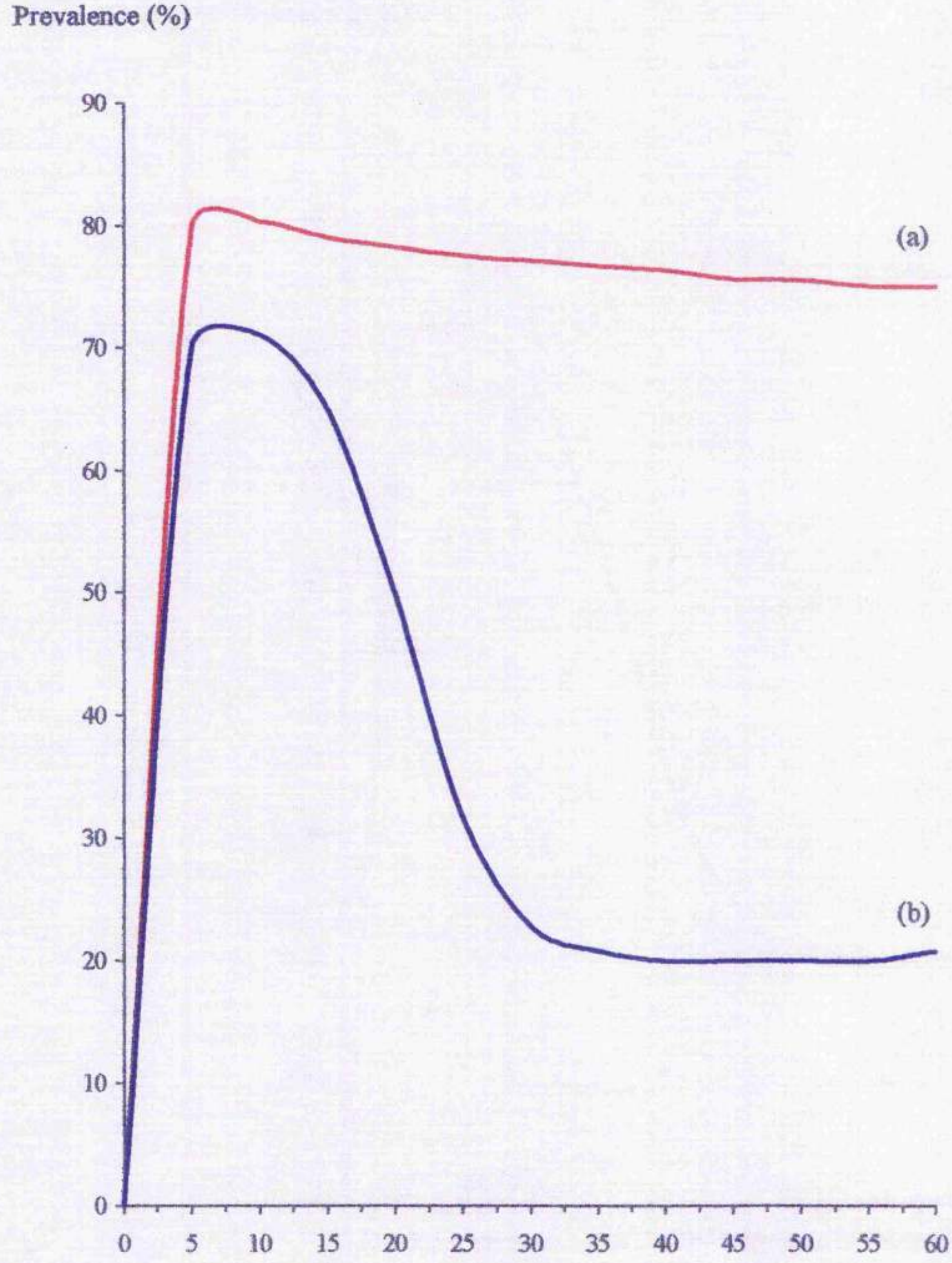


Figure 1.1 Typical age-prevalence profiles of *Ascaris* and *Trichuris* infections showing: a) an asymptotic and b) a convex pattern.



In terms of morbidity, the average intensity of infection in a community may have a greater bearing than the prevalence rate. Intensity of infection can either be estimated by quantifying the density of eggs in a sample of stool or by direct counting of worms expelled after administration of an effective antihelminthic drug. The latter may be a more accurate measure of intensity of infection but suffers from the major logistic drawback of requiring the collection of total stool output over 2-4 days and the painstaking task of recovering and counting the worms. The stool egg count despite its imperfection has therefore become established as the most frequently used index of worm intensity especially in large surveys. The age intensity profile of both ascariasis and trichuriasis is convex[1,14] and generally peaks during childhood.[1,9,14-18] Indeed the peak intensity often occurs in the 5-10 year age group[14,15,17-19] (fig 1.2). Only occasional departures from this pattern have been documented.[20] This further emphasises the importance of primary school children as a target for research into the effects of helminthiasis.

Another well recognised phenomenon of intestinal helminthic infection is the overdispersed frequency distribution of worms in the human host population. In simple terms, this means that a small proportion of any given community harbours most of the worms while the vast majority of inhabitants in an endemic area are either uninfected or harbour only a few worms.[9,17,19,20,21] This is schematically represented in figure 1.3.

Figure 1.2 Typical shape of the age-intensity curve for (a)*Ascaris* and (b)*Trichuris* infections

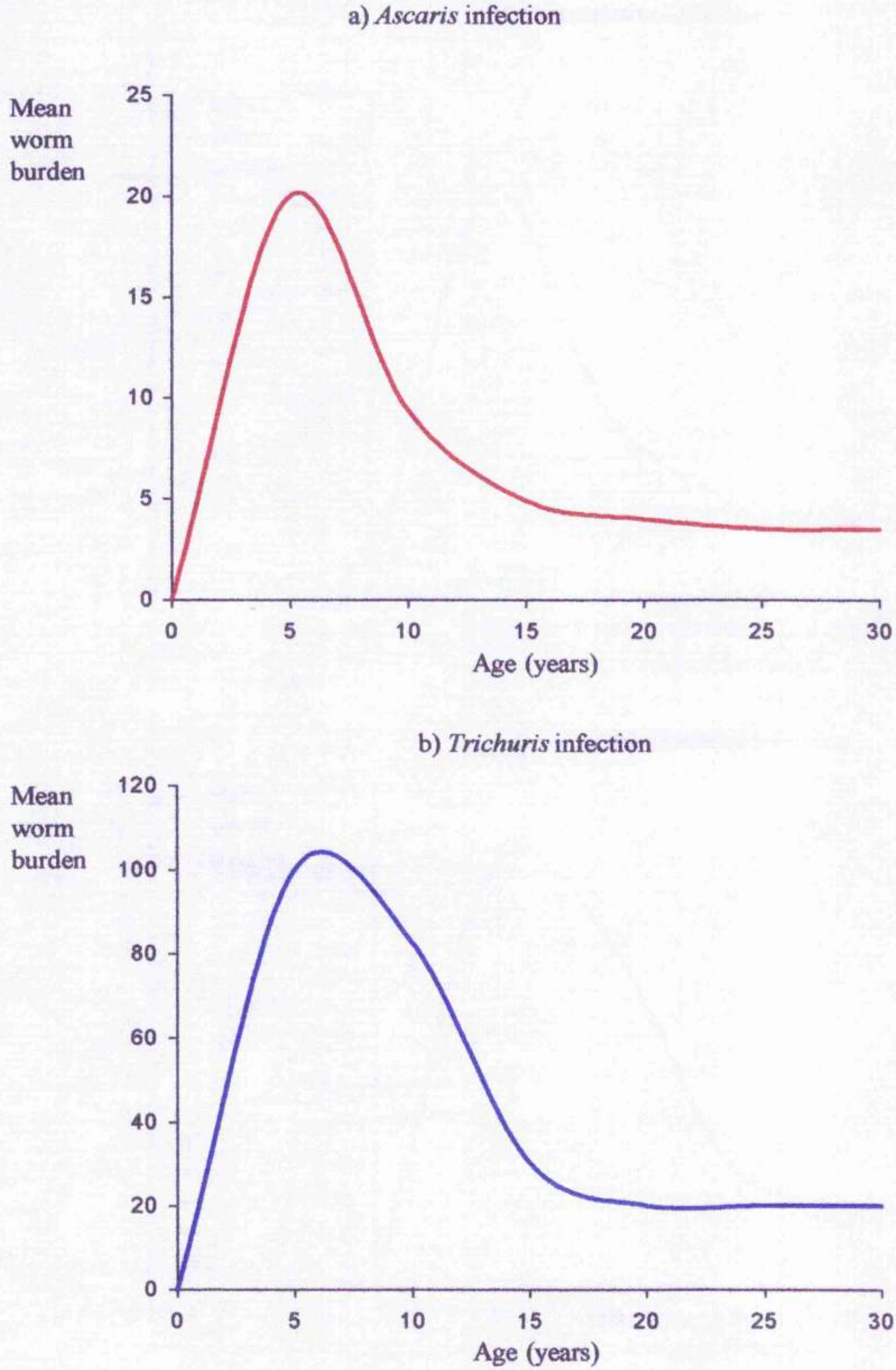
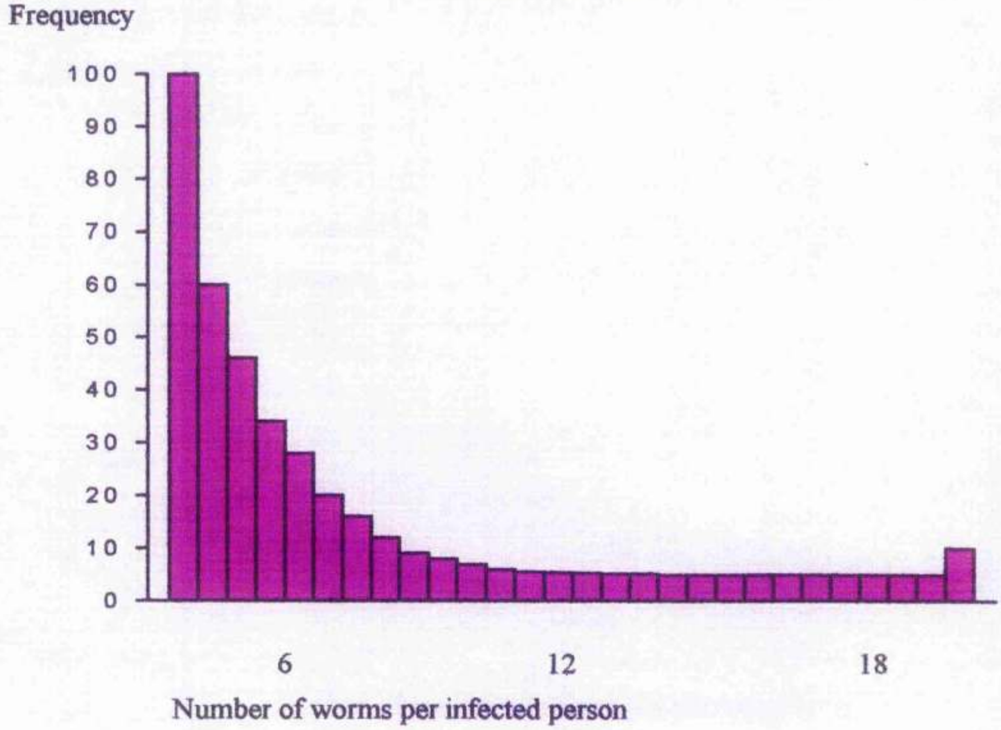
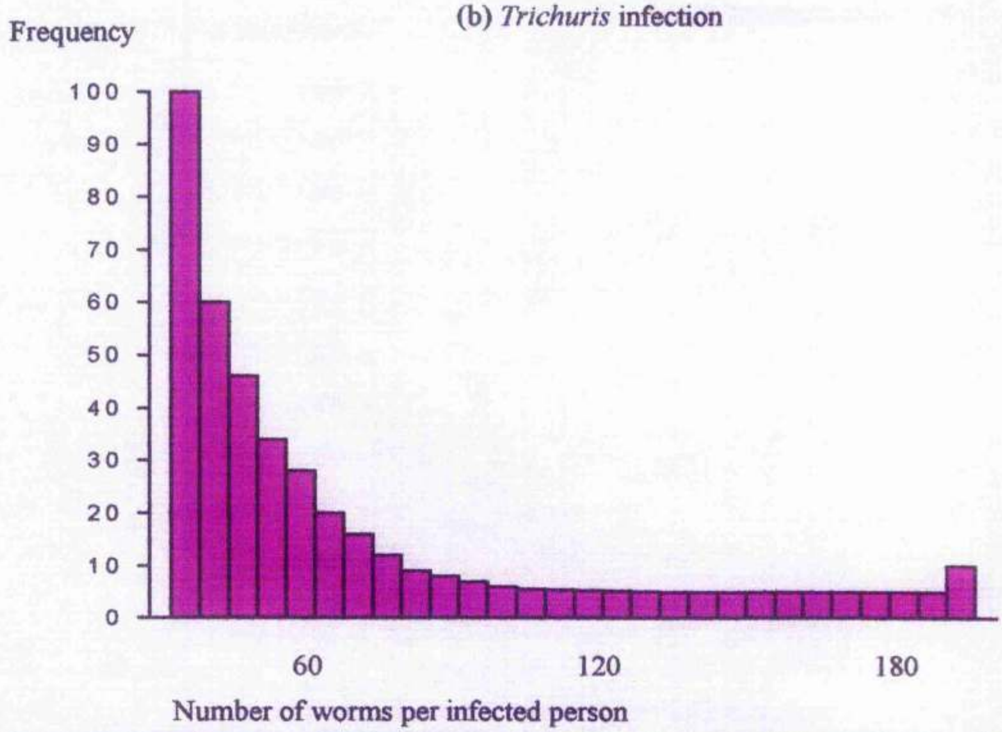


Figure 1.3 Typical shape of the frequency disribution curve of worm burden in the host population.

a) *Ascaris* infection



(b) *Trichuris* infection



Based on the negative binomial distribution probability model, the degree of overdispersion can be mathematically derived and expressed as the parameter κ; smaller values of which indicate greater overdispersion.[23] There is some evidence that κ is age dependent for both *Trichuris* and *Ascaris* infections and progressively increases in the first few years of life before stabilising at about 6-8 years of age.[24] It has also been suggested that the prevalence, intensity and degree of aggregation of infection in a given community may be related to each other in a predictable way.[25,26] Based on the assumption that heavily infected individuals are more likely to experience the morbid effects of infection, the relevance of these considerations is that the prevalence of infection per se in the community may also be used to predict the proportion at risk of helminth induced disease.

1.3 Prevalence data in Malaysia

A number of parasitological surveys have been undertaken in Malaysia. The data of community based studies with sample sizes of more than 500 subjects published in indexed journals since 1975 are summarised in table 1.1. It is notable that most surveys have focussed on populations in and around the Federal Capital, Kuala Lumpur. Furthermore, only one of the larger studies specifically reported that a random sample of the study population was selected for survey.[19] There may therefore have been some selection bias in most of the reported surveys. The data nonetheless provide some indication of the prevalence and pattern of helminthiasis. Ascariasis and trichuriasis are the commonest helminth infections, hookworm infection being consistently less common in all areas surveyed. *Trichuris* infection seems to be more common than *Ascaris* infection in Kuala Lumpur and its surrounding area (table 1.1). The large studies conducted in and around Kuala Lumpur reveal a couple of interesting trends. Firstly, the *Ascaris* and *Trichuris* prevalence rates are higher among children in urban slums than in rural or plantation communities.[13,19]

Secondly, there is evidence of racial predisposition to ascariasis and trichiuriasis.[8,13,24] Within urban slums, ethnic Indians are most likely to be infected and ethnic Chinese the least likely. Ethnic Malays seem to carry an intermediate risk of infection. The reason for the racial predisposition is unclear and may be be due to a complex of interacting sociocultural and genetic factors. Consistent with global trends, infection is commoner in children than adults. Where finely stratified age prevalence and age intensity data in large samples of children were available, the prevalence and intensity of *Ascaris* and *Trichuris* infections were shown to peak between 6 and 10 years of age,[13,19,24,27] the exception being a single survey from the west coast of Northern Peninsular Malaysia. In that particular study, the *Ascaris* prevalence and intensity were highest in the 20-40 year age group, while *Trichuris* prevalence and intensity peaked even later.[28] The compliance rate of stool submission in the various age groups in that study was not reported and the possibility of a selection bias cannot be excluded.

There are no systematic community based survey data from North-eastern Peninsular Malaysia reported in peer reviewed journals. The experience of clinicians working in the area however is that helminth infections are common. One survey of hospitalised adult patients in the area revealed *Ascaris* and *Trichuris* prevalence rates of 25% and 36% respectively, while hookworm rates were found to be lower at about 14%.[29]

Table 1.1 Summary data of community based Malaysian prevalence surveys published since 1975 with sample sizes of more than 500 subjects.

Author(s)	Year	Sample size	Population studied	Prevalence rates (%)		
				As.	*Tr.*	*Hk.*
Kan[8]	1982	25,246	Urban slum, plantation semirural village and urban flat dwellers in and around Kuala Lumpur.	18	33	7.1
George & Ow Yang[30]	1982	7,682	Urban primary schoolchildren in Kuala Lumpur	22	45	4.6
Kan[31]	1984	1,157	Indian primary schoolchildren in and around Kuala Lumpur.			
			Urban:	76	91	3
			Rural:	39	93	42
Kan & Poon[13]	1987	11,874	Children of urban slums and flats, semi urban residential areas, and plantations in and around Kuala Lumpur	19	36	3
Bundy *et al.*[24]	1988	1574	Urban slum children in Kuala Lumpur	50	63	5.3
Kan *et al.*[19]	1989	1499	Children of rural plantation community and urban slum, west coast Peninsular Malaysia	21	29	6
Kan[27]	1989	819	Indian plantation community	34	36	16
Rahman[28]	1994	706	Village communities in North-western Peninsular Malaysia	41	34	33

As. = Ascaris lumbricoides. Tr.= Trichuris trichiura. Hk.=hookworm .

1.4 Summary of life history and biology of the worms.

Ascaris lumbricoides, Trichuris trichlura and the hookworms belong to the Phylum Nematoda or roundworms. The nematodes share many basic similarities in anatomical structure[32] (fig 1.4 and 1.5). They are tubular, unsegmented worms with a dynamic exoskeleton consisting of a three zoned cuticle which is secreted by an underlying epidermal sheet of cells. The cuticle contains enzymes, is metabolically active and is selectively permeable to water and other substances. It is well adapted to serve as a functional interphase between the worms and their external environments. The epidermis separates the outer cuticle from the inner longitudinal rows of somatic muscle which lie between longitudinal epidermal cords (fig 1.4, 1.5a and 1.5b). It contains the energy reserves, enzymes and chemical substrate for protein and carbohydrate synthesis. The digestive system consists of a simple tube which opens anteriorly through a mouth and posteriorly through the anus. There is a triradiate muscular pharynx at the anterior end (fig 1.5a). The intestine is a simple tube of single layered epithelial cells with microvilli on the luminal surface. The worms are dioecious organisms which reproduce sexually. In addition to gonads, the worms have secondary sexual organs which demonstrate variability between species. The nervous system essentially consisists of a circumenteric nerve ring with processes synapsing with sensory end organs. The secretory-excretory system shows considerable varibility in complexity. The internal organs are bathed by fluid within the pseudocoelomic cavity which lies between the somatic muscles (fig 1.5a and b). The pseudocoelomic fluid contributes to the turgidity of the worms. The nematodes do not have specialised respiratory or circulatory organs. As a detailed discussion of the literature on the life cycles of the helminths is somewhat beyond the scope of this thesis, the brief account which follows is largely gleaned from authoritative reviews [14,32-35].

Fig 1.4 A simple schematic representation of the common anatomical features of nematodes shown in longitudinal section.

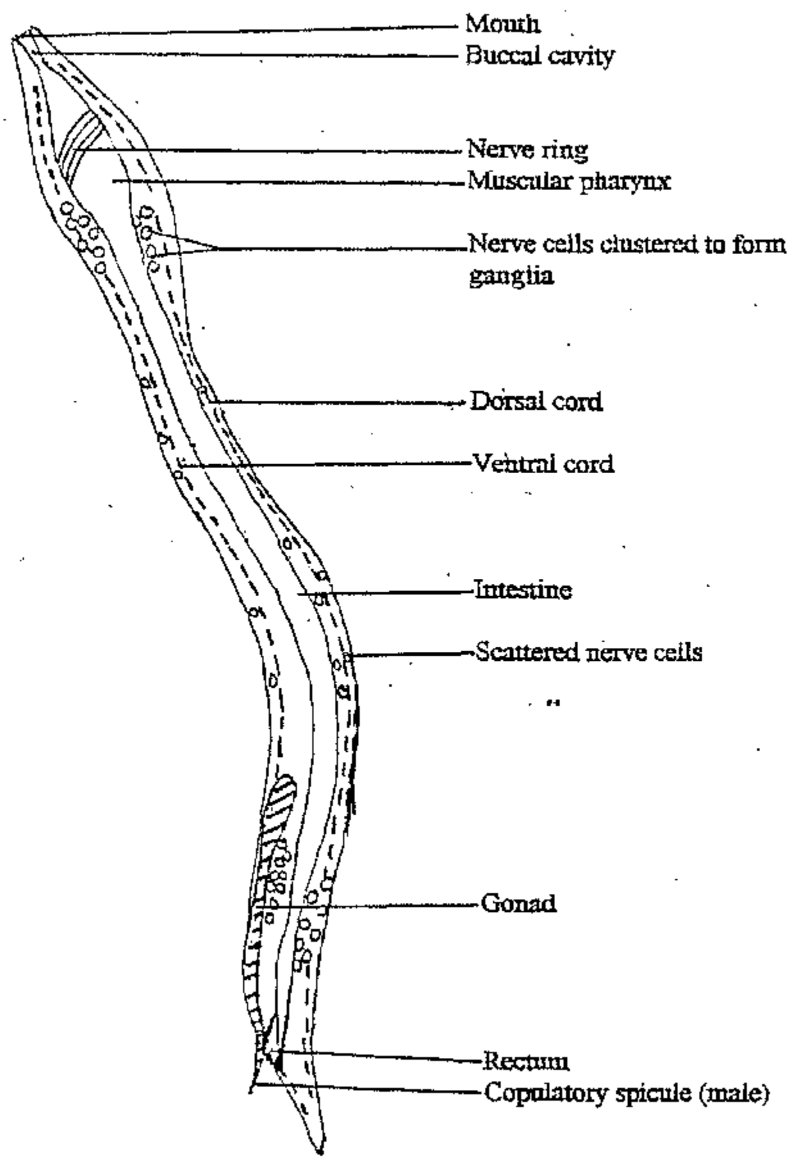
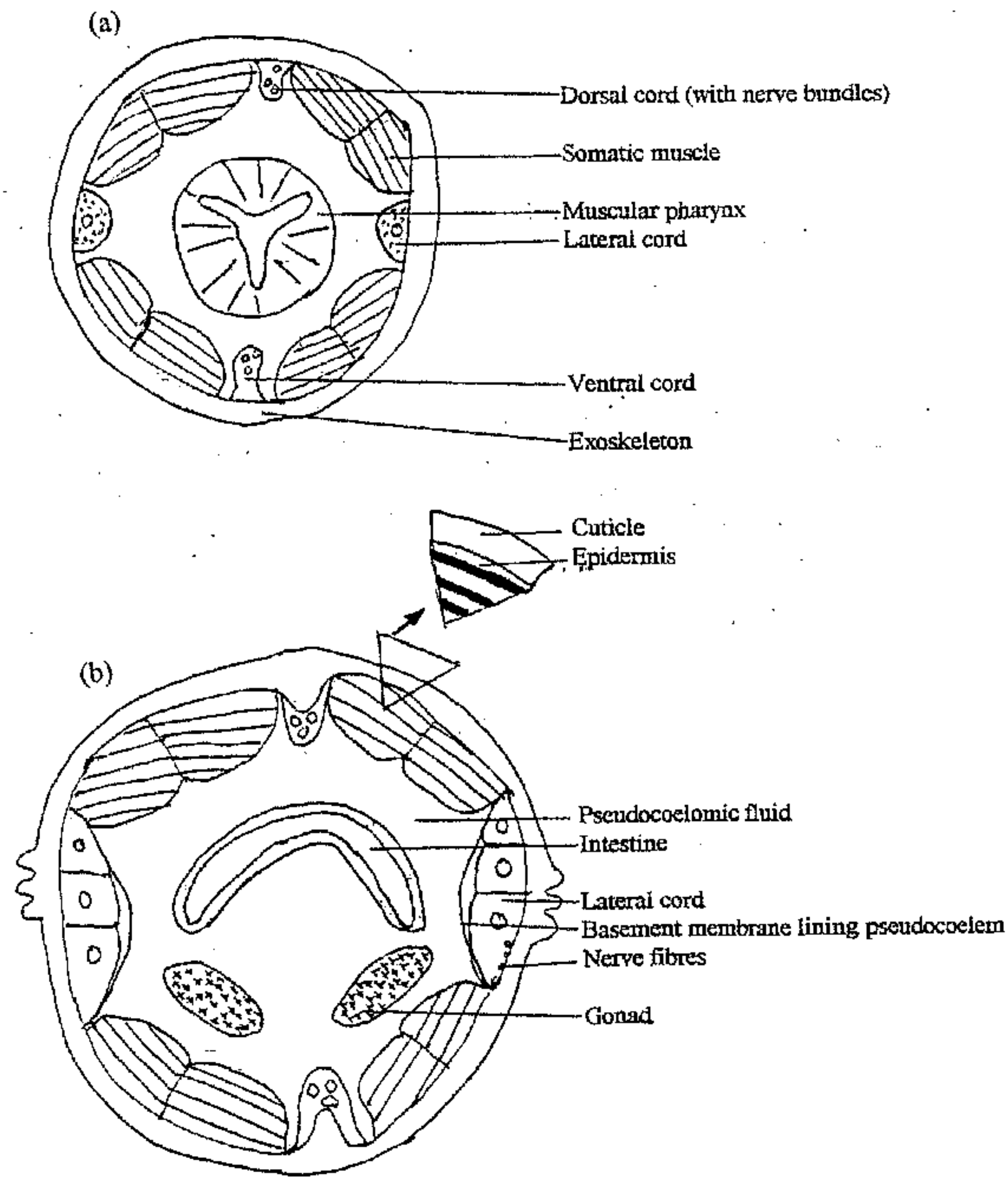


Fig. 1.5 Simple schematic representation of some common anatomical features of nematodes seen in cross-section at the level of (a) pharynx and (b) a more caudal level.



1.4.1 *Ascaris lumbricoides* [33,34]

This nematode belongs to the family *Ascaridae*. *Ascaris* denotes the genus, and *lumbricoides* the species. Mature adult female worms are typically about 300 mm long but have been known to acquire lengths of almost 500mm. The mature male worm is generally thinner and shorter averaging about 200 mm (fig. 1.6). Males are morphologically distinguished by a curved posterior end which houses the copulatory spicules. The worms are yellowish white and often have a pinkish transluscent hue due to the presence of haemoglobin in the body wall and pseudocoelomic fluid. Live worms have a tough and turgid feel. As reported by Makidono in his classic paper based on a large experience of barium examinations of the small bowel, the adult *Ascaris lumbricoides* mainly inhabits the human jejunum and ileum, with a ratio of 8 to 1 in favour of the jejunum.[36] Within the jejunum, the worms are most frequently found in the middle third. Makidono noted that 0.3% and 0.5% worms of the total number of worms were found in the duodenum and stomach respectively indicating that upward migration is relatively uncommon. Most worms appeared to maintain a stable position within the lumen against peristalsis. Occasional upward movement in a spiral manner is probably effected by contraction of longitudinal muscles found below the hypodermis. Through a small mouth, the worm is thought to feed on intestinal chyme which is pumped into the worm and propelled along its intestine by a muscular pharynx.

The ratio of male to female worms in the human host is roughly equal but there is sometimes a preponderance of females. The life expectency of the adult worm is thought to be about 1-2 years. Following copulation, each female worm produces between 150,000 and 350,000 eggs per day which are passed out in the faeces of the host.

Figure 1.6. Two adult worms among 6 which were removed endoscopically by the author from the bile duct of a patient who presented with cholangitis. The shorter worm with the curved posterior end is a male.

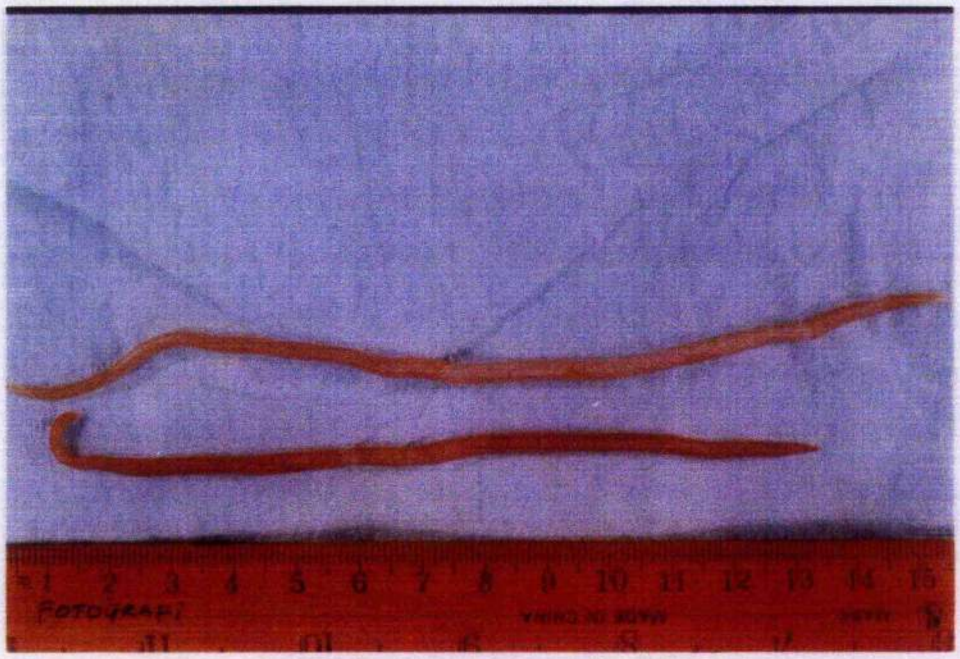
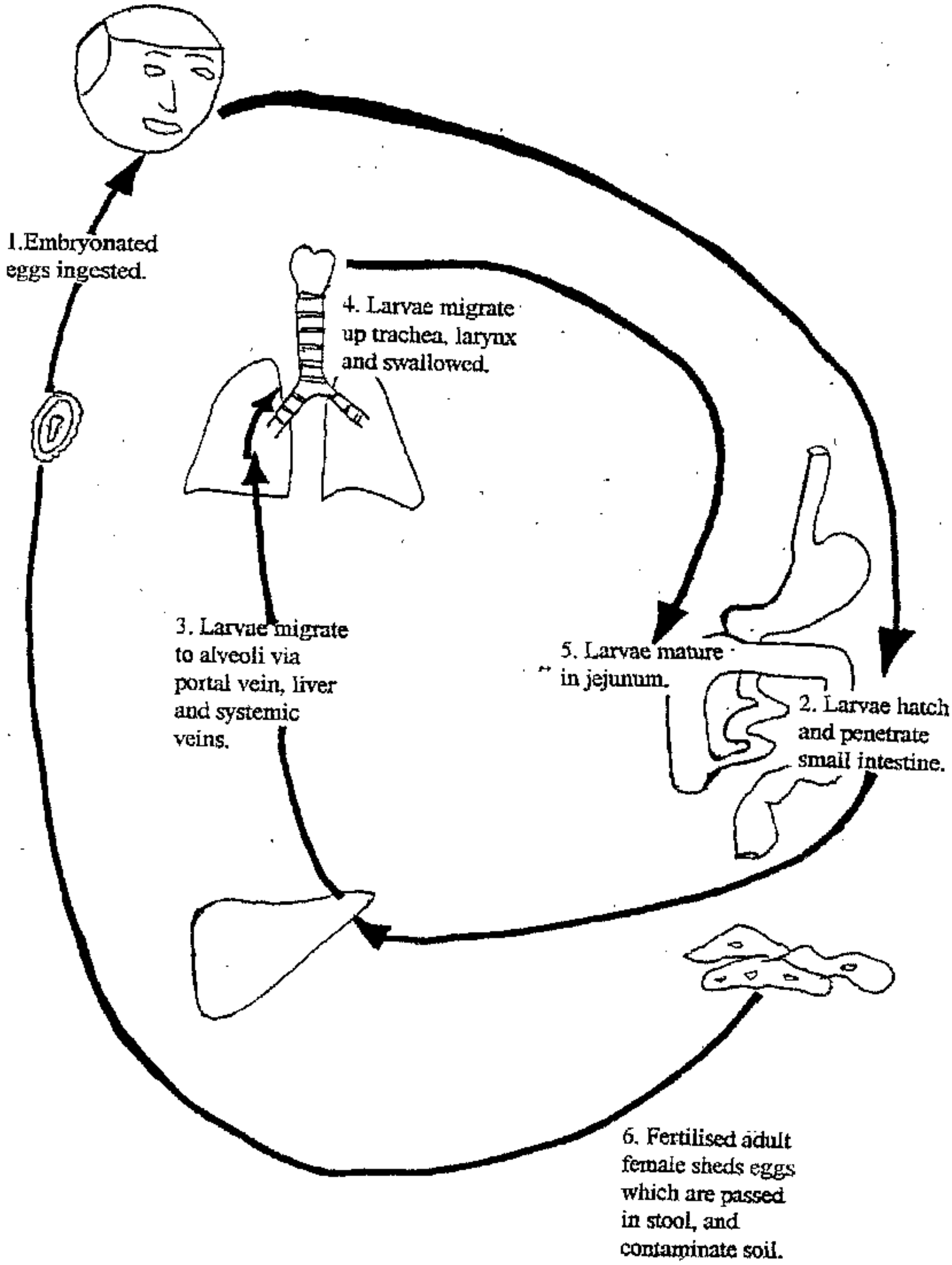


Figure 1.7. A photomicrograph (X 1000) of an *Ascaris* egg.

The life cycle of *Ascaris lumbricoides* is direct with no intermediate host. Knowledge of the life cycle of *Ascaris* has been obtained not only from data obtained from human infections but to a large extent from pig infections with the equivalent *Ascaris suum.* There was some debate in the past as to whether *Ascaris lumbricoides* and *Ascaris suum* are different species or the same species infecting two different hosts. Subtle morphological differences in the adult forms as well as antigenic differences between the two seem to suggest that they are indeed two different species. The life cycle of *Ascaris* broadly consisists of the fertilised egg, 4 larval stages and the adult stage of the worm. The fertilised eggs are elliptical and measure about 50-70 μm by 40-70 μm (fig 1.7). The zygote is encased in a complex egg shell composed of 4 layers which is responsible for its survival characteristics in the environment. When sanitary conditions are poor, the eggs passed in the faeces contaminate the soil. Under ambient conditions of warmth (28-32°C), moisture, oxygen and shade the eggs embryonate into the first and second stages of the larva within the egg shell. The second stage is infective and infection occurs by the oral route. Although infection can occur at any age, children are at particular risk as they frequently play in contaminated environments. Their hands are more likely to come into contact with contaminated soil and they are less likely to wash their hands before meals (fig 1.8). While eggs are destroyed by exposure to direct sunlight and desiccation, they have been known to survive for years within the soil if protected from these factors. It is thought that hatching occurs in the jejunum within a few hours of ingestion. The emergent second stage larvae then penetrate the intestinal mucosa and start their tissue migratory phase within the human host through the portal venous drainage to the liver, and then via the systemic veins and pulmonary arteries to the lungs. In the lungs the larvae moult into the third stage, migrate up the bronchial tree to the pharynx, are swallowed and reenter the gastrointestinal tract. The transformation from the third larval stage through the fourth and eventually to the adult phase occurs in the jejunum. The larvae in the lungs are about 500 μm long while the length just before the final moult in the intestine is about 1800 μm. Data on the tissue migratory phase which takes from 3 to 6 weeks has been derived from animal experimentation. After the final moult in the intestine, growth to the final adult size is rapid and the worms reach lengths of about 200mm within 4-6 months. Figure 1.9 summarises the life history of *Ascaris lumbricoides* schematically.

Figure 1.8. Children playing in close contact with potentially contaminated soil.

Fig 1.9 Diagrammatic summary of life history of *Ascaris lumbricoides*.



1.4.2 *Trichuris trichiura* [14]

This nematode belongs to the family *Trichuridae*. *Trichuris* denotes the genus and *trichiura* the species. Like *Ascaris lumbricoides* the life cycle of *Trichuris trichiura* is direct and infection of the human host is by the oral route. Unlike *Ascaris* however the natural habitat of the adult *Trichuris worm* is the colon. In light infections the adult worms are confined to the caecum but in intense infections the worms are found throughout the length of the colon and sometimes even in the terminal ileum. The adult worm which may be 3-5 cm in length is whip shaped with a thin anterior end and a thicker posterior end (fig 1.10). A simple mouth armed with a stylet and the oesophagus are found in the anterior end while the intestine and reproductive organs are housed in the thicker posterior end. The thin anterior end is embedded within the colonic epithelium while the posterior end lies free within the lumen. *Trichuris trichiura* is therefore considered to be a tissue parasite as opposed to the primarily luminal *Ascaris lumbricoides*. The female to male ratio of adult worms is generally a little over 1:1 and their life spans have been estimated to be between 1 and 3 years. The female worm passes between 3000 and 20,000 eggs per day.

The egg is barrel shaped, measures 50 μm by 22 μm and is composed of three layers (fig 1.11). The eggs embryonate into the infective second stage larva under ambient conditions of shaded warm moist soil. The optimum temperature for development of *Trichuris trichiura* eggs appears to be somewhat higher than that of *Ascaris lumbricoides* which may explain why *Trichuris* prevalence rates fall more steeply than *Ascaris* prevalence rates towards the Northern provinces of China.[7] The factors which predispose to transmission of *Trichuris trichiura* are in many ways similar to that of *Ascaris lumbricoides*, hence the tendency for children to bear the brunt of the *Trichuris* worm burden in an endemic community.

Figure 1.10. Colonoscopic view of an adult *Trichuris* worm attached to the caecal mucosa.

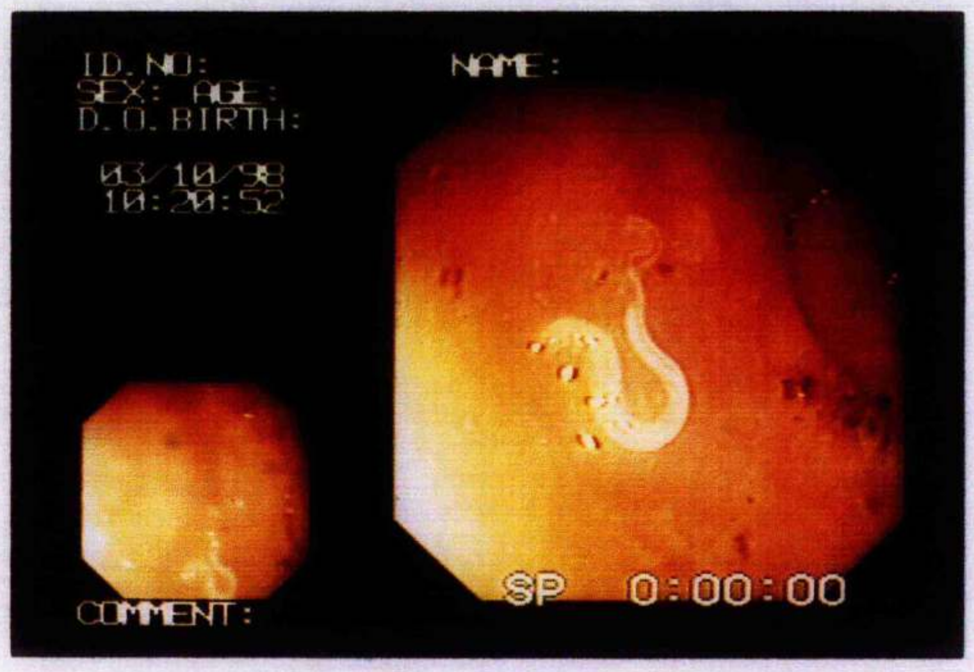


Figure 1.11. Photomicrograph of a *Trichuris* egg (X1000).

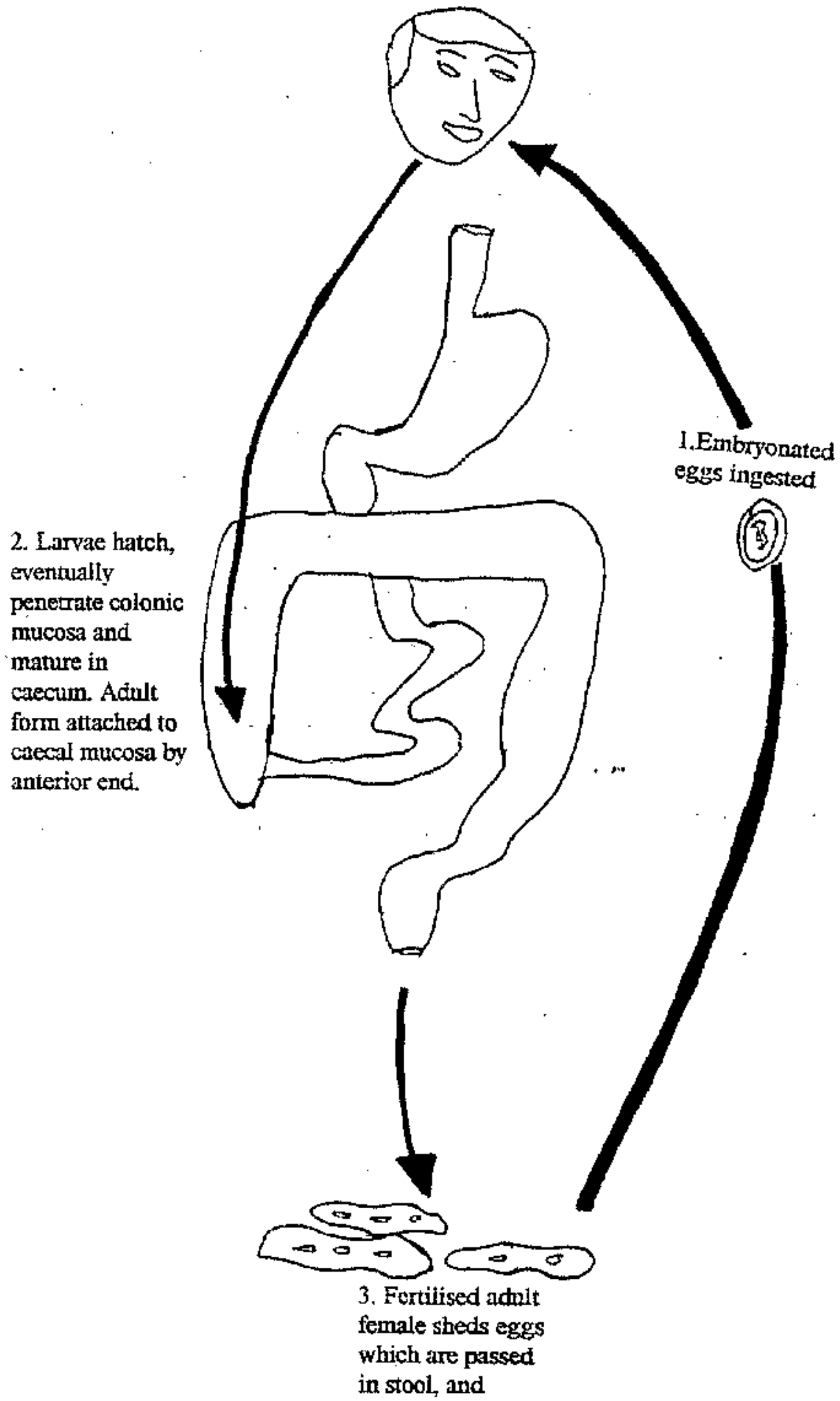


Fig 1.12 Diagrammatic summary of life history of *Trichuris trichiura.*

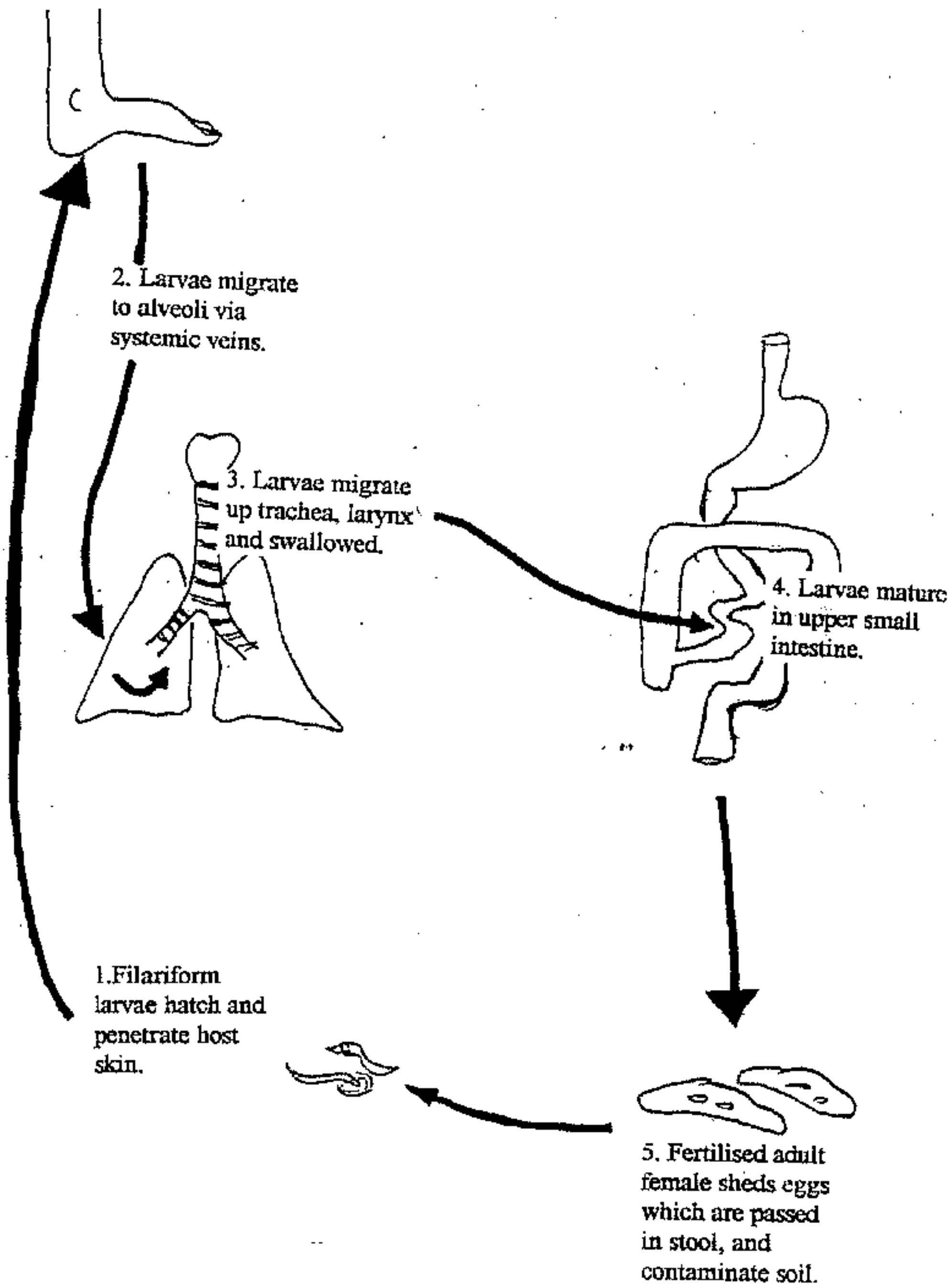
Much of the data on the developmental phase of *Trichuris trichiura* in the human host following ingestion of the embryonated eggs is derived from studies of *Trichuris suis* infection in pigs, *Trichuris muris* infection in mice and *Trichuris vulpis* infection in dogs. Some controversy surrounds the larval development phase in the human host. Certain animal studies suggest that the larvae hatch in the duodenum, transiently penetrate the intestinal mucosa, reemerge into the lumen a few days later, and eventually establish themselves in the mucosa of the caecum where development proceeds to completion. However some workers question the existence of this duodenal phase. It is nonetheless unanimously agreed that post-embryonic worms are found in the colonic epithelium and there is no evidence for a complex visceral migratory phase akin to that of *Ascaris lumbricoides*. The larvae are thought to penetrate the caecal epithelium in the crypts of Lieberkuhn and migrate up the crypts with the epithelium. The larvae as they grow and develop through four larval stages are completely enveloped by a sheet of epithelium and move by tunneling through the endocyte membranes. As the worm grows, the epithelial tunnel enlarges and eventually the thickened posterior end of the worm breaks through the epithelial lining and protrudes into the lumen. Figure 1.12 summarises the life history of *Trichuris trichiura* diagrammatically.

1.4.3 Hookworm[35]

Necator americanus and *Ancylostoma duodenale* are the two main hookworm species that infect man. Based on the available but albeit limited data, it would appear that *Necator americanus* is the predominant species in Malaysia.[37] The adult female *N. americanus* is between 9 and 11 mm long and the male is somewhat shorter at 5-9 mm. *Ancylostoma duodenale* is bigger with an adult female length of 10-13 mm. Both species have a subglobular buccal capsule which bear conical teeth in the case of *Ancyclostoma duodenale* and a pair of cutting plates in the case of *N. americanus*. The worms have a complete digestive system which lies in a fluid filled cavity and is enclosed in a muscular body wall covered by a tough cuticle. The posterior end of the female ends in a conical tip while that of the male ends in a copulatory bursa. The anterior end tapers to a dorsally bent hook which terminates at the mouth. In the human host, the adult worms are found mainly in the duodenum and jejunum but the distribution may vary in heavier infections. The large subglobular capsule and muscular pharynx enables the worm to suck in a plug of mucosa into the mouth and the well developed teeth or cutting plates facilitate anchorage to the mucosa. This results in rupture of the capillaries of the lamina propria and the worm is thought to feed on the extravasated blood, a process which is facilitated by the elaboration of anticoagulant substances by the worm. The worms eventually detach and move to another adjacent site. The female *N. americanus* adult produces between 5000 and 10,000 eggs per day while the *A. duodenale* produces twice that number. The life spans of *N. americanus* and *A. duodenale* in humans have been estimated to be in the order of 4 years and 1 year respectively.

The eggs are ovoid with thin colourless shells. The eggs of *N. americanus* measure 70µm by 40µm but the eggs of *A. duodenale* are a little smaller. They are in practice difficult to distinguish on microscopy. Once the eggs are passed in the faeces, embryonation and subsequent progress through three larval stages occurs outside the host. The first two stages consist of feeding rhabtidiform larvae and the third is the infective ensheathed filariform larva which under suitable conditions, migrates to the surface of the soil. Infection of the host is usually percutaneous. Another moult occurs during skin penetration and is followed by a visceral migratory phase which includes passage in the venous circulation to the lungs, migration up the bronchial tree and eventual reentry into the gastrointestinal tract.

Fig 1.13 Diagrammatic summary of life history of hookworm.



Infection may also occur via the oral route and this has been demonstrated in the case of *A. duodenale.* It has been suggested that the oral route may be an underestimated mode of transmission. It is recognised that development may become arrested during the tissue migratory phase followed by subsequent recovery and progression to complete maturation. This has been suggested as the basis for the possible transmission of *A. duodenale* in breast milk; the larvae accumulating in the mammary tissue due to arrested development. Figure 1.13 summarises the life history of hookworm diagrammatically.

1.5 Morbidity due to intestinal geohelminthiasis.

In any discussion of the morbidity of intestinal helminthisasis it is customary and indeed useful to distinguish between the acute potentially life threatening complications caused by the infection and the more insidious effects that infection may have on such parameters as nutritional state, growth, fitness and cognitive development.[2] The latter effects, while less dramatic, potentially affect millions of infected individuals and may exact a much higher cost on affected communities. Knowledge of the extent of morbidity in endemic areas is critical in developing health planning strategies. While the long term solution to the problem involves socioeconomic development which encompasses the provision of clean water supply, adequate sanitary facilities and better educational opportunities, it seems unlikely that this objective will be realised in the forseeable future in large parts of the third world. The availability of effective antihelminth drugs makes periodic mass chemotherapy and targetted health education programmes a realistic short term option to reduce the worm burden in endemic communities.[2,38] Detailed knowledge of the morbidity due to intestinal helminthiasis is therefore critical in calculating the cost-effectiveness of mass chemotherapy programmes. There have been a number of laudable efforts to quantify the morbidity in endemic areas based on knowledge of demographic data, worm prevalence rates and the the morbid effects of worm infection.[3,39,41] The consistently weak link in these models is the lack of precise empirical data on the effect of infection on parameters such as growth, nutritional state, fitness and cognitive development. These parameters are influenced by a myriad of factors both genetic and environmental, and there is therefore likely to be marked geographical variation in the magnitude of the independent effect of helminthiasis. This underscores the urgent need for careful studies to be undertaken under differing conditions in different localities before generalisations can be made on the burden of ill health attributable to intestinal helminthiasis.

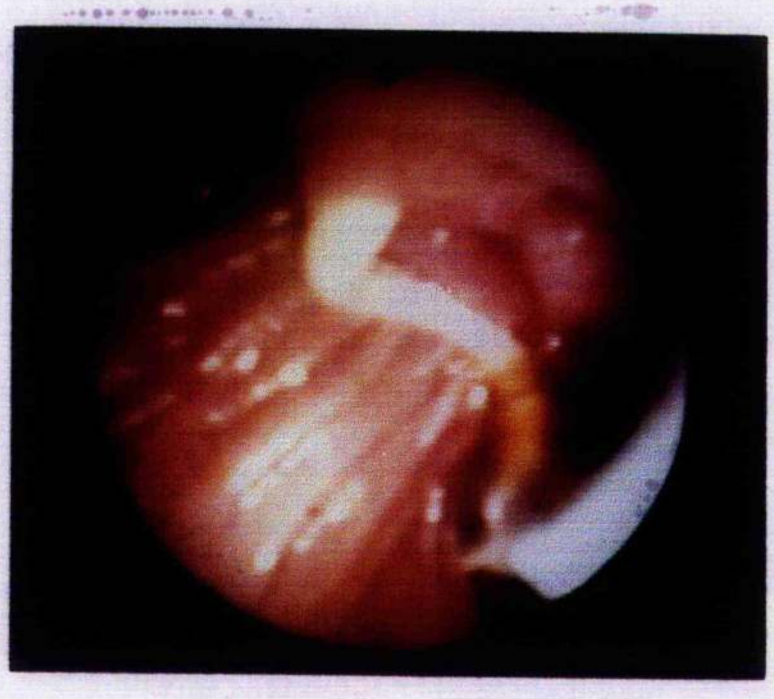
1.5.1 Acute severe complications of intestinal *Ascariasis*

Intestinal obstruction,[42-49] hepatobiliary ascariasis,[42,44-56] acute pancreatitis,[42,52-54,57-60] and acute appendicitis[42,45,48,49] are among the well described complications of intestinal *Ascaris* infection. While these are rare complications in industrialised countries observed occasionally among immigrants,[55,56] they are reportedly relatively common causes of acute gastroenterological emergencies in areas with a high prevalence of infection. Intestinal obstruction is in most reported series the commonest acute complication among children.[42,43-49] Obstruction of the small intestine which may be acute or subacute occurs in patients with large numbers of luminal worms which become entangled into an obstructing mass.[42,47,48] The sheer size of the worm bolus and the matting of adjacent loops of bowel due to surrounding inflammation are thought to precipitate obstruction.[42] Intussusception and volvulus of small bowel loops are often found at laparotomy and could either be a direct result of the worm bolus or be due to associated abnormalities which predispose to *Ascaris* induced intestinal obstruction.[42,46-48] Despite the fact that *Ascaris* induced intestinal obstruction has long been recognised, there is uncertainty on the number of luminal worms required to precipitate obstruction. de Silva *et. al.* after an exhaustive literature review gleaned 24 case reports in which the number of worms recovered at surgery, autopsy or after antihelminthics was documented.[61] There was a very wide range of worm burdens associated with obstruction; the complication reportedly occuring with as few as 4 worms or as many as 1978 worms; the median being 115 worms.[61] Authors in endemic areas with substantial clinical experience in dealing with this condition have chronicled distinctive clinical features which apparently aid in the diagnosis of *Ascaris* induced intestinal obstruction[42,47,48] Most cases respond to conservative measures while surgical intervention is required in between 7 and 35%.[42-46,49] Intestinal obstruction is predominantly a complication of pre-school children but older children may be affected.[42-49] The smaller size of the intestinal lumen in pre-school children may be the critical factor which places them at risk of this complication. Cases of peritonitis and peritoneal invasion by the worms have also been reported.[42,44-49] It is reasonably clear that obstruction, especially if complicated by volvulus or intussusception, may compromise the viability of the intestinal wall causing gangrenous bowel, perforation and severe peritonitis.[42,47,48] Perforation, peritoneal invasion and peritonitis have however also been reported in the absence of obstuction. Peritoneal granulomas have been observed, histological examination of which reveals granulation tissue containing ova and debris of dead worms.[47] This has resulted in a debate as to whether the worm causes the perforation or whether migration of the worms into the peritoneal cavity occurs in the aftermath of a perforation due to other causes, such as typhoid ulcers.[62-64] Effem[64] has made an eloquent argument that the latter is a more plausible explanation given that typhoid and non-specific ulcers are in themselves common entities in the tropics.[65] A number of series report acute appendicitis as a complication of *Ascaris* infection although the definition of this entity is rather unclear.[42,45,47-49] Appendicular ascariasis tends to be described in two contexts. The first is histologically demonstrable appendicitis in association with obstruction of the appendicular lumen by the worm; the relationship between worm migration into the appendicular orifice and appendicitis not unreasonably being presumed to be cause and effect.[42] The second is in the context of appendicular perforation and peritoneal *Ascaris* invasion[42,47] There is very little histological data on whether perforation was the result of appendicitis. Luow[42] described 2 cases of appendicular perforation and peritoneal ascariasis in the absence of histologically visible appendicular inflammation. This begs the question as to whether the appendicular perforation was coincidental and the worms merely escaped into the peritoneal cavity through a pre-existing hole. The other major group of *Ascaris* induced abdominal complications are due to migration of a single or multiple worms through the papillary orifice of the ampulla of Vater into the biliary or pancreatic ducts, the consequences of which include acute biliary colic, cholangitis, pancreatitis and hepatic abscesses.[42,44-60] The pathogenesis of these conditions is related to obstruction of the bile or pancreatic duct outflow and bacterial contamination of bile (fig1.14a and b). Until recently the literature on hepatobiliary ascariasis consisted of isolated case reports or small series. Taking advantage of more recently available imaging techniques such as ultrasonography and endoscopic retrograde cholangiopancreatography (ERCP), workers fron the Sher-i-Kashmir Institute of Medical Sciences in Srinigar-Kashmir, India, have undertaken systematic studies which have put into perspective the importance of *Ascaris* infection as a cause of hepatobiliary disease in an endemic area.[52-54,59,66]

Figure 1.14 Biliary ascariasis (a) An endoscopic cholangiogram showing linear filling defects in the bile duct and (b) a worm being removed endoscopically by the author using a basket.

(a)

(b)



About a quarter of cases of acute pancreatitis presenting to the institution in Kashmir was directly related to ascariasis[59] and up to 37% of all biliary and pancreatic diseases could be aetiologically linked to ascariasis.[52] Hepatobiliary ascariasis tends to affect adults more often than children.[52,53,59,60] Young adult females seem to be particularly at risk.[52,53,59,60] In North-eastern peninsular Malaysia there is strong circumstantial evidence that *Ascaris* infection is an important cause of acute pancreatitis.[67]

Ascaris infection has been putatively linked with recurrent pyogenic cholangitis. This is a distinct entity common in Southeast Asia and the Far-east which is characterised by recurrent episodes of cholangitis associated with multiple intrahepatic as well as extrahepatic brown pigment biliary calculi.[68,69] Microscopic examination of bile duct calculi recovered at surgery or necropsy have revealed *Ascaris* worm remnants and ova in the centre of the stones.[70-72] In the largest of these studies, *Ascaris* ova and/or worm debris were identified in 38% (11/40) of stones while *Clonorchis* ova alone were found within 15% (6/40) of the stones.[70] This has led to the theory that the eggs and debris of dead worms which had migrated into the biliary tree serve as the nidus for calculi formation. It has also been suggested that migration of the worm into the bile duct may result in damage to the sphincter of Oddi, the resultant bile stasis predisposing to stone formation.[73]

In a detailed analysis which entailed the integration of published data on *Ascaris* prevalence rates and *Ascaris* induced complications, de Silva *et al.* found the incidence of *Ascaris* induced intestinal obstruction to vary from 0.00 to 0.25 cases per year per 1000 population in endemic communities.[74] This is likely to be an underestimate as a substantial number of less severe cases of transient subacute intestinal obstruction, may not have reached hospital. Furthermore, even severe cases may not have been accounted for in areas remote from hospital facilities. It was noted that a three-fold rise in *Ascaris* prevalence from 30% to 90% was associated with a 15-fold rise in the incidence of intestinal obstruction.[74] The mean case fatality rate was calculated to be 5.7%.[74] The authors cautiously conclude that acute intestinal obstruction is the commonest acute complication of ascariasis while acknowledging that bilary ascariasis may be underestimated due to the limited availability of ultrasonography and ERCP. In fact early and serial imaging of patients presenting with acute biliary colic, cholangitis and acute pancreatitis in Kashmir revealed that the worms often move back out of the ducts into the duodenal lumen within 24-48 hours.[52,53,66] This may well have led to an underestimation of the role of ascariasis in previously reported series in the surgical literature. It is notable that in a random abdominal ultrasonographic survey of 1104 free living urban inhabitants in Kashmir, 5 subjects with biliary ascariasis were detected, all of whom were symptomatic.[75]

1.5.2 *Trichuris* Dysentery Syndrome

The *Trichuris* Dysentery Syndrome is an illness characterised by chronic diarrhoea, anaemia and growth retardation in association with very heavy *Trichuris* infection.[76-89] In contrast to the worms being found only in the caecum and ascending colon in light or moderately intense infections, they are present throughout the length of the colon in heavy infection.[14] When the *Trichuris* Dysentery Syndrome is suspected on clinical grounds, proctoscopy or sigmoidoscopy to directly visualise *Trichuris* worms on the rectal mucosa is considered the investigation of choice to confirm the diagnosis as rectal *Trichuris* worms are a consistent finding in this syndrome.[76-85] Assessment of the intensity of infection by worm expulsion has revealed worm burdens of between 130 and 10,000 per patient.[87] It is generally a disease of 4-7 year olds.[77-89] The duration of diarrhoea may be as short as one month or be as long as seven years.[79-81,83,85,87] Diarrhoea is bloody in most affected children.[77-85] Rectal prolapse is a feature in 30-70% of patients.[77-85] The frequency of associated finger clubbing is variable but has been observed in a high proportion of cases in at least two series.[81,84]

In addition to the presence of worms, sigmoidoscopy often reveals a rectal mucosal surface which bleeds readily and may be ulcerated.[77,79,83] It would seem likely that colonic mucosal bleeding contributes significantly to the chronic anaemia. Concurrent infection with other helminths, protozoa and pathogenic bacteria is common.[76,79-83,85] Gilman *et al.* reported that 70% of patients were coinfected with enteropathogenic bacteria or protozoa the commonest of which was *Entamoeba histolytica*.[85] This does raise the question as to whether the *Trichuris* worms cause the syndrome or are just innocent bystanders. The strongest evidence that they are causative is that successful elimination with antihelminth treatment consistently results in a clinical response.[79-83,85] This was particularly well demonstrated in the study by Kamath *et al.*[79] who treated patients with antihelminthics and the appropriate antimicrobials for coexisting amoebiasis and bacterial infection. Follow-up sigmoidoscopy revealed worms in all patients who had persistent dysentery but none in those who had responded clinically. Non-responders had been in most instances cleared of ameoba and pathogenic bacteria. Repeat antihelminthic treatment resulted in clinical cure in the non-responders. That the illness is chronic and due to trichuriasis is further evidenced by the presence of growth stunting which is reversed by antihelminthic treatment.[87-89] An acute dysenteric illness in comparison, may well cause wasting but is unlikely to cause stunting. Follow-up studies undertaken on children with the *Trichuris* Dysentery syndrome in Jamaica have shown dramatic increases in growth velocity in the months following treatment.[87-89] The height velocity post treatment was often more than two standard deviations above the mean gain for British children of similar age.[89] This clearly represented catch-up growth which could reasonably be attributed to antihelminthic treatment alone as the children were returned to their homes in the community without the institution of any other nutritional or environmental intervention.

Judging by the cumulative number of cases reported in clinical series, it would appear that the full blown *Trichuris* Dysentery Syndrome is a relatively uncommon event. Accurate statistics on prevalence and incidence rates in endemic areas are however elusive. In the only study of its kind, Cooper and Bundy measured disease prevalence directly in a Jamaican village and found that 3.5% of children between the ages of 6 months and 6 years had rectal prolapse in association with intense trichiuriasis.[86] Whether this is the case in other areas with comparable *Trichuris* prevalence rates is unknown. It is certainly curious that much of the available data on the syndrome is derived from Jamaica,[87-89] Malaysia[77-82,85] and South Africa.[83,84] Whether this reflects a heightened awareness and recognition of the condition in these areas or whether it reflects a true predisposition of the populations in these countries to the condition remains a matter for speculation. The Malaysian series are reported mainly from the Federal Capital, Kuala Lumpur and it is notable that children of Indian ethnic origin were overrepresented in the reported series.[77-82] This may simply be a function of the higher prevalence rates of trichuriasis among Indians in the area as previously discussed. Cooper and Bundy have suggested that the full blown syndrome may represent the tip of the iceberg and that up to 10% of infected children may have a milder form of colitis which results in underdetected growth impairment.[90]

The chronicity of the condition, the frequent occurence of finger clubbing and the associated growth retardation are reminiscent of ulcerative colitis and Crohn's disease.[91] Unlike inflammatory bowel disease however there is a paucity of histological changes in colonic biopsy specimens of patients with the *Trichuris* Dysentery Syndrome. The inflammatory infiltrate in affected children is mild and no more than that observed in uninfected children living in the tropics.[92] However, at least one immunohistochemical study of colonic mucosal specimens revealed increased numbers of tissue infiltrating monocytes and increased local production of tumour necrosis factor α ($TNF\alpha$).[93] The number of mucosal mast cells and cells with surface IgE were also found to be increased. The latter is associated with high levels of histamine release and suggests that infection causes an IgE mediated immediate hypersensitivity type mucosal response.[94] The significance of these changes in the pathogenesis of the condition remain unclear. That infection causes not only a local mucosal response but also a systemic response was illustrated by a study in which elevated serum levels of acute phase proteins were detected in patients with the syndrome.[95] A measure of success in the understanding of the pathogenesis of the disease has been achieved from studying infections of *Trichuris suis* and *Trichuris muris* in animal models. There is evidence from animal studies that heavy trichiuriasis predisposes to mucosal damage by other pathogens such as bacteria and protozoa.[96,97]

1.5.3 Intestinal helminthiasis in relation to growth and nutritional status of children.

The observation that malnutrition is highly prevalent in areas endemic for intestinal helminth infection is in itself not surprising. Malnutrition and helminthiasis are both associated with poverty, lack of clean water, poor sanitation and relative inaccessibility to nutritious food.[2] The key questions are whether the infection *per se* exerts an independent detrimental effect on the nutritional status of children and, perhaps more importantly, what the magnitude of that effect is. These questions are particularly pertinent with the availability of effective antihelminthic drugs which can be used in mass chemotherapy programmes.[2]

Human studies which have explored the relationship between helminthiasis and nutritional status have generally been of two types. The first type consists of observational studies either cross-sectional[98-106,86] or longitudinal[107-109] undertaken in endemic communities which have sought to identify the relationship between infection and a variety of anthropometric measurements such as weight, height and skinfold thickness. The second and more definitive type of study is the interventional study which documents changes in these anthropometric parameters after antihelminthic treatment.[110-128] Studies have in the main focussed on either pre-school [98,100,106,110-115,117,118] or primary school children,[99,101-103,105,107-109,119-128] although some studies have sampled a wider population and included adolescents.[104,116] While a number of studies support the hypothesis that helminthiasis is causally related to impaired nutrition and growth, this has by no means been a universal finding. In addition to obvious differences in study design, the often contradictory findings may reflect geographical differences in the pattern and intensity profile of worm infection, incidence of diarrhoeal diseases, underlying socioeconomic conditions and perhaps even genetic factors. In studying malnutrition it is important to distinguish between stunting which is reflective of chronic malnutrition and wasting which may occur when malnutrition is acute.[129] As polyparasitism is common, it is often dificult to determine the extent to which a particular type of worm infection contributes to malnutrition in a given community.

Cross-sectional studies in endemic areas have been more consistent in demonstrating an association between growth impairment and trichuriasis than with ascariasis. Trichuriasis has been associated with stunting and wasting in large samples of schoolchildren in Panama[101] and Indonesia.[103] Trichuriasis and stunting have also been positively associated among schoolchildren[102] and pre-school children[86] in the West Indies. On the other hand, among Zairian children aged 4 months to 10 years, ascariasis but not trichuriasis was associated with stunting while both infections carried a higher risk of wasting.[104] In a case-control study in India, ascariasis was associated with stunting but the study did not report the prevalence of other concomitant helminth infections.[106] In a Brazilian study of schoolchildren, cross-sectional data suggested that *Schistosoma mansoni* and *Trichuris* infections may act synergistically in causing malnutrition.[105] Some cross-sectional studies however have not detected an association between intestinal helminthiasis and malnutrition, among which were studies in Mexico and Louisiana.[98,130] In a comprehensive cross-sectional nutritional study, Blumenthal and Schultz undertook anthropometric measurements, examined for physical signs of vitamin deficiency and measured serum albumin, haemoglobin, serum transferrin saturation, serum levels of a host of vitamins and urinary riboflavin in children of seasonal farm workers in Louisiana.[130] They found no statistically significant differences in anthropometric parameters between *Ascaris* infected and matched uninfected children. Indeed, infected children tended to be somewhat taller but more wasted than controls, although the differences did not reach statistical significance. Nasolabial seborrhoea was however encountered more frequently in infected children raising the possibility of riboflavin deficiency. This was associated with a statistically non-significant reduction in urinary riboflavin levels. Considering that the numbers involved were only in the order of 30 pairs, this may represent a type two error. Ascariasis was however associated with lower serum albumin levels. Children with moderate to heavy worm burdens were found to have lower plasma vitamin C levels. It should be noted that the prevalence of trichiuriasis in the Louisiana population was also high. In matching *Ascaris* infected children the authors gave no indication that they had controlled for *Trichuris* infection. A more recent Mexican study of pre-school children in collective farms detected no association between either ascariasis or trichiuriasis and impaired nutritional status in a population with a high prevalence of malnutrition.[98]

Longitudinal observational studies investigating the change in anthropometric parameters associated with helminthiasis have been reported less frequently.[107-109] In a frequently cited paper, Cerf *et al.*[107] followed up children drawn from two different sub-populations in a Balinese village and found a significant negative correlation between nutritional status and *Ascaris* worm burden only in a sub-population characterised by lower nutritional intake and lower utilisation of healthcare services.

In a 12 month follow-up of schoolchildren in Zanzibar, ascariasis which coexisted with schistosomiasis was found by multivariate analysis to account for only a small part of the variability of weight gain.[109] In Madagascar, 4 to 10 year old children were treated and then followed up for a year.[108] Growth status and velocity were found not to be correlated with *Ascaris* worm burden as measured by the worm expulsion method. Observational studies suffer from the obvious limitation that an association between infection and malnutrition does not equate to a cause and effect relationship. Cross-sectional studies have an added disadvantage of potentially producing a false negative effect. As highlighted by Stephenson,[131] helminth infection in a community is a dynamic process; children becoming infected at different times during a given period and being rendered uninfected by *ad hoc* treatment or spontaneous expulsion. Growth impairment on the other hand is likely to be a chronic, insidious process. At any given point in time therefore, there may be no detectable difference in anthropometric parameters between infected and uninfected children.

A number of human interventional studies have been undertaken since the late seventies. The earlier studies spanning the decade from the late seventies to the late eighties, were almost exclusively conducted on pre-school children and tended to focus on the effect of *Ascaris* infection[110-115,117] Indeed data on the prevalence of the other common helminths in the studies reported during that period was scarce. In the nineties the focus seemed to have shifted to children of primary school going age, perhaps due to the realisation that as a group they bear the highest worm burdens and also constitute a group at whom healthcare packages can be easily targetted.[119-128] Furthermore schoolchildren are relatively more accessible to investigators through the school set-up and therefore easier to study; a factor which may partly explain the shift of interest to this group. The results of these studies have to be interpreted in the light of differences not only of the geographical location, demographic characteristics of the study population and the profile of the different worm infections but also variations in the drug regime employed and the period between treatment and outcome measurement. Randomised, double blind, placebo controlled drug treatment designs are ideal from a scientific point of view. In reality however, ethical considerations relating to withholding treatment particularly in heavily infected children and the acceptability to members of the local community of the concept of receiving placebo treatment require some compromise to the study design.[132] Blinding of subjects to the treatment is particularly difficult as a visible result of treatment is worm expulsion. Randomising different villages to treatment or non-treatment and using uninfected children within the same community as controls have been among the strategies adopted to overcome the ethical and logistic problems involved.

A review of the interventional studies undertaken in samples consisting exclusively or predominantly of pre-school children reveals some trends.[110-117] Two studies from Bangladesh[114] and Brazil[115] respectively which employed single doses of antihelminths and measured growth parameters at 10 months and a year post-treatment respectively, have not shown any beneficial effect. This may be due to high reinfection rates as the prevalence of *Ascaris* infections at the end of the study period in the treated groups remained high. Another study from Ethiopia also yielded a negative result.[112] In that study, repeat anthropometric measurements were undertaken after just one month, a period which may well have been too short to detect any growth benefits.[112] On the other hand two studies from India[110] and Tanzania[111] on pre-school children using tetramisole and levamisole respectively at 3-4 monthly intervals revealed treatment benefits in terms of gain in weight for age. In both these studies the *Ascaris* prevalence rates in the intervention groups were substantially lower than in the control groups at the end of the follow-up period. No information was given however on the prevalence and intensities of other parasites. In a short term study over 14 weeks, *Ascaris* infected Kenyan pre-school children treated with levamisole gained more weight than uninfected controls.[113] In a large study done in Myanmar (Burma), involving 2-12 year olds, the subset of *Ascaris* infected children aged 2-5 years grew 0.6 cm more than the control group at the end of one year; a difference which was statistically significant.[116] It was the first and perhaps only study which demonstrated that antihelminthic treatment produced a height gain in children of pre-school age. In this Myanmarese study however, evidence of significant weight gain was only detected after two years.[116] It should be noted that nealy all children in the Myanmarese study were infected with *Ascaris* whereas the *Trichuris* and hookworm prevalence rates were less than 1%. The notion that success or failure of mass chemotherapy in pre-school chhildren is simply a function of the frequency of treatment and effectiveness of reducing the *Ascaris* worm burden in the community is however refuted by the results of two studies from Guatemala[117] and Bangladesh[118] which used two-monthly regimes. In the Guatemalan study which was conducted on a population with a relatively high prevalence of giardiasis, the investigators designed the study to distinguish the relative contributions of piperazine and metronidazole in effecting improvements of nutritional status.[117] Children who received metronidazole benefitted both in terms of linear growth and weight gain but piperazine, which is an antihelminth, had no effect on growth despite reducing the aggregate *Ascaris* prevalence from about 60% to 34%. In the large Bangladeshi study involving 1402 children, despite dramatic reductions in the prevalence of both *Ascaris* and *Trichuris* prevalence rates at the end of the 18 month follow-up, no benefit on growth parameters in the treated group was detected.[118] Several reasons have been proposed to explain the dichotomy in the findings. The possibility of inadequate parasite clearance has already been alluded to but this is unlikely to be the case in the Guatemalan[117] and more recent Bangladeshi[118] studies. Another suggestion is that morbidity is a function of worm load and the mean intensity of infection may not have been high enough in some areas for a benefit to be seen. However the prevalence rates of *Ascaris* in the latter two studies were no lower than in the earlier studies which yielded positive results; and a reasonable correlation has been shown to exist between prevalence and mean intensity of infection in a community.[40] Another putative explanation is that the effect of worms on nutrition becomes critical only where food availability is already low to begin with. As most studies are undertaken in areas of poverty, evidence for this assumption is rather slim. Indeed it is possible that reversibility of growth deficit is maximum when food availability is sufficient.

The same considerations in the interpretation of interventional studies on pre-school children also apply to studies undertaken on primary school children. However the studies on the latter are somewhat more recent and generally provide more comprehensive information on the prevalence rates and intensities not only of *Ascaris lumbricoides* but also of other common worm infections.[119-128] At the time of writing, a Medline search of the literature published in peer reviewed journals revealed only ten interventional studies investigating the effects of antihelminth treatment on growth parameters which focused exclusively on primary school children and were conducted in areas endemic for the geohelminths *Ascaris, Trichuris* and hookworm but not schistosomiasis.[119-128] In addition to these was the study from Myanmar (Burma) already referred to which included children of primary school going age.[116] Despite the global ubiquity of intestinal geohelminthiasis, the sum total of the published data is accrued from only 6 different locations in the world, namely the Rangoon Division in Myanmar,[116] the coast province of Kenya,[119-122] Freetown in Sierra Leone,[125] Ujung Pandang in Indonesia,[127,128] the area in and around Kuala Lumpur in Malaysia,[124] Santa Maria de Jesus in Guatemala,[126] and two areas in Jamaica.[123]

The strongest positive data has emanated from a series of four randomised double blind placebo controlled studies from the coast province of Kenya which is characterised by *Trichuris* and hookworm prevalence rates of almost 100% and an *Ascaris* rate of about 50%.[119-122] The first of these showed marked improvements in linear growth, weight gain and increase in skin fold thickness six months after a single dose of albendazole.[119] Similar anthropometric improvements were reproduced in a four month post-treatment follow-up study in which quantifiable improvements in physical fitness and appetite were also recorded.[120] The same group in another study comparing the effects of a single dose of albendazole against two doses delivered 6 months apart, showed that both types of dosing resulted in gains in weight for age and weight for height but not in linear growth.[121] The shortest post-treatment follow-up period in the series of Kenyan studies was nine weeks at the end of which improvements in weight gain and skin fold thickness but not linear growth were detected,[122] accompanied by a concomitant increase in quantifiable physical activity. Multiple regression analysis showed that hookworm intensity was consistently the strongest predictor of gains in growth parameters.[119-122]

Outside of Kenya, another African study conducted in Sierra Leone showed detectable gains of statural and ponderal growth in urban and rural primary school children six months after albendazole treatment.[125] The study included placebo control groups but suffered from a rather high dropout rate of more than 20% in the treatment groups. The urban group had moderately high prevalence rates of *Ascaris* and *Trichuris* but lower hookworm rates. Conversely the rural group had relatively high rates of *Ascaris* and hookworm but a low *Trichuris* rate. Analysis of the 6-10 year old subset in the large treatment study in Myanmar which employed three monthly levamisole showed that a significant gain in height was detected only after 18 months and weight gain only after 24 months.[116] The Indonesian studies both of which were randomised double blind and placebo controlled were undertaken on samples of urban slum children with high *Ascaris* and *Trichuris* infection rates but revealed somewhat divergent main outcomes.[127-128] In the earlier of the two studies, significant weight gain accompanied by improved appetite was detected as early as 3 weeks and seven weeks after treatment with pyrantel pamoate.[127] The beneficial effects were ascribed by the authors to the reduction in the *Ascaris* worm load as pyrantel is effective against *Ascaris* but not *Trichuris*. In a study published the following year, the same group compared the effects of pyrantel, albendazole and placebo administered at baseline and six months in the same population of children. Rather disappointingly, measurements at the end of one year did not reveal any treatment benefit on growth parameters for either drug.[128] The investigators managed to detect a significant association between ascariasis and height gain as well as between trichuriasis and increase in mid-arm circumference but only after post-hoc adjustments for confounding factors.

The Jamaican study sought specifically to examine the effects of treating *Trichuris* infected children. An increase in body mass index was detected only in children with light *Trichuris* infection.[123] The studies on Mayan children in Guatemala[126] and on ethnic Indian children in Malaysia[124] were also disappointing in their outcome. The Malaysian investigators in a randomised double blind placebo controlled study with a two year follow-up period employed three monthly regimes of mebendazole and pyrantel, and could find no evidence that treatment improved growth.[124] A problem with that study was that at the end of the follow-up period, less than half the children submitted stool samples for evaluation of worm eradication rates. Consistent with previous survey data from the area, the prevalence rates of *Trichuris* and *Ascaris* infections were high but hookworm prevalence was low.[124] In the Guatemalan study albendazole was administered at baseline and at 12 weeks while final anthropometric evaluation was undertaken at 6 months[126] Treatment resulted in marginal weight gain in a population with very high *Ascaris* and *Trichuris* infection rates but no detectable hookworm infection. The drugs used were very effective against *Ascaris* and hookworm infections but the effect on *Trichuris* was modest. Indeed, in the Guatemalan study, there was little difference in *Trichuris* worm burdens between treatment and placebo groups at the end of the follow-up period.[126] In contrast, *Trichuris* clearance rates appeared to be have been good in the Malaysian study alluded to earlier,[124] despite which the results were negative. However, the reported effectiveness of *Trichuris* clearance in the Malaysian study has to be interpreted with caution in the light of the poor compliance in submission of follow-up stool samples. It has been demonstrated that infected children are less likely to comply with requests for submission of repeated stool samples, hence resulting in an underestimation of infection rates.[133]

It is quite striking that the most impressive results were from Kenya where the hookworm prevalence rates were much higher than in the other areas studied. It is certainly tempting to postulate that hookworm may have a greater effect on growth than the the other worm infections. Variations in the prevalence of baseline malnutrition is unlikely to explain the divergent findings as all the studies on primary school children were done in areas where malnutrition was clearly prevalent. There may be other as yet unrecognised factors which determine the magnitude of the growth response and are of variable influence from one place to another. This merely serves to underscore the danger of extrapolating the results obtained in one area to others. More data is clearly needed from many different endemic areas before a clearer picture emerges of the undoubtedly complex interaction between helminthiasis and other factors in influencing growth and before the effects of treatment on growth can be predicted with any consistency.

While the discussion so far has focused on growth and overall nutritional status, there is concern that helminthiasis may also cause disease states resulting from deficiency of specific nutrients. The data on whether ascariasis impairs vitamin A absorption is contentious and will be discussed in the next section. Data on the link between ascariasis and xerophthalmia, the specific disease state due to vitamin A deficiency is however scarce. In a case-control study in Nepal, clinical cases of xeropthalmia were more likely to be infected with *Ascaris* than matched controls.[134] This does not of course prove a cause and effect relationship. No discussion of nutritional status is complete without touching on anaemia. It has been established beyond doubt that hookworm infection causes a chronic iron deficiency aneaemia.[2] In a recent cross-sectional study in Zanzibar involving a sample of almost 4000 schoolchildren in a population with a high prevalence of hookworm, *Trichuris*, *Ascaris*, and *Schistosoma haemotobium*, measurements were made of haemoglobin, serum ferritin and erythrocyte protoporphyrin.[135] Using multivariate analysis it was estimated that 25% of all anaemias, 35% of iron deficiency anaemias and 73% of severe anaemias could be attributed to hookworm infection.[135] Anaemia is also a feature of patients with the overt *Trichuris* Dysentery Syndrome. The question which requires clarification is the extent to which ascariasis and trichuriasis contribute to anaemia in the vast majority of infected populations. Cross-sectional studies have shown an association between heavy trichuriasis and lower haemoglobin levels although the clinical significance of this may not be critical.[136-138] In the large study in Zanzibar, ascariasis was associated with 10% of the attributable risk of anaemia.[135]

1.5.4 Intestinal helminthiasis, school attendance rates, academic performance and cognitive function.

There has been a recent rejuvenation of interest in the extent to which chronic intestinal helminthiasis adversely affects learning ability and academic achievement.[139] It has been suggested that helminthiasis may mediate this effect either indirectly by inducing malnutrition or by some direct effect on cognitive function. Malnutrition in itself could contribute to poor school performance by predisposing to ill health and school absenteeism, by impairing cognitive function, or by inducing inattentiveness and lassitude. There is reasonable evidence both from observational and interventional data that iron deficiency causes impairment of cognitive function which is at least partially reversed by iron supplementation.[140-142] Hookworm infection which is a major cause of chronic iron deficiency[2] is by implication probably an important cause of impaired cognition. The data linking ascariasis and trichuriasis with underachievement at school is however still somewhat sparse. Studies addressing this issue have broadly measured either school attendance rates,[102,123,143] functional school performance as assessed by indicators such as reading, vocabulary, spelling and arithmetic skills[102,123,143,144] or more specific aspects of cognitive functioning using a battery of neuropsychological tests.[145,146-151]

Observational data on Jamaican schoolchildren have revealed an association between moderate to heavy trichuriasis and reduced school attendance rates[133] as well as reduced academic achievements[102,144] In one of these studies, which was primarily designed to examine the effects of *Trichuris* infection, significant associations were observed between ascariasis, lower school achievement, and absenteeism even after controlling for socioeconomic status.[102] Another such study from South Africa which was conducted on a sample with a relatively high prevalence of schistosomiasis, trichiuriasis and ascariasis suggested that parasitic infections act in synergy with undernutrition to impair cognitive function.[145] Although testing was repeated in that particular study after antihelminthic treatment, the effect of worm clearance was deemed insufficient by the authors to enable meaningful inferences.[145]

In an Equadorian study of primary schoolchildren which employed not only neuropsychological tests but electroencephalography (EEG), multivariate analysis revealed a consistent and strong association between malnutrition and impaired test scores. Parasite infection with protozoa and helminths was found to have a significant main effect on only one of the tests.[146] An interaction between parasites and level of nutrition was demonstrated for two of the tests. There was however a correlation between *Ascaris* intensity and test scores in two of the tests. Notably a third of the children had an abnormal EEG. When only helminth infected children were analysed, an EEG had a main effect on only one of the test scores. The *Ascaris* intensity in the Ecuadorian sample was low and the *Trichuris* prevalence was only 5.8%, leading the authors to speculate that this might be the reason for the weak association between helminthiasis and impaired cognitive function. The study nonetheless illustrates the strong association between malnutrition and impaired cognitive function.

The first of a series of interventional studies from Jamaica reported a dramatic improvement in psychomotor development over a period of one year among 19 pre-school children treated for the *Trichuris* Dysentery Syndrome which paralleled the equally dramatic improvement in growth.[152] The rate of improvement was compared against a group of matched controls. The same year saw the publication of two papers from Jamaica based on the same data, reporting impressive improvements in auditory short-term memory, and the scanning and retrieval of long term memory in 9-12 year old children 9 weeks after treatment.[147,148] Multiple regression analysis suggested that removal of *Trichuris* was a more important determinant than removal of *Ascaris*. This interesting study employed not only a randomised placebo control arm but also an uninfected control group. Significantly, at the end of the study period, there was no difference between the treated infected group and uninfected controls.

Over the following four years, two more studies examining the effects of light to moderate burdens of *Trichuris* infection on cognitive function found no benefit of treatment.[149,150] Both these studies were very similar in design to the earlier Jamaican study except for differences in the battery of neuropsychological tests used. Indeed in one of the two studies the infected group had comparable baseline scores to the uninfected group.[150] In the other study, infected children tested worse at baseline than uninfected controls and some post-treatment benefit was observed only in the subset with low weight for age.[149] It has been suggested that the discrepancy in the findings of these Jamaican studies may have been due to differences in worm load.[147-150]

In a study from Zaire which was rather difficult to interpret, half the sample were treated with iron supplements and half the children with *Ascaris* or hookworm infection were randomised to antihelminths.[151] There was a suggestion that antihelminthic and iron supplementation may have acted synergistically to raise the haemoglobin concentration with attendant improvements in cognitive scores.

Two recent randomised double blind placebo controlled studies have measured the effect of antihelminthic treatment on the functional outcomes of school attendance and school performance.[123,143] The first of these again from Jamaica[123] measured school performance indicators as well as growth, serum ferritin and haemoglobin concentrations on primary school children. As in the previous Jamaican studies, *Trichuris* infection with a minimum intensity of 1,200 eggs per gram of faeces was a criterion for enrolment into the study. There was no overall difference between placebo and treatment groups six months after treatment in any of the school achievement tests nor in school attendance. However multiple regression analysis did show that subsets of children with heavy trichuriasis or stunting benefitted from treatment. There was a significant treatment by intensity interaction for spelling which was one of three indicators of school achievement. There was also a treatment by stunting interaction with school attendance. The second of these studies[143] was conducted by the same group which reported on the effects of antihelminth treatment on the growth of Mayan schoolchildren in Guatemala.[126] No improvement in school attendance or performance tests was observed in response to treatment.[143] It was noted that mean worm burdens of the placebo group based on sequential stool egg counts undertaken during the follow-up period correlated weakly with some of the performance tests. As mentioned in the previous section, the effect of treatment was a marked reduction in the *Ascaris* burden but only modest reductions in *Trichuris* burdens. While it seems reasonable to test for changes in specific aspects of cognitive function and even school attendance within weeks or months after treatment, it is perhaps questionable if indicators of academic performance such as reading, spelling and vocabulary which are quite complex tasks are responsive to such rapid changes.[139] The elucidation of the relationship between helminthiasis, scholastic underachievement and school absenteeism is far from complete.

1.5.5 Intestinal helminthiasis and intestinal mucosal injury or dysfunction.

The relevance of investigating the effects of intestinal helminthiasis on intestinal mucosa relates to two of its main functions. The first function is nutrient absorption, derangement of which has obvious implications on nutritional status and growth. The second function is the provision of a protective barrier between the body and the lumen of the intestine. Mucosal damage leading to a breach in the integrity of this protective barrier exposes the body to the potentially noxious contents of the intestinal lumen which could result in ill effects.

Human studies investigating the effects of *Ascaris* infection on the small intestine have generally assessed intestinal absorptive function of protein, fat, xylose, lactose, vitamin A and iron. There are fewer reports on the effects of ascariasis on the histology of the intestinal mucosa and on intestinal transit time. *Ascaris suum* infection of pigs has provided a useful animal model particularly for histological study.

In one of the earliest quantitative studies published in 1953, Venkatachalam and Patwardhan[153] measured 24 hour faecal nitrogen in 9 *Ascaris* infected Indian children before and 4 days after antihelminthic treatment. The children were noted by the authors to be physically underdeveloped for their age. There was a 40% fall in mean 24 hour faecal nitrogen after treatment which represented about 6% of the daily nitrogen intake. This led the authors to conclude in their paper that ascariasis causes protein malabsorption. In that study no decrease in faecal nitrogen was observed in two children without *Ascaris* infection who also received vermifuge treatment.

In a study published almost 20 years later, Tripathy and others[154] evaluated nitrogen balance, fat absorption and xylose absorption before and after treatment in 12 *Ascaris* infected Colombian children aged between 5 and 10 years. Most of the children also harboured *Trichuris trichiura* and two had hookworm infection. Faecal nitrogen was measured over a 12 day period before treatment and repeated over a similar period after a 5 day antihelminthic regime. Tripathy *et al.* [154] found a significant improvement in dietary protein absorption of a magnitude similar to the earlier Indian study. In addition they found that the mean faecal fat excretion had decreased by an amount equivalent to 9% of the daily dietary fat intake. There was a trend towards an improvement in xylose absorption.

The same group in another study[155] of five Colombian children aged 3-7 years with heavy *Ascaris* infection found similar improvements of protein and fat absorption after treatment but did not detect any change in xylose absorption. Tripathy *et al.* [155] examined jejunal biopsies and found changes which included broadening and shortening of villi, elongation of crypts and round cell infiltration. These changes were noted to have improved after treatment. The authors did not specify the time lag between treatment and repeat biopsies but the reader is left with the impression that they were undertaken at about the same time as repeat absorption studies.

In a similar study in Bangladesh, Brown *et al.* sudied 13 *Ascaris* infected children aged from 3 to 7 years who were considered free of recent diarrhoea and other symptomatic infections.[156] The authors did not report the comprehensive data regarding concomitant helminth infections but did state that the hookworm intensity in the sample was light and implied that trichiuriasis was also present. Children with asymptomatic enteric infections and iron deficiency were included in the study after treatment of the conditions. The subjects were generally undernourished as judged by height and weight for age. Seven day balance studies were performed before treatment and about a week after treatment. The mean protein absorption improved by an equivalent of 9% of the daily intake in children with heavy *Ascaris* worm loads. Mean fat absorption in the subset improved by 3% of the daily intake. Protein and fat absorption did not however improve in children with light *Ascaris* loads. Post-treatment xylose absorption remained unchanged.

A paper by Bray[157] reported in 1951 is often cited as evidence against *Ascaris* induced protein malabsorption. However close examination of the results of this small study on 8 Gambian children, 4 of whom were infected with *Ascaris*, reveals that treatment of ascariasis was in fact associated with reductions in faecal nitrogen excretion.

It has been suggested that the magnitude of protein malabsorption demonstrated in these studies may be of consequence in children from poor socioeconomic backgrounds whose protein intake is already marginal.[156]

The results of the studies mentioned thus far[153-157] have to be interpreted with caution for a number of reasons. Firstly, the numbers investigated are small; a result perhaps of the logistic difficulties encountered in conducting nutritional balance tests which involve hospitalisation, laborious stool collection and dietary monitoring. Secondly the studies involved admission to metabolic wards away from the natural habitat of the infected children often for weeks at a time. Worm expulsion was therefore not the only intervention undertaken. Correction of malnutrition in itself may reverse malabsorption and histological abnormalities.[158] The lack of controls in most of the studies imposes a major constraint on the conclusions that can be drawn. Venkatchalam and Patwardhan included only two uninfected controls.[153] Brown *et al.* in the Bangladeshi study did at least have as controls children who were lightly infected.[156] However it can only be presumed that the arbitrary cut off between heavy and light infection in that particular study was determined prior to the study rather than after analysis of the data. It is well known that mild to moderate histological abnormalities of the small intestinal mucosa as defined by standards of Western industrialised societies are in fact the norm in the tropical setting; hence the term tropical enteropathy.[159,160] The possibility cannot be dismisssed that transfer to a hospital setting for a period of weeks away from the natural environment of the children investigated may in itself reverse some of the histological changes independent of antihelminthic treatment.

Interestingly, in the only double blind controlled study conducted within the community, treatment of *Ascaris* was found to have no effect on nitrogen, fat and xylose absorption in.[112] In that study by Freij *et al.*, 13 *Ascaris* infected Ethiopian boys aged 1.5 - 4 years were matched into pairs and allocated to treatment or placebo groups. Children found to have any other infection on stool examination were excluded. Faecal nitrogen and fat excretion were measured at baseline and a fortnight after treatment on 3 day stool collections which were handed to trained field nurses. In addition, jejunal biopsies were taken before and after treatment. As expected, most of the biopsies showed mild abnormalities but no improvement occured with treatment. Mucosal disaccharidase levels were assayed in a few of the subjects. The levels were lower than would be expected in European children but treatment did not result in an increase in the levels. It should be noted however that the intensity of

Ascaris infection in the Ethiopian boys was low; a factor which may account for the negative result.

The studies on nitrogen balance and ascariasis have focused predominantly on pre-school children and it cannot be assumed that the same findings apply to children of primary school going age.

Another parameter of small intestinal function which has been assessed in relation to ascariasis is lactose digestion. Two studies on pre-school children, both conducted in Panama, provide strong evidence that ascariasis impairs lactose digestion.[161,162] The first of the studies by Carrera *et al.* involved two experiments which employed different measurement techniques.[161] In the first experiment, the rise in blood glucose was measured after an oral lactose load. In the second experiment serial breath hydrogen levels were measured after the lactose load; a high breath hydrogen level indicating lactose maldigestion. Between the two experiments, 67 infected children and 56 uninfected controls were investigated. *Ascaris* infection was found to be associated with symptoms of milk intolerance and measurable lactose malabsorption. Repeat measurement three weeks after treatment resulted in marked improvement.

In the second study, Taren *et al.* conducted hydrogen breath tests on 23 *Ascaris* infected and 9 uninfected pre-school children using a similar study design and their findings were equally persuasive.[162] There was also a good correlation between intensity of infection and the breath hyrogen index. In addition Taren *et al.* utilised the hydrogen breath test after oral lactulose to assess mouth to caecum transit time in 15 *Ascaris* infected and 26 uninfected children whose mean age was about 6.7 years. Although there was a trend towards shorter transit times in infected subjects the difference did not reach statistical significance. When only infected children were considred there was a negative correlation between intensity and transit time after log transformation of both variables.

A third study from Gabon which employed the breath hydrogen test, arrived at the conclusion that ascariasis had no independent effect on lactose digestion.[163] The study differed from the earlier Panamian studies in two respects. It was essentially a cross-sectional study and comparisons were made between *Ascaris* infected and unparasitised subjects. Furthermore the subjects studied were schoolchildren and young adults as opposed to pre-school children who were the subjects of the Panamian studies.

Vitamin A deficiency is a major problem in the third world.[164] It is therefore not surprising that much effort has been devoted to investigating the effect of ascariasis on vitamin A absorption. Three Indian studies conducted in the seventies are still widely quoted.[165-167] Sivakumar and Reddy administered radiolabelled retinyl acetate orally and subsequently measured radioactivity in 24 hour collections of faeces to estimate the amount of unabsorbed vitamin A.[165] The study was conducted on 6 children with ascariasis and 5 uninfected controls. Mean vitamin A absorption in the uninfected group was almost 100% while infected children only absorbed 80% of the radiolabelled dose. Repeat absorption tests on two of the infected subjects after treatment showed improved absorption. Sivakumar and Reddy also estimated the amount of the radiolabel in homogenates of worms recovered after treatment and could only find trace amounts.[165]

Mahalanabis *et al.* in an Indian study of 28 *Ascaris* infected adults and 12 apparently healthy controls assayed vitamin A levels in serum serially after an oral dose.[166] The peak serum vitamin A concentration was lower in infected adults than controls although there was some overlap. Fourteen infected subjects who were deemed to have abnormally low peak levels underwent repeat absorption studies 48 hours after worm expulsion and showed a consistent and statistically significant increase in peak levels.

In yet another study from India by Mahalanabis *et al.*, vitamin A absorption studies were undertaken on 15 children with ascariasis and 4 uninfected controls.[167] The principles of the measurement of absorption was similar to the previous study. Eight of the *Ascaris* infected children had significant giardiasis diagnosed on jejunal aspirate. Absorption studies were done at baseline and after treatment with a combination of metronidazole and piperazine to cover both giardiasis and ascariasis. Peak serum vitamin A levels at baseline in children with giardiasis were universally low and treatment resulted in marked improvement. Among seven who were infected with *Ascaris* but not with *Giardia,* only three had depressed peak levels before treatment. Although the peak levels in all three of these patients rose after treatment the small numbers involved made any meaningful statistical analysis virtually impossible.

A more recent study by Ahmed *et al.* on Bangladeshi children of primary school going age in contrast to the Indian studies did not yield any evidence that ascariasis causes vitamin A malabsorption.[168] The approach taken in that study was to measure the vitamin A concentration directly in a 48 hour stool collection after a single oral dose of vitamin A administered in the form of retinol. At baseline, 80% of the sample of 24 children had low serum retinol levels as opposed to none of five uninfected children drawn from a higher socio-economic environment. Less than 1% of the ingested dose was recovered in the faeces, leading the investigators to conclude that ascariasis did not result in vitamin A malabsorption. Furthermore, they could not detect retinol in the expelled worms. They did acknowledge that retinol metabolism by luminal bacteria may have resulted in underestimation of the amount recovered in stool. The authors however defended the validity of their methodology by arguing that they had been able to recover more than 50% of the administered dose in cases of cystic fibrosis using the same technique. The intensity of *Ascaris* infection in the study by Ahmed *et al.* was not particularly high and the possibility that heavier infections cause vitamin A malabsorption cannot be discounted. The studies of vitamin A absorption are not only few and far between but have involved small numbers of subjects. The issue of whether ascariasis causes significant vitamin A malabsorption can only be regarded as unresolved.

In the only study which addressed iron absorption in ascariasis, Islek *et al.* conducted iron absorption tests on 26 *Ascaris* infected children and 21 controls.[169] There was no evidence of depressed iron absorption in *Ascaris* infected children. As expected the children with iron deficiency in the control group had the highest absorption.

Small intestinal mucosal damage is but one of the potential mechanisms by which ascariasis may interfere with nutrient absorption. It has been proposed that the adult worms may interfere with luminal digestion of nutrients. Venkatachalam and Patwardhan in their seminal paper suggested that protein malabsorption may be due to substances elaborated by the adult worms which inhibit proteolytic digestive enzymes.[153] They based this on a review of the data available at the time on the antienzyme activity of *Ascaris* extracts. In 1967, Green[170] confirmed the existence of anti-trypsin and anti-chymotrypsin activity in *Ascaris lumbricoides* extracts while others later reported similar findings in extracts of *Ascaris suum*.[171,172] In the study on vitamin A malabsorption by Mahalanabis *et al.*[166] the rapid improvement in vitamin A absorption after treatment makes it unlikely that mucosal damage was the mechanism of malabsorption. Factors elaborated by the *Ascaris* worms could well have a direct effect on vitamin A within the lumen, hence interfering with its absorption. The albeit equivocal findings of Taren *et al.*[162] suggests that reduced intestinal transit time may contribute to nutrient malabsorption by reducing both the time for luminal digestion and contact time with the mucosal surface. The relative contributions and significance of these mechanisms remain speculative. Although the xylose absorption test is a direct measure of mucosal function, test results are affected by a host of other factors such as transit time, renal function, degree of metabolism by the body and completeness of urine collection.[173] Another non-invasive test of mucosal integrity which has been used extensively in recent years is the dual sugar intestinal permeability test. This test circumvents some of the limitations of the xylose absorption test. Before the publication of the results of the work undertaken for this thesis, there was only one paper which studied the effect of helminthiasis on intestinal permeability.[174] Northrop *et al.* used the dual sugar permeability test on 104 malnourished Bangladeshi children aged 2 to 9 years with mixed infections of *Ascaris, Trichuris* and hookworm. Permeability tests were done at baseline and repeated 9-14 days after antihelminthic treatment. There was neither a difference in permeability between *Ascaris* positive and negative groups at baseline nor a change in permeability after treatment. It has been suggested however that repeat permeability testing may have been undertaken too soon after treatment for changes to be detected. The investigators did find that ascariasis was associated with lower plasma albumin concentrations at baseline and noted a significant increase in concentration after treatment; prompting speculation that protein malabsorption may occur by mechanisms other than direct mucosal damage.

Animal studies have provided some insight into the effects of ascariasis on intestinal morphology and function. Stephenson *et al.* [175] and Forsum *et al.*[176] in a series of experiments on young pigs infected with the larvae of *Ascaris suum* found at necropsy that the wet and dry intestinal weights of infected pigs were significantly higher than that of uninfected controls. There was a good correlation between intestinal weight and worm burden.[175] Histology of intestinal sections showed a striking increase in the tunica muscularis layer.[175,176] The muscular hypertrophy seemed to affect the entire length of the small intestine despite the fact that the worms are usually found in the jejunum. Furthermore, Stephenson *et al.* reported that histological examination showed deeper crypts and lower villus height to crypt depth ratios in infected pigs.[175] Forsum *et al.* made no specific comment regarding mucosal changes but did report that lactase levels of the upper and mid small intestine were lower in heavily infected pigs.[176] The investigators made the plausible suggestion that this may be a direct mechanical effect of worms on the mucosal brush border as lactase is the most superficially located of the disaccharidases. Curiously however sucrase levels in the lower small intestine were increased in infected pigs.

Neither Stepheson *et al.*[175] nor Forsum *et al.*[176] found consistent abnormalities in nitrogen absorption in *Ascaris* infected pigs. Forsum *et al.* did however report a degree of fat malabsorption in infected pigs.[176] Martin *et al.* using electron microscopy, observed ultrastructural changes on the mucosal surface of three protein deficient pigs infected with *Ascaris suum* which were not present in an uninfected pig.[177]

It is quite obvious that substantial controversy still surrounds the issue of ascariasis, mucosal damage and malabsorption in children. Age of the host, baseline level of nutrition and intensity of infection are among the variables that may explain some of the contradictory findings. Although the discussion thus far has concentrated on malabsorption it should be borne in mind that decreased food intake per se may be an important factor contributing to helminth induced malnutrition. There is certainly evidence from both human and animal experiments that helminthiasis depresses appetite and food consumption.[120,127,175,176,178]

Trichuris trichiura usually inhabits the colon although in heavy infections the worms have been found to extend proximally into the terminal ileum. There has understandably been little work undertaken on the effect of trichiuriasis on small intestinal integrity. Northrop *et al.* did examine this to some extent in their study on intestinal permeability and found no difference in permeability between groups infected and uninfected with *Trichuris.*[174] As treatment of trichiuriasis was unsuccessful, they were unable to draw any conclusions on the effects of removing trichuriasis on intestinal permeability. Mathan and Baker undertook a study of intestinal function, jejunal histology and rectal histology on three children with the *Trichuris* Dysentery syndrome and found little abnormality except for increased cellular infiltration of the rectal mucosa.[179] The abnormalities of colonic histology in the *Trichuris* Dysentery Syndrome have been discussed in an earlier section. There is virtually no human data investigating the intestinal effects of trichiuriasis in the absence of the overt *Trichuris* Dysentery Syndrome.

Studies investigating the effects of hookworm infection on intestinal absorption have generally been conducted on adults and the results have been contradictory. Some have reported a link between hookworm infection, malabsorption and jejunal mucosal damage[180,181] but others have refuted this.[182-184] The hookworm studies have also suffered from the design limitations of similar studies on ascariasis and malabsorption as outlined earlier.

1.5.6 Helminthiasis and gastrointestinal bleeding.

The link between hookworm anaemia and chronic intestinal bleeding is well established. Direct assessment of bleeding using radiolabelled red cell studies and more recently, faecal measurement of haem and haem derived porphyrin have established that hookworm infection causes chronic blood loss that correlates well with worm intensity.[185-187] Overt rectal bleeding and anaemia are also frequent features of the *Trichuris* Dysentery Syndrome. The issue which requires clarification is whether trichuriasis causes occult bleeding in the absence of the overt *Trichuris* Dysentery Syndrome. It is not implausible that the overt *Trichuris* Dysentery Syndrome represents the tip of the iceberg and less intense infections may cause milder bleeding. Very few attempts have been made to directly estimate intestinal bleeding in trichuriasis. Four radiolabelled red cell studies on *Trichuris* infected children reported in the English literature have yielded conflicting results.[186,188-190] The radiolabelled red cell technique in essence involves tagging of the subject's red cells with

Cr^{51}, reinjection of the tagged blood into the circulation and measurement of radioactivity in a three day faecal collection. One of the studies[188] was conducted by the group which was responsible for elucidation of the mechanism of hookworm anemia. Layrisse *et al.* performed radiolabelled red cell studies on 9 *Trichuris* infected girls aged 3 to 14 years and found a mean daily faecal blood loss of 4.1 ml.[188] Eight of the 9 subjects lost more than 1.5 ml of blood a day in the faeces, the level which was regarded by their laboratory as the upper limit of normal. After treatment, the faecal blood loss in all but one of the subjects fell to below 1ml a day. They reported a good correlation between daily blood loss and the number of worms recovered after treatment. While the calculated blood loss per worm was less than that due to hookworm infection, Layrisse *et al.* nonetheless considered it sufficient to cause an iron deficiency anaemia in heavily infected individuals. Cho in a Korean study[190] also found a strong correlation between *Trichuris* intensity and faecal blood loss although the magnitude of loss was less than that reported in the Venezuelan study of Layrisse *et al.*

On the other hand, Lotero *et al.* employing both a radiolabelled red cell technique and a chemical method of detecting faecal haemoglobin found no evidence that trichiuriasis causes occult gastrintestinal bleeding in a study of 6 children aged 3.5-10 years.[189] Blood loss in all six children was less than 1 ml a day. There is an important difference between the studies which merits consideration. In the study by Layrisse *et al.*, 6 of the 9 subjects were anaemic and 6 had diarrhoea.[188] The investigators did not specify the criteria of selection but may well have included children with the *Trichuris* Dysentery Syndrome. Lotero *et al.* in comparison, specifically excluded children with dysentery or chronic diarrhoea.[189]

Mahmoud performed similar studies on a group which included 13 patients with trichuriasis and found daily blood losses of 0.06 ml to 1.35 ml which were within the normal range of the laboratory.[186] Mahmoud did not state the ages of the subjects studied. Lotero *et al.*[189] in their paper cited a study in the Spanish literature which apparently also arrived at the same negative conclusion as them. The radiolabelled red cell studies all suffered from the limitations of small numbers and the absence of controls.

In a more recent cross-sectional study of 203 Zanzibari schoolchildren, Stoltzfus *et al.* used a commercially available test kit which measures intact and degraded haem in faeces.[187] The test is a highly sensitive measure of occult faecal blood. There was an association between trichuriasis and faecal haemoglobin which disappeared after adjusting for hookworm intensity. The authors however conceded that the high prevalence of hookworm in the population may have obscured a *Trichuris* effect on intestinal bleeding.

Using ^{59}Fe labelled erythrocytes, Beer *et al.* demonstrated a good correlation between worm burden and intestinal blood loss in pigs infected experimentally with *Trichuris suis.*[191] At necropsy, red blood cells which had presumably leaked from damaged mucosal capillaries were found in the large intestinal lumen. More work is clearly required to clarify the role of *Trichuris* infection in occult gastrointestinal bleeding.

Chapter 2. Geography of the study area

Geography of the study areas

2.1 Climate and geophysical characteristics.

The subjects of the studies reported in this thesis consisted of children drawn from two primary schools located in and around the town of Kota Bharu which is the administrative capital of the state of Kelantan in North-eastern peninsular Malaysia (fig. 2.1). Kota Bharu town is 102°17' east of Greenwich and 6° 10' north of the equator.[192] The town is within the greater district of Kota Bharu (fig 2.1).

The state of Kelantan covers an area of 14,922 square km[193] and sustains a population of approximately 1.4 million.[194] It shares a border with Thailand to the north and has an eastern coastline facing the South China Sea.[192] From a geographical point of view, the state of Kelantan is conveniently divided into two areas. An inland mountainous area to the south which occupies 75% of the land area of the state is covered by tropical rain forests and is relatively sparsely populated except in certain pockets.[194] The northern coastal area consists of flat land which has been developed, is more intensely cultivated and sustains some 70% of the population of the state of Kelantan.[194] Kota Bharu and the adjoining districts are located within this northern coastland in the delta of the Kelantan river and its tributaries.[192] The centre of Kota Bharu is 14 km from the coastline.

The first of the study schools, Tapang primary school, is located within the municipal limits of Kota Bharu town (fig. 2.2a and b). The second, Tawang primary school, is located 5.7 km outside the town limits in the adjoining district of Bachok (fig. 2.3a and b). Tawang school is approximately 9 km east of Tapang school and is nearer the coast. Topographical maps obtained from the survey and mapping department of Kelantan, place Tapang and Tawang schools at altitudes of approximately 5 - 7 metres above sea level.

Figure 2.1
Map showing the location of the study areas, Tapang and Tawang

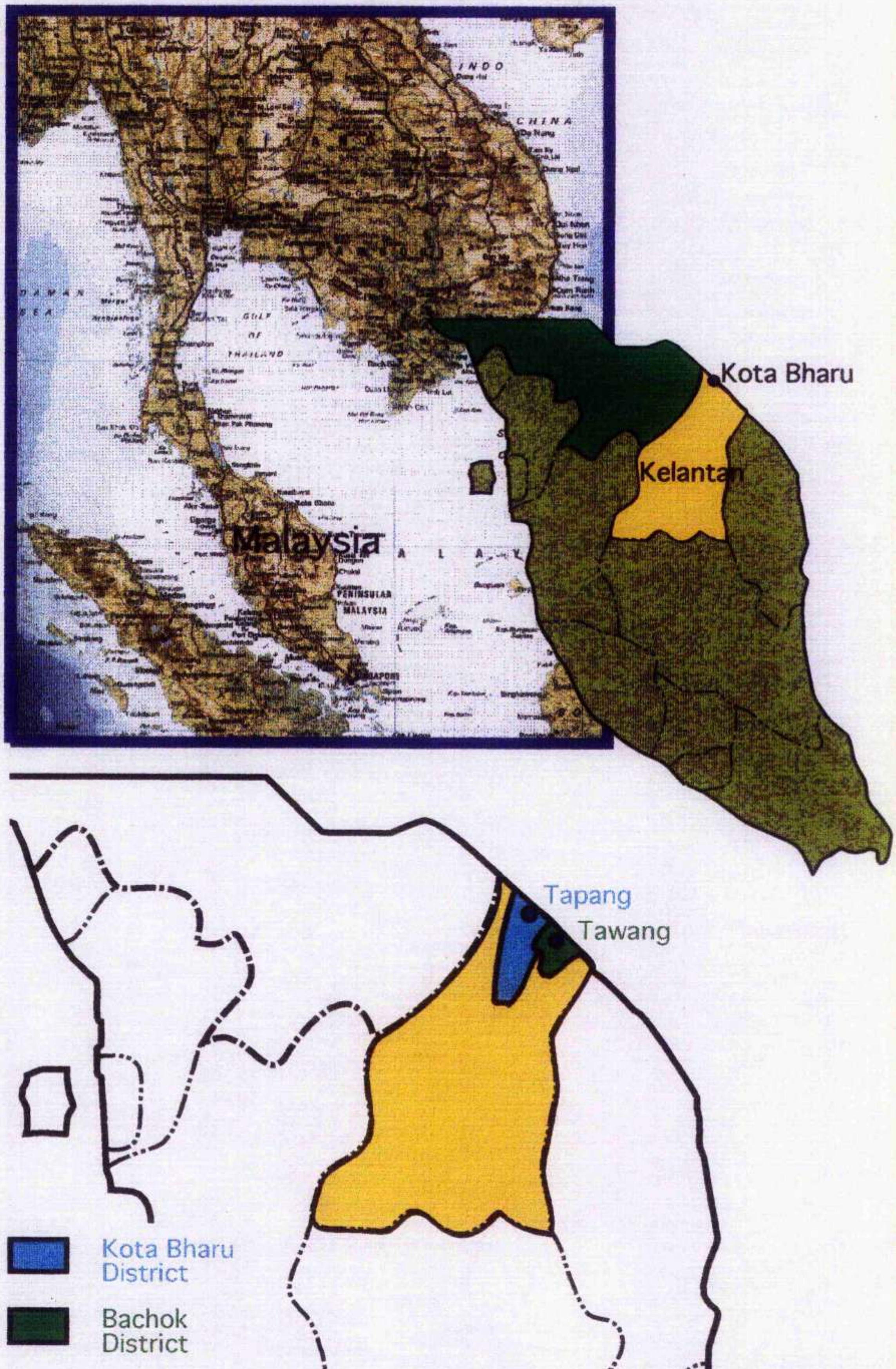
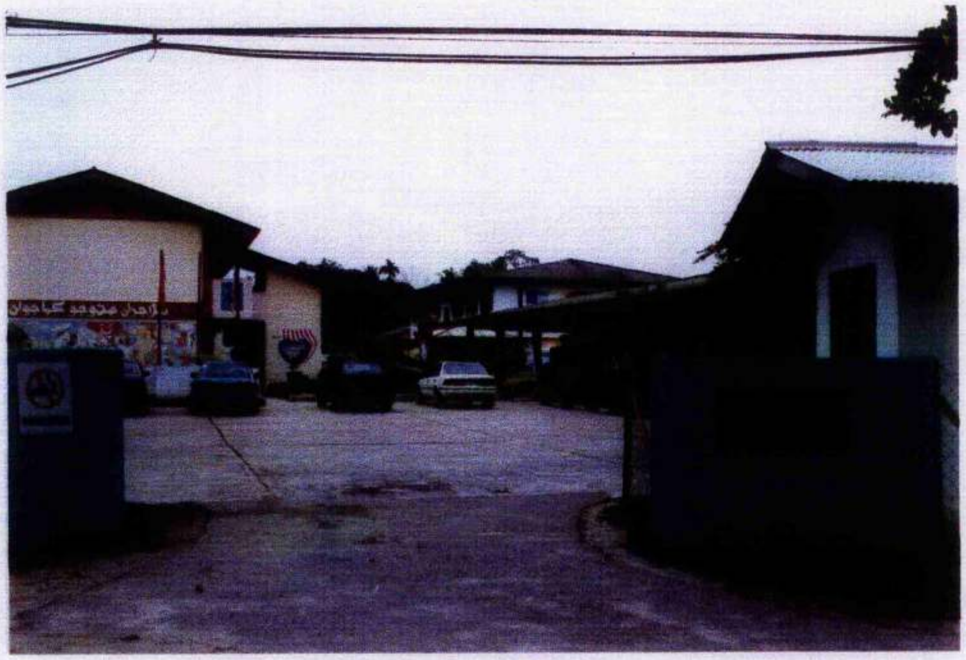


Fig 2.2 (a) and (b). Views of the school building at Tapang which is located within the town limits of Kota Bharu.

(a)



(b)

Fig 2.3 (a) and (b) Views of the school building at Tawang which is located in Bachok district on the outskirts of Kota Bharu town.

(a)

(b)

The mean annual rainfall of Kota Bharu is in the order of 2,700 mm.[195] Although there is rain throughout the year, the wettest months are November and December during the season of the North-east monsoon which blows in from the South China Sea bringing with it heavy rainfall. The mean monthly temperature in Kota Bharu generally varies within the narrow range of 25-28°C.[195] The minimum and maximum recorded temperatures in the last 30 years were 18.3°C and 36.5°C respectively. The area enjoys approximately 2400 sunlight hours a year.[195] Geologically the bedrock in the study areas consists of sedimentary rock.[192] The soil in Kota Bharu where Tapang school is located is predominantly clay while the soil in Tawang which is nearer the beach tends to be sandy.[196]

2.2 Demography

The population of the state of Kelantan consists predominantly of ethnic Malays who constitute 93% of the 1.4 million inhabitants.[194] Indeed all the children who participated in the studies documented in this thesis are Malays. Based on projections from a population census undertaken in 1991, the mid 1996 population of Kota Bharu district (which extends beyond the town limits) has been estimated to be 434,537, of which 13.7% is in the 5-9 year old age group.[194] The population density of Kota Bharu district is 1062 per square km. The population of Bachok district (which includes Tawang) is 116,547 and 15.3% of this population is in the 5-9 year old age group. The population density of Bachok district is 441.5 per square kilometre which is less than half that of Kota Bharu district.

2.3 Economic activities and indicators

The main economic activity in both Kota Bharu and Bachok districts is agriculture. Sevent-nine percent of Kota Bharu district and 57% of Bachok district are used for agricultural purposes.[197] Farming is generally conducted on small family farms or smallholdings. Rubber, rice, tobacco, vegetables, coconuts and other fruits are grown for cash.(Fig. 2.4a and b) It is estimated that in 1990 about 40% of the workforce was employed in the agricultural, forestry and fishery sectors.[198] Direct enquiry revealed that the proportion of children from Tawang school drawn from families engaged directly in agricultural activities was slightly more than half, whereas just over a tenth of children in Tapang school were from farming families. The latter is a reflection of the fact that Tapang school is located near the heart of Kota Baru town and draws children from a more urban community. While development since the 1960's has undoubtedly brought a measure of progress to the state of Kelantan the average income in the state is in relative terms, among the lowest in the country. A number of economic indicators as published in 1995 are shown in table 2.1.[197] In 1995 the per capita gross domestic product in Kelantan was 2,207 Malaysian ringgit (US 550) which was 40% of the national average and the lowest among all the states in Malaysia.[199] The mean monthly household income in 1990 was 726 ringgit (US 180) which was about half of the national average.[199] In 1995 there were 68 telephone lines per 1000 population in Kelantan as compared to the national figure of 166 per 1000 population (table 2.1).[197,200] The infant mortality rate in Kelantan in the same year was 11.4 per 1000 live births as compared to the national rate of 10.4 per 1000 live births[201]. The 1995 infant mortality rate in Bachok district (12.0 per 1000) was higher than in Kota Bharu district (9.6 per 1000).[201] An estimated 65.8% of households in Kelantan had piped water supply in 1995 as compared to 89% in Malaysia as a whole.[197,200]

Fig 2.4 (a) and (b) A typical owner occupied tobacco farm in Tawang area.

(a)

(b)

Table 2.1 Kelantan state: Economic indicators as published in 1995.[197]

Indicator	
Registered cars and motorcycles	180.5[a]
Telephones	68[a]
Television licences	56.6[a]
Literacy rate	84%
Population with piped water supply	65.7%
Population with electricity supply	100%
Length of paved roads	1.7 km/10 km^2

a- Number per 1000 population

2.4 Type of housing in the study areas.

The majority of the children who were studied live in individual wooden houses with variable amenities. The amenities are on average somewhat better in Tapang, Kota Bharu than in Tawang, Bachok. Figures 2.5 a-d illustrate some typical houses in the study area. Many houses especially in Tawang have outdoor pit latrines (fig. 2.6). Some of the houses in Tapang located near the main roads have the benefit of refuse disposal services provided by the town municipal council. Almost all the residents in Tawang however have to find their own means of disposing garbage.

The selected schools are therefore located in areas where conditions favour a relatively high transmission rate of intestinal helminth infection. This is especially true of Tawang school.

Figures 2.5a - 2.5c The spectrum of houses in the study areas varying from one with modern amenities to another with relatively poor facilities.

(a)

(b)

(c)

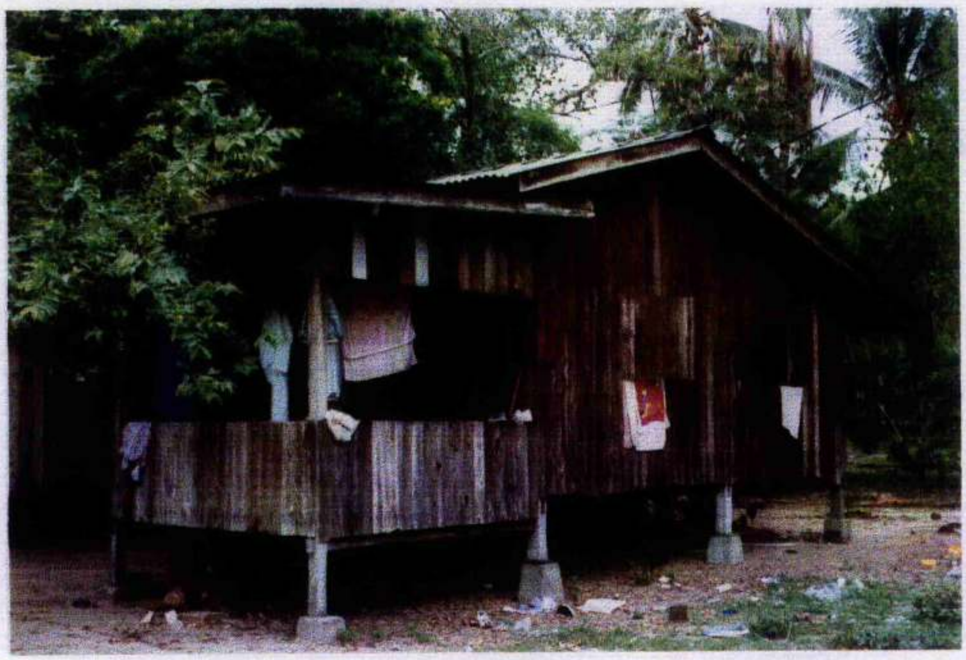
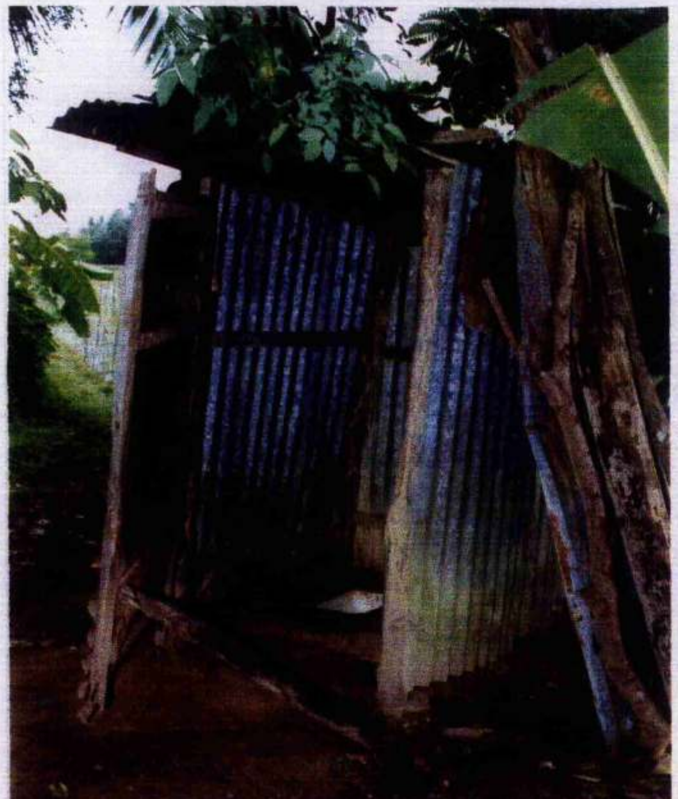


Fig 2.6 A pit latrine used by a family living in Tawang.



2.5. The Universiti Sains Malaysia, School of Medical Sciences.

The author of this thesis is based at the Universiti Sains Malaysia, School of Medical Sciences, the third Medical faculty to be established in Malaysia. The medical school complex which includes a 675 bed modern teaching hospital is located within Kota Bharu (figure 2.7). The medical school was established in 1980 and produced its first batch of medical graduates in 1986. In addition to running the undergraduate programme, the faculty has established post-graduate programmes in internal medicine, paediatrics, psychiatry, surgery and the laboratory disciplines. The teaching hospital has been operational since 1984 and has support laboratory services in chemical pathology, haematology, blood banking, histopathology, microbiology and immunology

The hospital provides a tertiary referral service for North-eastern peninsular Malaysia in neurosurgery, oncology, radiotherapy and therapeutic endoscopic cholangiopancreatography (ERCP).

Fig 2.7 The Universiti Sains Malaysia teaching hospital

Chapter 3. Effect of helminthiasis on small intestinal permeability.

Effect of helminthiasis on small intestinal permeability.

3.1 Aim and summary of study design.

The aim of this study was to determine the effect of intestinal helminthiasis on small intestinal permeability as assessed by the lactulose and mannitol differential sugar absorption test. The design essentially involved the non-invasive assessment of small intestinal permeability in children of two schools, a proportion of whom were infected with intestinal helminths. Following treatment of infected children, repeat measurements of small intestinal permeability were undertaken both on children who were infected at baseline and on uninfected controls. The study tested the hypothesis that helminthiasis is associated with abnormal intestinal permeability and that the latter is restored towards normal after treatment.

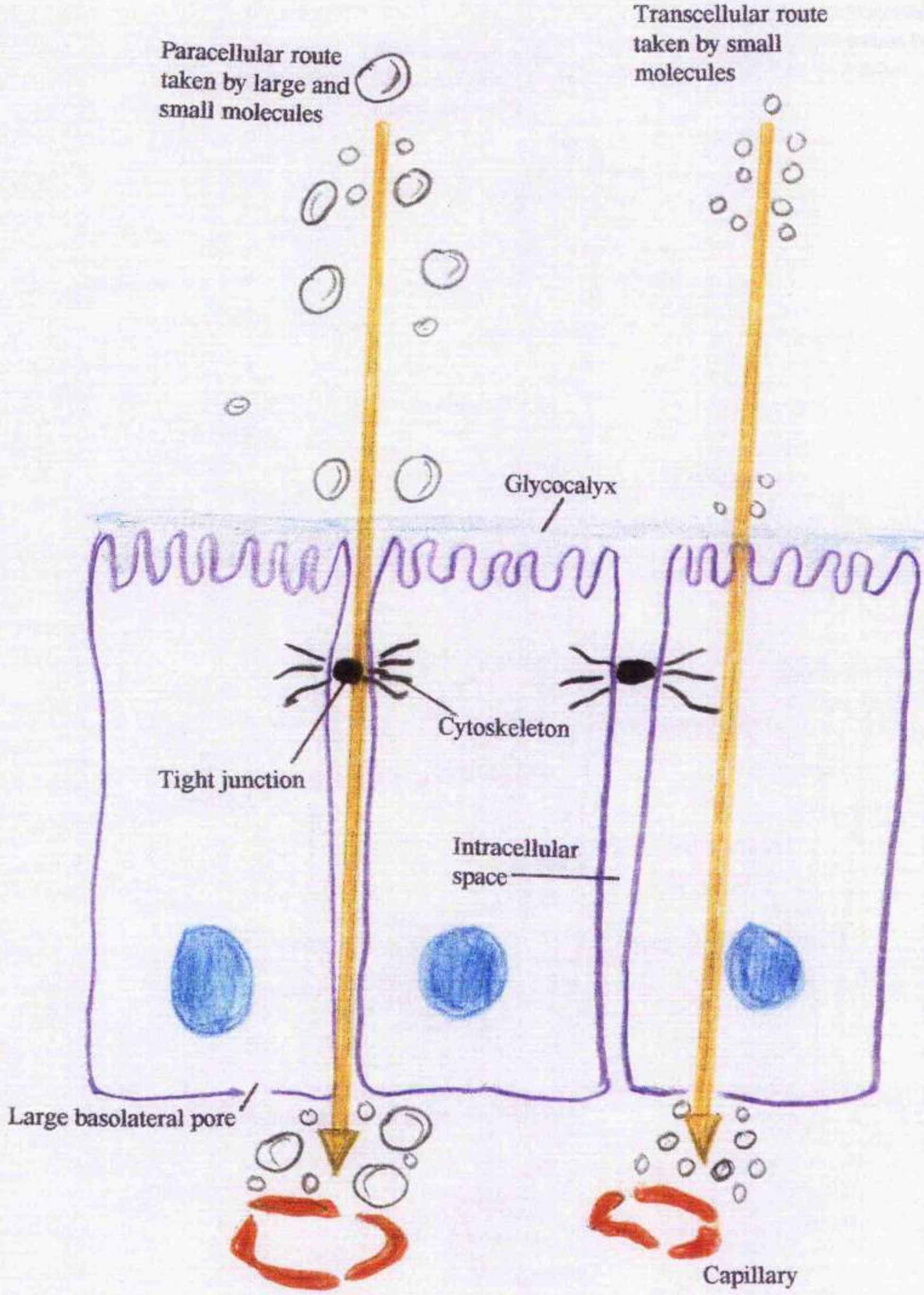
3.2 Materials and methods

3.2.1 Principle of the intestinal permeability test

Passage of certain solutes such as glucose, amino acids and ions across the small intestinal mucosa is facilitated by active transport systems. The unmediated passage of other "inert" molecules is dependent on the physicochemical properties of the solute among which are size and lipid solubility.[202-204] In health, absorption of these inert water soluble molecules is inversely related to the molecular mass or more precisely to the molecular diameter. Although there is still considerable uncertainty about the precise anatomical channels which facilitate the unmediated passage of water soluble solutes across the epithelial layer of the intestine, current concepts based on indirect evidence suggest that smaller molecules (<4nm in diameter) traverse relatively small aqueous transcellular channels while larger molecules (>5nm in diameter) pass through a paracellular route the main site of resistance of which is the tight junction[202,203] (fig 3.1). In disease states such as coeliac disease, the permeability of smaller molecules is reduced; a phenomenon attributed to the reduction in total surface area available for absorption through the transcellular route while the permeability of larger molecules is greater due to the increased "leakiness" of the intercellular tight junctions which "guard" the paracellular route.[205-207] An alternative explanation is based firstly on the observation that the paracellular tight junctions vary in tightness, being leakier in the crypts and tighter towards the tip of the villus and secondly on the assumption that all inert solutes large and small traverse the paracellular route.[204] According to this hypothesis, villous atrophy makes the "leakier" crypt junctions more accessible to larger molecules hence enhancing permeation while the reduction in the number of the paracellular routes results in diminished permeation of smaller molecules. Whatever the actual mechanism, the differential permeability has been exploited in the non-invasive assessment of small intestinal mucosal integrity.

The oral administration of a solution of two inert molecules, one small and one large followed by measurement of their recoveries in urine provides a convenient way of assessing the state of the small intestinal mucosa. In disease states, the ratio of the urinary recoveries of the larger to the smaller molecule is increased.[203,205] The ideal probe molecules should be rapidly and completely excreted in the urine, not be metabolised in the body and not be produced endogenously. A number of probe molecules fulfill these requirements sufficiently to have found wide clinical application. Among them are the non-metabolised monosaccharides mannitol and L-rhamnose which are used as small molecular probes, non-metabolised disaccharides such as lactulose which serve as the larger molecular weight probes, ethylene glycol polymers of widely ranging molecular weights; and radiolabelled non-degradable chelates.[204] The ready accessibility to mannitol and lactulose, their palatability and the availability of relatively simple enzymatic assays for the two sugars made them the probes of choice in this study. Kinetic studies have shown that 24 hour urinary recovery of lactulose after intravenous injection approaches 100% indicating that it does not undergo significant metabolism and renal excretion is near complete.[208,209] The 24 hour urinary mannitol recovery after intravenous administration has been shown in two detailed studies to approach 100%, [208,210] although a third study reported a lower recovery of 80%.[211] In one of these studies, negligible levels of radioactive carbon dioxide were detected in exhaled air after intravenous administration of ^{14}C labelled mannitol, confirming that mannitol is not metabolised in the body.[208] The body may produce endogenous mannitol but in amounts which are negligible compared to that absorbed after the commonly administered test doses. Not only are the rates of excretion of mannitol and lactulose very similar, but the distribution volumes of both sugars have been shown to be almost identical.[208] Lactulose and mannitol are therefore suitable markers of intestinal permeability as the ratio of the urinary recoveries of the two sugars after ingestion are primarily influenced by intestinal mucosal absorption, circumventing the problem of variations in gastric emptying, renal function or incomplete urine collection. The completeness of urine collection may however influence the recovery ratio to some degree. The ratio is lower during the first 2 hours of urine collection as the rate of mannitol absorption rises and falls rapidly, while lactulose absorption rises more slowly.[208] The differential sugar absorption test is a well established method of assessing intestinal permeability and has been widely used in clinical practice for over a decade.

Fig 3.1. Schematic diagram of the unmediated permeation of molecules through the intestinal epithelial layer: transcellular and paracellular routes.



3.2.2 Significance of altered intestinal permeability

Abnormal intestinal permeability occurs in virtually any disease affecting the small bowel mucosa including coeliac disease,[205-207,212-215-217] small bowel Crohn's disease,[218-221] rotavirus and other gastrointestinal infections,[222-229] tropical sprue,[230] small bowel bacterial colonisation,[231] cystic fibrosis,[232-234] drug induced enteropathy,[235-243] food allergy,[244,245] HIV enteropathy,[246-249] and other immunodeficiency states.[250] Intestinal permeability tests have been utilised not only to screen for active disease but also to monitor response to treatment. There is some evidence that intestinal permeability tests are more sensitive to abnormalities of small intestinal mucosa than conventional histology.[251,252] In one study, a subset of coeliacs with normal jejunal histology under light microscopy but abnormal cellobiose/mannitol permeability tests had demonstrable abnormalities when the biopsies were subjected to computed jejunal morphometry.[251] In another study, electron microscopic abnormalities were found to correlate well with abnormalities in permeability.[252] In yet another study of patients with a wide array of intestinal mucosal abnormalities, abnormalities of permeability were detected not only in cases with obvious histological abnormalities such as villous atrophy but also in cases where a subtle increase in mucosal lymphocyte infiltration was the only abnormality.[253]

Lactulose/mannitol recovery ratios have been shown to be higher in residents of the tropics or immigrants from the tropics than in natives of developed industrialised countries[254-256]. This is in keeping with the observation that the intestinal mucosal histology of asymptomatic residents in the tropics are often abnormal by Western standards. The villi are generally broader and shorter with some degree of lymphocyte infiltration. The term tropical enteropathy that has been used to describe these abnormalities denotes a state quite distinct however from the symptomatic disease tropical sprue.[159,160, 257-259] Tropical enteropathy is considered to be the norm in many parts of the third world where poverty and poor sanitary conditions are thought to influence the bacterial and parasitic microflora of the gut. [159]

Abnormalities in intestinal permeability have been implicated in the pathogenesis of Crohn's disease,[260-264] rheumatic diseases,[265-267] IgA nephropathy,[268,269] and even neurological conditions such as migraine,[270] autism,[271] and multiple sclerosis[272]. It is postulated that altered permeability lays the intestinal mucosa open to the passage of a myriad of potentially noxious luminal macromolecules that stimulate immunological and inflammatory responses resulting in tissue damage. Intestinal permeability has been reported to be altered in a proportion of asymptomatic first degree relatives of patients with Crohn's disease[260,262,264] leading to the suggestion that impaired intestinal permeability is a primary defect which may be genetically or environmentally determined. Altered permeability in patients with IgA nephropathies have not only been correlated with circulating levels of IgA containing immune complexes but also with the risk of deteriorating renal function[268,269] Patients with certain forms of arthropathy have been shown to have altered permeability even in the absence of non-steroidal antiinflammatory drug (NSAID) consumption.[267] Although the cause and effect relationship between altered permeability and these disease states remains speculative and far from proven, the suggestions are not entirely implausible.

In the context of the current study therefore, assessment of the effect of helminthiasis on intestinal permeability is relevant not only because it serves as a marker of mucosal damage with attendant implications on intestinal absorptive capacity of nutrients but also because of the possible consequences of an impairment in the protective mucosal barrier function. It is not totally inconceivable that breakdown of the intestinal mucosal barrier function in itself could adversely affect the general well being of the millions of infected children and significantly compromise their functional capacity independent of any effect on absorption of nutrients.

3.2.3 Lactulose assay

Lactulose and mannitol levels in the urine can be measured by a number of techniques including gas liquid chromatography,[273,274] paper chromatography[275], high pressure liquid chromatography[276] and automated enzymatic assays.[277-279] The last of these methods was employed for the purpose of this study as the enzymatic assays are relatively simple to perform, inexpensive and in terms of equipment require only a centrifugal spectrophotometric analyser such as the Cobas Bio which is used in the routine clinical biocemistry laboratory at the institution where the author of this thesis is based.

A validated manual enzymatic assay for lactulose was first described in 1984 by Behrens *et al.* from the Dunn Nutritional Laboratory, Cambridge, United Kingdom.[277] Northrop *et al.*, also from the Dunn Nutritional Laboratory adapted the technique to developed a rapid automated spectrophotometric assay.[278] The modified automated assay had the additional advantage of adjusting for the presence of lactose, glucose and fructose in urine. The principle biochemical reactions upon which the assay is based are as follows:

1. Lactulose $\xrightarrow{\text{ß-galactosidase}}$ fructose + galactose,
2. Fructose + ATP $\xrightarrow{\text{hexokinase}}$ fructose-6-phosphate + ADP,
3. Fructose-6-phosphate $\xrightarrow{\text{phosphoglucoisomerase}}$ glucose-6-phosphate,
4. Glucose-6-phosphate + NADP $\xrightarrow{\text{glucose-6-phosphate dehydrogenase}}$ gluconate -6-phosphate + NADPH

The concentration of the end product NADPH is determined by measuring increased absorbance at 340nm. An aliquot of 50ml of urine is incubated with β–galactosidase in a suitable buffer for 2 hours at 37°C. Hexokinase, glucose-6-phosphate dehydrogenase, ATP and NADP in buffered solution constitute the main reagent cocktail in the autoanalyser while phosphoglucoisomerase is the starter reagent.

If lactulose is the only sugar present in the urine, the concentration of NADPH generated would be equimolar to lactulose. However fructose, glucose or lactose in the urine, can enter the reaction at various steps to generate additional amounts of NADPH. In the assay described by Northrop *et al.* [278] contamination of the urine with lactose, glucose or fructose is corrected for by two manouvres. Firstly, separate assays were done on two aliquots of each urine specimen, one after incubation with β-galactosidase and the other after incubation in the absence of the hydrolysing enzyme. The second manouvre was to perform each assay in two stages by placing phosphoglucoisomerase in the starter reagent cup of the Cobas bio analyser such that change in absorbance was recorded before and after the action of phosphoglucoisomerase. Figure 3.2 is a schematic representation of the concept. The concentration of lactulose can be calculated by subtracting the absorbances due to the contaminating sugars.

Figure 3.2 Schematic representation of the steps involved in the lactulose assay:

First assay (Sample not incubated with β-galactosidase)	**Second assay** (Sample incubated with β-galactosidase)
1st absorbance reading[a] **[Glucose]**	1st absorbance reading[c] **[Glucose] + [Lactose]**
2nd reading (after PGI added)[b] **[Glucose] + [Fructose]**	2nd reading (after PGI added)[d] **[Glucose] + [Fructose] + [Lactose] +[Lactulose]**
[Lactulose absorbance] = (d + a - c - b)	

PGI - Phosphoglucoisomerase

Northrop *et al.* reported a linear relationship between lactulose concentration and change in absorbance up to concentrations of 3000 mg/L.[278] The assay was found to be reliable at concentrations as low as 25 mg/L. The precision of the assay was reported to be extremely good with coefficients of variations in the order of 0.5-1.2% over the concentration range of 125-1000 mg/L. Spiking urine with a host of other sugars did not seem to interfere with the lactulose assay.[278]

The assay protocol as described by Northrop *et al.* including the recommended sample and reagent volumes, and settings of the Cobas-Bio analyser[278] was adhered to in the present study. Before embarking on analyses of the urine samples, the assay was tested on aqueous solutions of pure lactulose (Sigma, USA) and the linear relationship between concentration and change in absorbance was confirmed. The intra and inter-assay coefficients of variation were 5% and 1.4% respectively at 500mg/L. This was slightly poorer than the precision reported in the original paper but was within acceptable limits for the purpose of this study.

3.2.4 Mannitol assay

In 1989, Lunn *et al.*, also from the Dunn Nutritional Laboratory, described an automated enzyme technique for measuring mannitol concentration in urine.[279] The technique utilises a bacterial enzyme, mannitol dehydrogenase which catalyses the following reaction:

$$\text{Mannitol} + \text{NAD} \xrightarrow{\text{mannitol dehydrogenase}} \text{fructose} + \text{NADH}.$$

The end product NADH, is generated in equimolar amounts to mannitol and is measured by the change in absorbance at 340 nm using a spectrophotometric analyser such as the Cobas-Bio centrifugal analyser. Mannitol dehydrogenase was originally extracted from a culture of the bacterium *Leuconostoc* *mesenteroides* but can now be extracted from other bacteria. In the original paper, Lunn *et al.* (83J) reported that the the change in absorbance with mannitol concentration was virtually linear over the range from 62.5 mg/L to 1000mg/L, with slight curving at the extreme upper and lower ends of the range.[278] The reported inter-assay precision was remarkably good with a coefficient of variation of less than 1% over the range of 250-1000 mg/L.[278] The intra-assay precision was only marginally poorer at lower concentrations with a coefficient of variation of less than 2.5%. The presence of other sugars in the urine does not appear to interfere with the assay.[278] In 1992, the group reported some modifications to improve the performance of the assay after accumulating experience in assaying thousands of samples.[280] The revised protocol increased the effective range of concentration over which the assay could be used and circumvented the occasional problem ecountered due to acidic urine or the presence of mannitol dehydrogenase inhibitors.[280]

The revised protocol was used in the present study. The enzyme mannitol dehydrogenase was obtained from two sources. The first batch was a kind gift from Dr Peter Lunn at the Dunn Nutritional Laboratory who pioneered the technique. A second batch of commercially available mannitol dehydrogenase extracted from a *Pseudomonas* species bacterium was purchased from Biocatalysts Ltd (U.K). The performance of the assay in aqueous solutions of manitol was tested in the local laboratory before proceeding with analyses of the test urine samples. Linearity of the response up to 1000mg/L of mannitol was confirmed. The precision of the assay as reported in the original description was confirmed; intra and inter-assay coefficients of variation being less than 1% at a concentration of 500 mg/L.

3.2.5 Quantification of intensity of helminth infection.

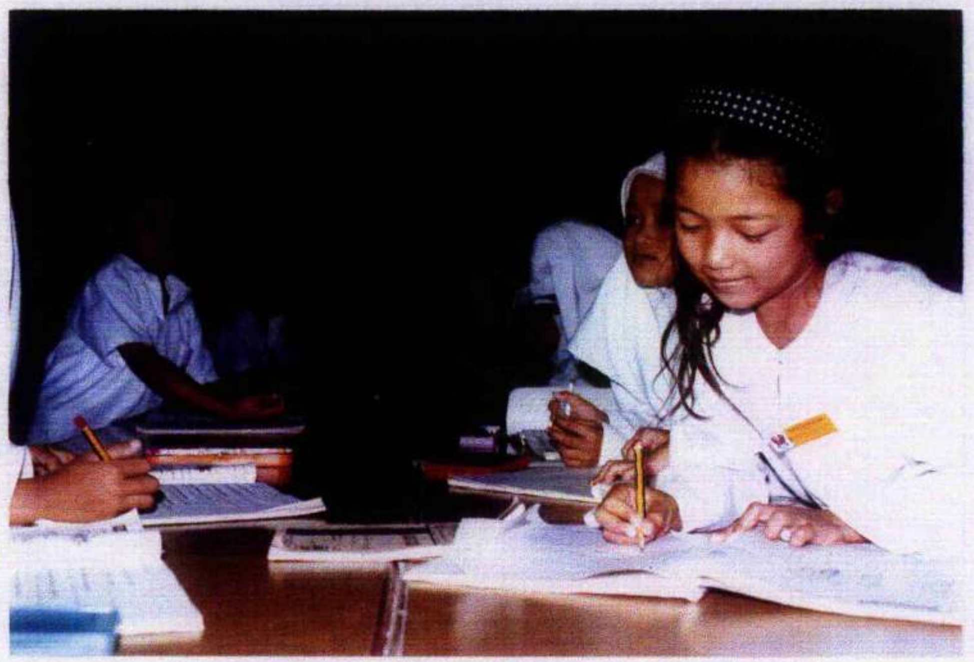
Measurement of the intensity of worm infection can be undertaken in two ways. The more widely applied method is to quantify the egg counts in the stool. A more direct method is to count the number of worms passsed in total faecal collections after administration of efficacious antihelminthics. The latter technique by virtue of its tediousness, unpleasantness and logistic limitations is in most instances unfeasible. Stool egg quantification is however an imperfect estimation of worm burden. Egg counts may vary to some degree due to non-uniformity of the distribution of eggs in the stool, day to day variations in the numbers of eggs produced by each worm and the phenomenon of density dependent fecundity.[281-283] The latter refers to the observation that as the number of worms infecting the host increases, there is a trend for the fecundity per female worm to decrease. Despite these limitations, the egg count remains a reasonable and widely accepted index of intensity of infection. In the case of *Ascaris* at least, there is a reasonably good correlation between egg counts and the number of worms expelled after treatment.[9,284] In the present study, stool samples were quantified by the commonly used modification of Stoll's method which essentially involves dilution of a small quantity of stool in 0.1M sodium hydroxide solution before microscopic examination of a small volume of the resulting suspension on a glass slide.[285] The total number of eggs on the slide are counted and a correction factor multiplied to adjust for the consistency of the stool.[285] Heavy *Ascaris* and *Trichuris* infections were defined by counts of more than 50,000 and 10,000 eggs per gram (e.p.g.) of faeces respectively.[38]

3.2.6 Subjects and study protocol

The 249 subjects of the study consisted of all children in the first grade at Tapang primary school and in the second grade at Tawang school (fig 3.3). The geographic characteristics of the study area have been described in the previous chapter. The children were all Malays.

Figure 3.3 Classroom pictures of children at a) Tapang school and b) at Tawang school.

(a)



(b)

Investigations were undertaken at Tapang between August and October of 1994 while the children at Tawang school were investigated between March and May of 1995. Stool parasitological examination was undertaken during the week prior to permeabilty testing. With the assistance of parents (which was sought by way of written communication), a specimen of stool from each child was placed in a standard plastic container using a wooden spatula and the specimen handed in at school. Stool samples were transported to the laboratory within a few hours of being received and examined for helminth eggs on the same afternoon or were sometimes stored refrigerated before being examined the next day. The specimens were initially examined using a standard zinc sulphate concentration technique.[285] If positive, the egg counts were subsequently quantified by the commonly used modification of Stoll's dilution method.[285] Stool examination was conducted by two field assistants trained in the technique and supervised by the author. The author sought the assistance of an experienced parasitologist to recheck random specimens to ensure quality control. Stool samples were collected from the subjects in batches and examination of the samples took about a week to complete at both schools. Intestinal permeability tests were conducted over a period of 4-5 days at each school. Parents were informed of the date of the permeabilty test in a letter and instructed to keep their children fasted from bedtime the night before. Urine collections always started in the morning immediately after school began. An iso-osmolar test solution which contained 5g/100ml of lactulose (Duphulac, Duphar, Netherlands) and 2g/100ml of mannitol was prepared by the hospital pharmacy the day before the test. An oral dose of 2ml/kg of the solution was administered under direct supervision. The children were encouraged to drink plain water throughout the 5 hour period of urine collection. Urine was collected in plastic containers which contained a few drops of 0.5% chlorhexidine gluconate to prevent bacterial growth. The children were given a meal of rice or noodles after at least 2 hours had lapsed from the time of ingestion of the test solution. Urine volumes were measured at the school immediately after collection and aliquots of urine were transported to the laboratory within 1-2 hours. The samples were stored immediately at -20^{o}C until the time of analysis. All helminth infected children were given a supervised dose of 400 mg of albendazole (SmithKline and Beecham, U.K.) within a few days of completing the permeability tests.

Repeat stool examination and intestinal permeability tests were conducted six to eight weeks later. At Tapang, repeat investigations was undertaken on all infected children and a randomly selected subset of 26 uninfected children. At Tawang, post treatment investigations were conducted on all children irrespective of baseline infection status. Analysis of the urine samples were performed over the course of 2 weeks at the end of 1995 using the enzymatic assays as outlined above. Enzyme buffer cocktails were freshly prepared each day. The author was directly involved in the sample assays and had the benefit of the assistance of a skilled laboratory technician familiar with the Cobas-Bio autoanalyser as well as the advice and guidance of a trained biochemist.

Lactulose and mannitol recoveries were calculated based on the concentrations of the two sugars in the urine, the total urine volumes and the dose administered. Change in the lactulose to mannitol recovery ratio between visits (ΔL/M) was defined as the lactulose/mannitol recovery ratio at the first visit (L/M1) minus the ratio at the second visit (L/M2). A positive value for ΔL/M therefore indicates a fall in the lactulose/mannitol ratio and signifies an improvement in the intestinal permeability.

Information about the occupation of the parent, the source of water for domestic use, the type of sanitation facility and the number of siblings in the family was obtained by means of a questionnaire which was conveyed to the parents by the children. It is likely that parents who were illiterate would have completed the questionnaires with the assistance of older children, relatives or friends. Based on the limited information available, a crude socioeconomic index was constructed and each child categorised to one of three socioeconomic groups; group three being the most disadvantaged. Weight and height were measured at the first visit. Height for age, weight for age and weight for height was computed using an anthropometry programme in the Epiinfo software package (World Health Organization, Geneva, Switzerland and Centers for Disease Control, Atlanta, Georgia, USA).

The study was approved by the research and ethics committee of the School of Medical Sciences, Universiti Sains Malaysia. Written consent was obtained from the parents or guardians of all children.

3.2.6 Statistical analysis.

If the distribution of a quantitative variable was skewed, the degree of dispersion was generally indicated by the quartile deviation (QD) which is half the interquartile range. Non-parametric tests were generally used to assess differences in permeability data between groups. Two tailed significance values were quoted in all instances. Statistical computations were performed with the aid of the Epiinfo version 6 program (World Health Organization, Geneva, Switzerland,Centers for Disease Control, Atlanta, USA).

3.3 Results

3.3.1 Demographic data and baseline infection rates.

Consent for the study was obtained from all 249 children who were targetted for the study of whom 108 were infected (tables 3.1a and b). *Ascaris lumbricoides* and *Trichuris trichiura* infections were common but hookworm infection was uncommon (tables 3.1a and b). During the visits to the schools it was immediately obvious that the socioeconomic backgrounds of the children at Tawang were generally lower than at Tapang. Sixty-eight percent of the subjects at Tawang but only 41% of the children in Tapang fell into socioeconomic category 3. Three children were drawn from socioeconomic group 1, all of whom attended Tapang school. Furthermore, 23.8% (25/105) of the children at Tawang were stunted as defined by a height for age z-score of less than -2.0, whereas the frequency of stunting at Tapang was only 14.6% (21/144).

The helminth infection rate was higher at Tawang than at Tapang (tables 3.1a and b). Infected and uninfected children were comparable in terms of age and sex at both schools (tables 3.1a and b). As the subjects in each school were all in the same grade there was little variation in age within the school; the standard deviation being in the order of 0.2-0.3 years (tables 3.1a and b).

At Tapang, 45% (22/49) of infections were mixed and all but one of the 27 children with single infections were positive for *Trichuris*. At Tawang, 78% (46/59) of infections were mixed. The mean *Ascaris* intensity was substantially higher at Tawang due largely to the higher prevalence of ascariasis rather than a tendency for infected children to harbour more worms. When *Ascaris* infected children were considered separately, the mean intensities at Tapang (n=23) and Tawang (n=50) were similar at 61,600 e.p.g. (range 1,600-235,200) and 72,300 e.p.g. (range 3,200-356,400) respectively. Among *Trichuris* infected children, the mean *Trichuris* intensities at Tapang (n=48) and Tawang (n=55) were 11,200 e.p.g. (range 200-137,000) and 8,500 e.p.g. (range 800-39,000) respectively. Ten children at Tapang had heavy ascariasis (>50,000 e.p.g) and 16 had heavy trichuriasis (>10,000 e.p.g.). The frequencies of heavy ascariasis and trichuriasis at Tawang were 23 and 17 respectively.

Table 3.1 (a). Baseline characteristics and infection status (pre and post-treatment) at Tapang school.

Baseline infection status	Infected (n=49)	Uninfected (n=95)
Height for age, z- score [a]	-1.1(0.6)	-1.0 (0.5)
Weight for age, Z-score[a]	-1.8(0.7)	-1.6(0.5)
Weight for height, Z-score[a]	-1.3(0.5)	-1.4(0.6)
Age, years[b]	7.2 (0.3)	7.2 (0.3)
Sex ratio (M:F)	1.3 : 1	0.8 : 1
Ascaris infection rate:		
baseline	46.9% (23/49)	-
follow-up	2.1% (1/47)	7.7% (2/26)
Trichuris infection rate:		
baseline	98.0% (48/49)	-
follow-up	36.2% (17/47)	3.8% (1/26)
Hookworm infection rate:		
baseline	2.0% (1/49)	-
follow-up	2.1% (1/47)	0.0% (0/26)
Ascaris intensity[c]:		
baseline	28.9 (0.0 - 235.2)	-
follow-up	3.5 (0.0 - 165.6)	0.4 (0.0 - 6.0)
Trichuris intensity[c]:		
baseline	11.0 (0.0 - 137)	-
follow-up	3.2 (0.0 - 34.4)	0.2 (0.0 - 6.0)

[a]Median (quartile deviation in parentheses).

[b]Mean (standard deviation in prentheses).

[c]Mean intensity (range in parentheses); expressed in eggs/g stool x 10^3.

Table 3.1(b). Baseline characteristics and infection status (pre and post-treatment) at Tawang school.

Baseline infection status	Infected n=59	Uninfected n=46
Height for age, Z- score [a]	-1.5 (0.5)	-1.4 (0.6)
Weight for age, Z-score[a]	-1.4 (0.6)	-1.4 (0.4)
Weight for height, Z-score[a]	-0.7 (0.7)	-0.8 (0.6)
Age, years[b]	7.8 (0.2)	7.8(0.3)
Sex ratio (M:F)	1.2 : 1	0.9 : 1
Ascaris infection rate:		
baseline	84.7% (50/59)	-
follow-up	3.4% (2/58)	17.8% (8/45)
Trichuris infection rate:		
baseline	93.2% (55/59)	-
follow-up	17.2 % (10/58)	28.9% (13/45)
Hookworm infection rate:		
baseline	28.9% (17/59)	-
follow-up	3.4% (2/58)	6.7% (3/45)
Ascaris intensity[c]:		
baseline	61.2 (0.0 - 356.4)	-
follow-up	0.1 (0.0 - 3.6)	7.6 (0.0 - 83.2.0)
Trichuris intensity[c]:		
baseline	8.0 (0.0 - 39.0)	-
follow-up	1.6 (0.0 - 38.0)	3.1 (0.0 - 44.4)

[a]Median (quartile deviation in parentheses).

[b]Mean (standard deviation in prentheses)

[c]Mean intensity (range in parentheses); expressed in eggs/g stool x 10^3

3.3.2 Infection rates and intensities at the second visit

At Tapang, stool samples for re-examination were submitted by all but 2 children who were infected at baseline and 26 randomly selected uninfected children. At Tawang all but two children submitted stool samples for repeat examination. The elimination of *Ascaris* infection at both schools was near complete whereas elimination of *Trichuris* was less complete (tables 3.1a and b). Of the 26 uninfected children at Tapang selected for repeat testing, 2 were found to be infected at follow-up. At Tawang, 15 initially uninfected children were found to be infected at re-examination (tables 3.1a and b).

3.3.3 Intestinal permeability data

Baseline intestinal permeability tests were successfully completed on all children at Tapang and on 97% (102/105) of the children at Tawang. At Tapang, repeat test results were available on 94% (46/49) of the children infected at baseline and on the 26 randomly selected controls. At Tawang, follow-up permeability data was obtained on 94%(96/102) of children who had been tested at baseline. The small number of missing data was due to absenteeism, poor compliance in urine collection or technical mishaps such as loss of the sample due to accidental spillage.

Intestinal permeability in relation to infection status analysed by school.

At Tapang, helminth infection was associated with a higher baseline lactulose mannitol ratio (L/M1) (table 3.2a). This difference disappeared at follow-up. However there was no difference between infected and uninfected groups in terms of change in lactulose mannitol recovery ratio (ΔL/M) as shown in table 3.2a. At Tawang on the other hand, there was no difference between infected and uninfected groups in either L/M1 or ΔL/M (table 3.2b).

Table 3.2. Intestinal permeability in relation to infection status at:

(a) Tapang

	Infected[b]	Uninfected[b]	*p*
First visit:	[n=49]	[n=95]	
Lactulose recovery, %[a]	0.5 (0.3)	0.4 (0.3)	*0.05*
Mannitol recovery, %[a]	14.9 (4.0)	14.0 (4.1)	*0.95*
L/M1[a]	0.042 (0.018)	0.031 (0.023)	*0.04*
Follow-up:	[n=46]	[n=26]	
Lactulose recovery, %[a]	0.4 (0.2)	0.3 (0.2)	*0.61*
Mannitol recovery, %[a]	12.3 (3.5)	12.4 (3.3)	*0.61*
L/M2[a]	0.033 (0.017)	0.025(0.016)	*0.47*
	[n=46]	[n=26]	
ΔL/M[a]	0.009 (0.021)	0.003 (0.013)	*0.48*

(b) Tawang

	Infected[b]	Uninfected[b]	*p*
First visit:	[n=57]	[n=45]	
Lactulose recovery, %[a]	0.7 (0.4)	0.6 (0.3)	*0.19*
Mannitol recovery, %[a]	10.9 (4.9)	10.9 (5.0)	*0.35*
L/M1[a]	0.065 (0.032)	0.047 (0.013)	*0.26*
Follow-up:	[n=56][c]	[n=42]	
Lactulose recovery, %[a]	0.4 (0.2)	0.4 (0.2)	*0.37*
Mannitol recovery, %[a]	7.6 (2.8)	7.4 (3.20	*0.78*
L/M2[a]	0.046 (0.023)	0.044 (0.015)	*0.36*
	[n=54]	[n=42]	
ΔL/M[a]	0.005 (0.015)	0.005 (0.023)	*0.91*

L/M - Lactulose/Mannitol ratio (1 and 2 indicate first and follow-up visits respectively).

Δ L/M - Change in L/M between visits (LM1- LM2).

[Number of subjects in square brackets] .

a - Median, (quartile deviation in parentheses).

b- Refers to infection status at baseline.

c-Two children absent at first visit but tested at follow-up.

Intestinal permeability in relation to Ascaris and Trichuris infection status analysed by school.

A comparison was made between *Ascaris* infected and *Ascaris* uninfected children. Although *Ascaris* infection *per se* was associated with a higher L/M1 at both Tapang and Tawang; at neither school was there a difference between *Ascaris* infected and uninfected groups in terms of ΔL/M (tables 3.3a and b). A similar comparison was made between *Trichuris* positive and *Trichuris* negative groups. The difference in L/M1 between *Trichuris* positive and negative groups at Tapang approached significance ($p=0.08$) but the difference between the groups in terms of ΔL/M was clearly not significant (tables 3.3a and b).

The correlation between baseline *Ascaris* intensity and L/M1 at Tapang was modest ($r=0.41$) while the correlation was rather weaker at Tawang ($r=0.25$). There was no significant correlation between the fall in *Ascaris* intensity and improvement in lactulose mannitol recovery ratio (ΔL/M) at either school (tables 3a and b). There was a weak correlation between baseline *Trichuris* infection and L/M1 at both schools but at neither school was there a significant correlation between fall in *Trichuris* intensity and ΔL/M (tables 3.3a and b).

Table 3.3. Intestinal permeability in relation to type of infection at:

(a) Tapang school

	Ascaris		*Trichuris*	
	Infected[c]	Uninfected[c]	Infected[c]	Uninfected[c]
	[n=23]	[n=121]	[n=48]	[n=96]
L/M1[a]	0.045 (0.035)	0.031 (0.020)[d]	0.039 (0.019)	0.032 (0.024)
	[n=21]	[n=51]	[n=45]	[n=27]
ΔL/M[a]	0.015 (0.039)	0.004 (0.018)	0.009 (0.020)	0.003 (0.022)
Correlation coefficients:				
Intensity and L/M1[b]	0.41 (0.26-0.54)		0.24 (0.08-0.39)	
ΔIntensity and ΔL/M[b]	0.15 (-0.09-0.37)		0.22 (-0.02-0.43)	

(b) Tawang school.

	Ascaris		*Trichuris*	
	Infected[c]	Uninfected[c]	Infected[c]	Uninfected[c]
	[n=48]	[n=54]	[n=53]	[n=49]
L/M1[a]	0.071 (0.034)	0.047 (0.014)[d]	0.065 (0.031)	0.047 (0.014)
	[n=45]	[n=51]	[n=50]	[n=46]
ΔL/M[a]	0.004 (0.025)	0.005 (0.016)	0.004 (0.023)	0.005 (0.015)
Correlation coefficients:				
Intensity and L/M1[b]	0.25 (0.06-0.42)		0.20 (0.01-0.38)	
Δ Intensity and ΔL/M[b]	0.08 (-0.13-0.27)		-0.12 (-0.31-0.08)	

[Number of subjects in square brackets]

L/M - Lactulose/Mannitol ratio (1 and 2 indicate first and follow-up visits respectively).

Δ L/M - Change in L/M between visits (L/M1 – L/M2).

ΔIntensity - Change in intensity (baseline intensity – follow-up intensity).

a- Medians (quartile deviation in parentheses).

b-95% confidence interval in parentheses.

c-Refers to infection status at baseline.

d- $p<0.05$.

Intestinal permeability in relation to Ascaris, and Trichuris infection analysed by socioeconomic status.

Within socioeconomic group 2, L/M1 was higher among *Ascaris* infected than *Ascaris* negative children (table 3.4a). More importantly, treatment of ascariasis was associated with a significantly higher ΔL/M (table 3.4a, fig 3.4). Within the same socioeconomic group, trichuriasis was also associated with a higher L/M1 but there was no difference in ΔL/M between *Trichuris* positive and negative subsets. There was a modest correlation not only between baseline *Ascaris* intensity and L/M1 but more importantly between decrease in *Ascaris* intensity and ΔL/M (table 3.4a).

The relationship between permeability changes and infection in socioeconomic group 3 was quite different (table 3.4b). Ascariasis was associated with a higher L/M1 but not with a higher ΔL/M (table 3.4b). There was no difference between *Trichuris* infected and uninfected groups with respect to either L/M1 or ΔL/M (table 3.4b). There was a weak correlation between baseline *Ascaris* intensity and L/M1 but none between fall in *Ascaris* intensity and ΔL/M.

Influence of urinary lactulose recovery on L/M ratio

It is notable that wherever a significant difference in lactulose to mannitol recovery ratio between groups was detected, the difference was largely due to a difference in the urinary lactulose recovery rather than the mannitol recovery (tables 3.2a). The improvement in ΔL/M observed in *Ascaris* infected children within socioeconomic group 2 was also predominantly due to a fall in urinary lactulose recovery post-treatment. The median decrease in urinary lactulose recovery in that subset was 0.4% as opposed to a median decrease of only 0.1% in *Ascaris* negative children within the same socioeconomic group (p=0.005).

Table 3.4 Intestinal permeability in relation to type of infection in:

(a) socioeconomic group 2.

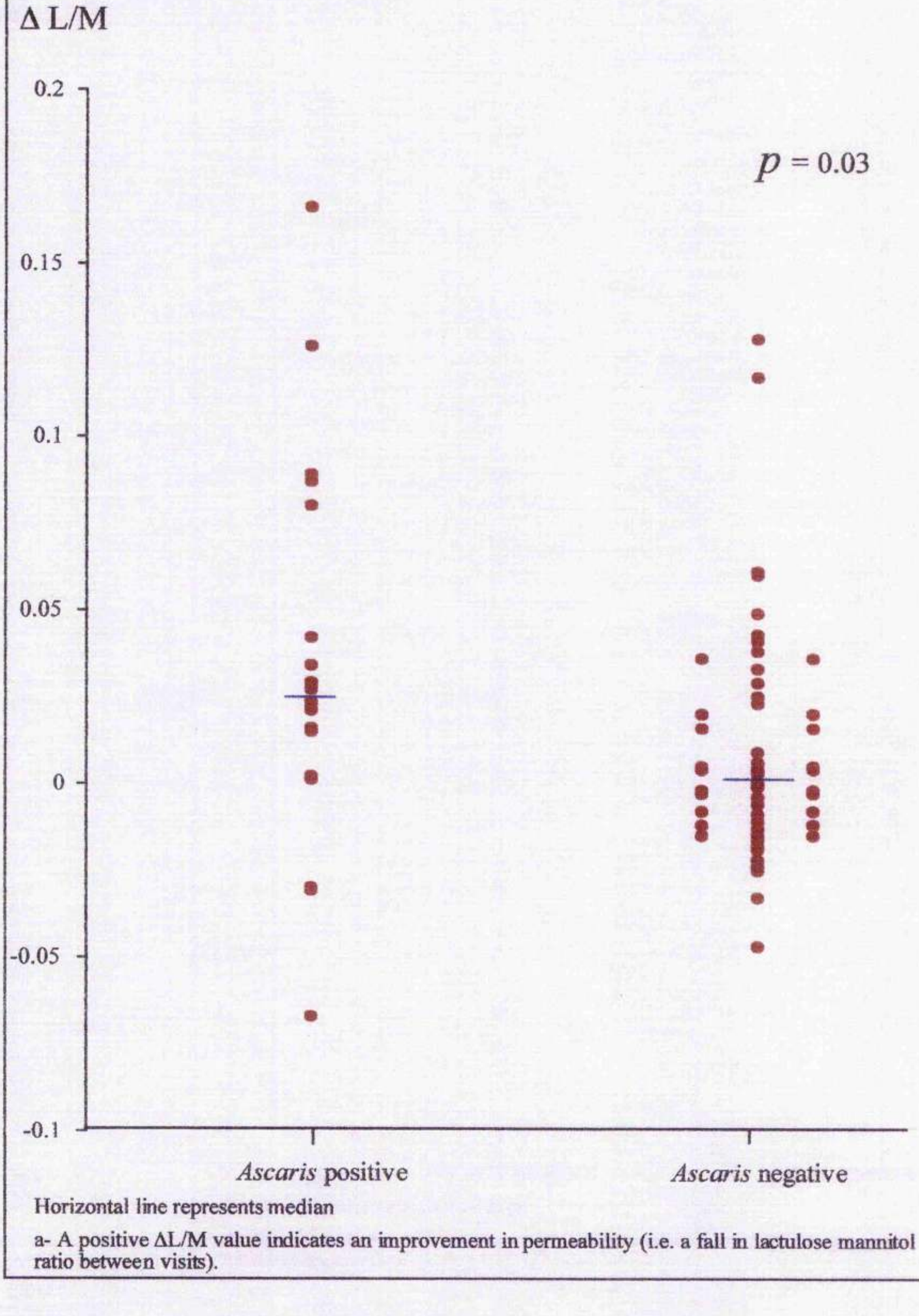
	Ascaris		*Trichuris*	
	Infected[c]	Uninfected[c]	Infected[c]	Uninfected[c]
	[n=19]	[n=96]	[n=35]	[n=80]
L/M1[a]	0.067 (0.049)	0.034 (0.022)[d]	0. 045 (0.037)	0.036 (0.023)[d]
	[n=18]	[n=52]	[n=34]	[n=36]
ΔL/M[a]	0.025 (0.039)	0.002 (0.026)[d]	0.016 (0.021)	0.002 (0.021)
Correlation coefficients:				
Intensity and L/M1[b]	0.43 (0.26-0.50)		0.38 (0.21-0.52)	
Δ Intensity and ΔL/M[b]	0.46 (0.25-0.63)		0.38 (0.16-0.57)	

(b) socioeconomic group 3.

	Ascaris		*Trichuris*	
	Infected[c]	Uninfected[c]	Infected[c]	Uninfected[c]
	[n=52]	[n=76]	[n=66]	[n=62]
L/M1[a]	0.059 (0.030)	0.043 (0.019)[d]	0.047 (0.028)	0.043 (0.018)
	[n=48]	[n=49]	]n=61]	[n=36]
ΔL/M[a]	-0.001 (0.028)	0.009 (0.014)	0.003 (0.021)	0.009 (0.011)
Correlation coefficients:				
Intensity and L/M1[b]	0.28 (0.11-0.43)		0.06 (-0.12-0.23)	
Δ Intensity and ΔL/M[b]	-0.10 (-0.29-0.01)		-0.13(-0.32-0.07)	

[Number of subjects in square brackets]
L/M - Lactulose/Mannitol ratio (1 and 2 indicate first and follow-up visits respectively).
Δ L/M - Change in L/M between visits (L/M1 – L/M2).
ΔIntensity - Change in intensity (baseline intensity – follow-up intensity).
a- Medians (quartile deviation in parentheses).
b-95% confidence interval in parentheses
c-Refers to infection status at baseline
d- $p<0.05$

Figure 3.4 Change in lactulose mannitol ratio (ΔL/M)[a]: Comparison between *Ascaris* positive and negative groups within socioeconomic group 2.



Horizontal line represents median

a- A positive ΔL/M value indicates an improvement in permeability (i.e. a fall in lactulose mannitol ratio between visits).

3.4 Discussion

It is acknowledged at the outset that the ideal design for such a study would have entailed the random allocation of infected children to treatment or placebo arms and the measurement of intestinal permeability in a double blind fashion. However a number of considerations precluded such a design. There was the ethical consideration of witholding treatment in children diagnosed to harbour worm infections, a dilemma which is particularly pertinent in relation to heavy infections which may, albeit rarely, result in life threatening complications such as *Ascaris* induced intestinal obstruction or the *Trichuris* Dysentery Syndrome. To proceed with a randomised double blind study after excluding children with heavy infection would have been an unsatisfactory solution as the ability of detecting an effect which is intensity dependent would have been diminished. The second and more important obstacle to the double blind design relates to the acceptance of the community to receiving placebo treatment. Worm infection is perceived as unpleasant and the author had reservations about the response of the community to the possibility of being denied immediate treatment. The nature of the study was such that the goodwill and cooperation of the community were critical to its success. The expectation of treatment had probably contributed towards securing the cooperation of all concerned. Throughout this and the subsequent studies it was a policy to administer treatment to all children upon detection of worm infection. Hence children who were initially uninfected but subsequently found to be infected were given treatment after the repeat studies had been completed. Furthermore, blinding the patient in studies involving antihelminth treatment is not always easy. An obvious consequence of treatment is expulsion of worms which in the case of patients harbouring many *Ascaris* worms is an event which does not go unnoticed by the patient. One approach used in other studies was to allocate different villages to treatment and non-treatment arms.[116] This however does not circumvent the problem of confounding factors linked to locality which in a study such as this one may be crucial. Furthermore antihelminth drugs are easily available at the primary health care level, and are often prescribed on just the suspicion of worm infection. The examination of stool samples without subsequent treatment may prompt the procurement of antihelminth treatment from other sources. For the purpose of this study, a compromise was therefore struck by using uninfected children as controls.

It was obvious that there were important differences in the socioeconomic settings from which the students of the two schools were drawn. The children at the urban Tapang school were on average better off than those attending Tawang and this factor may explain some of the differences observed in relation to the permeability results. Helminthiasis in general and ascariasis in particular were associated with altered intestinal permeability at baseline as reflected by a higher lactulose/mannitol recovery ratio (L/M1). This was more easily discerned at Tapang school and corroborated by the better correlation between baseline *Ascaris* intensity and L/M1 at Tapang (table 3.3a). After treatment, there was no longer a difference in the recovery ratios at Tapang indicating that there had been some improvement in the lactulose mannitol ratio of the infected group as a whole (table 3.2a). Despite this, comparison of the magnitude of the post-treatment fall in the lactulose mannitol ratio (ΔL/M) between infected and uninfected groups revealed no difference. At Tawang there was even less evidence that treatment improved intestinal permeability.

There was no correlation between the decrease in worm intensity and ΔL/M at either school. The possibility exists of course that the relationship between fall in intensity and improvement in intestinal permeability is non-linear. However regression analysis after log transformation of egg counts, ΔL/M or both parameters did not improve the correlation (data not shown).

The main findings of this study therefore have to be regarded as negative in that the data provide no convincing evidence that intestinal helminthiasis exerts an important influence on intestinal permeability. The consistent association between ascariasis and altered permeability at baseline is probably because ascariasis is a marker for a host of other factors such as other gastrointestinal infections which may be responsible for the intestinal mucosal damage.

However the observation could not go unnoticed that at Tapang there was at least a hint that treatment may have restored the intestinal permeability to some extent while the findings at Tawang were unequivocally negative in this respect. As there was a major difference between the schools with respect to the prevailing socioeconomic conditions, the possibility was entertained that this may be an important determinant of the outcome. This prompted a comparison of the permeability data within socioeconomic groups. The trends as far as the change in lactulose mannitol ratio (ΔL/M) was concerned were markedly different in the two socioeconomic groups. While treatment was associated with significant improvements of permeability among the children in socioeconomic group 2, no such trend was seen among the more disadvantaged children of socioeconomic group 3. It was equally notable that the change in *Ascaris* intensity accounted for 21% of the variation in ΔL/M (ie. $r=0.46$, $r^2=0.21$) within socioeconomic group 2.

How then can some of these seemingly opposing trends be incorporated into a unifying hypthesis? The most plausible explanation is that intestinal helminthiasis exerts a small affect on small intestinal permeability, the relative impact of which in children of lower socioeconomic status is negligible in relation to the cumulative effects of a multitude of other noxious stimuli to which these children are exposed. It is difficult in the presence of a high prevalence of mixed infections to be sure of the relative contributions of the individual species of worms, but based on the more consistent association between ascariasis and altered permeability it would seem that the albeit minor effect on intestinal permeability is largely an *Ascaris* rather than a *Trichuris* mediated effect. This is not surprising as the adult *Ascaris lumbricoides* dwells in the small intestine while *Trichuris trichiura* is predominantly a colonic parasite. The prevalence of hookworm infection in this study was too low to enable meaningful conclusions on the effect of hookworm infection on small intestinal permeability.

The *in vitro* precision of the sugar assays in the local laboratory was comparable to that achieved by the workers who had previously validated the assays. It is possible however that some precision may have been lost during the field studies. In a sense, the consistent association between ascariasis and impaired permeability at both schools as shown in tables 3.3a and b, attests to the precision of the assays to some extent. The issue is whether even a small loss in precision may have resulted in failure to detect changes in permeability post-treatment. That this may have been a contributory factor when comparing *Ascaris* positive and negtive groups at Tapang (table 3.3a) cannot be entirely excluded as there was a trend towards an improvement in permeability after treatment which was not statistially significant. However this is unlikely to be the case at Tawang school or for that matter within socioeconomic group 3, where there was not even a hint that intestinal permeability improved with antihelminthic treatment (tables 3.3b and 3.4b). In any case, this consideration does not detract from the argument that the independent effect of ascariasis is likely to be modest and reaches negligible proportions among children of lower socioeconomic backgrounds who constituted the majority at Tawang school.

Direct comparison of the mean values of lactulose mannitol ratios between studies is hampered by variations in the doses of the sugars administered, the osmolarity of the solutions used and in the assay technique. Test solutions are either iso-osmolar (200-300 mosmol/kg) or hyperosmolar (1350-1500 mosmol/kg).[203,204] Hyperosmolarity is achieved by adding an osmotic filler such as glycerol or sucrose. The rationale of using hyperosmolar solutions is based on the observation that hyperosmolar stress increases the permeability of disaccharides to lactulose. This occurs both in disease and health but to a greater degree in disease and has led to the suggestion that hyperosmolarity improves the discrimination between normals and coeliacs. The use of hyperosmolar solutions however may produce false positive results[203] and it has been argued that it is unnecessary in practice.[204] While it is possible that the use of hyperosmolar solutions in this study may have accentuated differences between groups that were already detectable, it is unlikely to have changed the observed trends.

Having acknowledged the potential pitfalls of direct comparisons of lactulose mannitol ratios between studies, a useful insight is provided by cautious comparison of the results of this study with those of an Italian study in which identical doses of lactulose and mannitol were used.[214] In the Italian study, the median lactulose to mannitol recovery ratio of 0.038 in coeliac patients picked up during screening was as expected significantly higher than the median of 0.014 observed in healthy controls. The subjects in the Italian study were adolescents and therefore somewhat older than the children studied in Tapang and Tawang. Nonetheless it is noteworthy that the median lactulose mannitol ratios of subsets of children at Tapang and Tawang were comparable to that of coeliacs in the Italian study and certainly higher than in healthy Italian controls. While this confirms the existence of a tropical enteropathy in Northeastern peninsular Malaysia, the data suggests that *Ascaris* infection contributes only marginally towards the enteropathy.

Another point of interest to emerge from this study is that the difference in lactulose mannitol ratio between groups was generally due to increased lactulose permeability rather than reduced mannitol permeability. In this respect it is pertinent that the improvement of the lactulose mannitol ratio in *Ascaris* infected children of socioeconomic group 2 was also largely a consequence of the reduction in lactulose permeability after treatment. Extrapolating from first principles it would seem that there is little variation in the total mucosal surface area among children in the area but exposure to the various noxious stimuli probably results in increased "leakiness" of the paracellular tight junctions.

As discussed in chapter 1 the bulk of the earlier work investigating the effects of ascariasis on small intestinal absorptive function was undertaken on pre-school children [153-156,161,162] and suffered from design limitations such as small numbers and the absence of controls. As mentioned in chapter 1, the most persuasive evidence that ascariasis causes small intestinal damage is derived from the two controlled studies of lactose absorption on Panamian pre-school children which showed that ascariasis was associated with lactose malabsorption which reversed on treatment[161,162]. However the study on lactose absorption conducted in older well nourished children in Gabon did not reveal an association between ascariasis and lactose intolerance, an outcome which is compatible with the findings of the present study.[163] The differing lactose absorption results could relate to the size of the intestinal lumen. Lactase is the most superficial of the brush border disaccharidases and Forsum *et al.* have suggested that the reduction in lactase levels observed in experimentally infected pigs is due to a direct mechanical effect of the *Ascaris* worms on the mucosal surface.[176] The smaller luminal space and mucosal surface area of pre-school children could conceivably result in greater direct mechanical contact between the worms and mucosal surface than in older children. It is important to emphasise that the results of the present study are applicable to early primary schoolchildren in Northeastern peninsular Malaysia and cannot be extrapolated to pre-school children.

In summary, the results of this study suggest that ascariasis exerts a marginal effect on small intestinal permeabilty of 7-8 year old schoolchildren in Northeastern Peninsular Malaysia, the impact of which on children of lower socioeconomic backgrounds is negligible in comparison to the cumulative effects of other noxious factors.

Chapter 4 Trichuriasis and occult colonic bleeding.

Trichuriasis and occult colonic bleeding

4.1 Aim and summary of study design

The aim of this study was to determine if trichuriasis predisposes to occult colonic bleeding in children of early primary school going age in the absence of the overt *Trichuris* Dysentery Syndrome. Stool samples of children in the second grade of Tawang school were examined for helminth eggs and occult blood. A comparison was made between *Trichuris* infected children and *Trichuris* negative controls with respect to the stool occult blood positive rate. The design provided for repeat assessment after treatment if an association between trichuriasis and occult bleeding was detected at baseline. Justification for the study lay not only in its implications on iron balance and chronic iron deficiency in infected children but also in the fact that *Trichuris* induced bleeding if it were shown to exist, provides indirect evidence of colonic mucosal injury.

4.2 Materials and methods

4.2.1. The guaiac impregnated paper test as a tool for detecting faecal occult blood.

Occult gastrointestinal bleeding can be detected by a number of methods. The most reliable method involves radiolabelling of autologous red blood cells with ^{51}Cr, reinjection of the radiolabelled cells intravenously into the subjects, and measurement of radioactivity in the total faecal output over a period of a few days.[286-288] While this method has the advantage of being very sensitive, it does not differentiate between colonic and extracolonic bleeding. More importantly it is an involved procedure which virtually precludes its use in field studies involving relatively large numbers. A number of chemical and immunological tests exist which are based on the detection of haem, haem derived porphyrins or intact heemoglobin in stool.[289-293] Among these tests, the one that has found most widespread use is the guaiac test.[294-301] A number of commercial guaiac test kits are available, most of which essentially consist of windows of guaiac impregnated paper mounted on test cards (figure 4.1). A thin smear of stool is made on the guaiac impregnated paper and two drops of a test reagent solution added to the faecal smear. The presence of occult blood results in the initially colourless guaiac acquiring a blue colour of varying intensity. In chemical terms, the colour change is due to the oxidation of a phenolic compound, in this case guaiac to a coloured quinone. Oxidation is catalysed by the pseudoperoxidase activity of haem in the presence of hydrogen peroxide which is a component of the test reagent. The use of the guaiac test has been popularised largely in mass screening programmes for colorectal carcinoma which involve periodic examination for faecal occult blood.[295-301] A number of randomised controlled trials have shown a decrease in mortality from colorectal carcinoma as a result of mass fecal occult blood testing employing the guaiac test, which at least to some extent attests to the effectiveness of the test.[295,298,299] The test has a number of advantages which made it suitable for the purpose of the present study. It was a convenient test for field studies and could be performed and read by someone with little training. It was also relatively inexpensive. There is a voluminous literature on the performance of the test, most of which relates to the detection of colorectal carcinoma and colonic adenomas.[294,297,300] In the interpretation of the data on the performance of the test it is important to distinguish between the performance of the test in detecting colorectal carcinoma or colonic polyps and its performance in detecting colonic bleeding. For the purpose of this study it is the latter which is more relevant.

Figure 4.1 A guaiac impregnated stool occult blood test card

Figure 4.2 Guaiac reactions showing (a) positive, (b) weakly positive and (c) negative reactions.

a b c

The presence of peroxidase activity in stool upon which the test depends is not caused exclusively by bleeding from the gastrointestinal tract. The presence of haem in rare red meat, and peroxidase like activity in certain uncooked vegetables and fruits may cause false positive results.[289, 302] This has led to dietary restrictions being incorporated into the protocols of trials on faecal occult blood screening for colorectal carcinoma[295,298] and have subsequently been encouraged but not overemphasised in recent North American recommendations on occult blood testing with the guaiac test.[300,301] In what is arguably the most detailed and carefully performed study on the effects of diet on faecal occult blood testing, Macrae *et al.* showed that rare red meat had the greatest effect of all the food groups in increasing the test positive rate.[302] When fresh fruit and uncooked vegetables were allowed but not rare red meat the occult blood positive rate did not increase appreciably.[302] Dietary factors are unlikely to have interfered in the testing of the cohort of primary school children in the present study. Red meat is not commonly consumed in large quantities by the local community. Furthermore, local mores dictate that meat is well cooked. It has also been shown that consumption of pharmacological doses of vitamin C, a reducing agent, interfere with the test and produce false negative results.[303] Again, this is unlikely to have impacted significantly on the present study. Aspirin and other non-steroidal anti-inflammatory drugs by inducing bleeding from the stomach or small intestine could theoretically produce false positive results. Although recent North American guidelines recommend that these drugs be discontinued temporarily before testing stool for occult blood,[300,301] there is some evidence to suggest that in practice this may not be necessary.[304,305] In any case this is unlikely to have been a problem in the current study as the main oral analgesic and antipyretic prescribed to children in the area is paracetamol which is obtained from small government run health clinics or private primary care practitioners.

For obvious reasons, bleeding in any part of the gastrointestinal tract, if it occurs at a sufficient rate, could potentially result in a positive guaiac test result. However, haem as it passes down the gastrointestinal tract is converted to other non-haem porphyrins which lack pseudoperoxidase activity.[306] Occult upper gastrointestinal bleeding is therefore less likely to be detected than colonic bleeding of the same magnitude.[307,308] This to some extent enhances the value of the guaiac test as a test of colonic bleeding. *In vitro* studies have shown quite clearly that the propensity of the guaiac test to produce a positive result is related to the concentration of haemoglobin in the faeces.[309-311] Haemoglobin concentrations in the order of 4mg/g of faeces or more have certainly been shown to produce a test positivity rate of 100% if tested within 48 hours.[309,311,312] Recent improvements in the test may have improved the sensitivity at lower concentrations as demonstrated in this study. Storage delay of more than 48 hours before testing appears to diminish the *in vitro* sensitivity of the test.[310] However rehydration of the faecal smear with a drop of water has been shown *in vitro* to restore the sensitivity of the test even after storage.[313] This has been corroborated by a careful *in vivo* study by Macrae *et al.*.[314] of patients with colorectal carcinomas and adenomas who underwent both radiolabelled red cell studies as well as guaiac faecal occult blood testing. Rehydration of slides improved the sensitivity of the guaiac test from 40% to 70% in the concentration range of 2-6 mg of haemoglobin per gram of faeces.[314] That rehydration improves the sensitivity was borne out in a large mass screening study by Mandel *et al.* who found that slide rehydration increased the percentage of positive tests from 2.4% to 9.8% which translated to an improvement in sensitivity of detecting colorectal carcinoma from 80.8% to 92.2%.[295] The mechanism of the improved sensitivity induced by rehydration is uncertain. It is thought to be due to dissolution of the dessicated haemoglobin and perhaps red cell lysis resulting in release of free haemoglobin, both of which enhance the peroxidase reaction. The improvement in sensitivity however is at the cost of some loss of specificity. In the dietary study by Macrae *et al.* already alluded to, rehydration resulted in a specimen positive rate of 6.3% among normal subjects on a full challenge diet consisting of daily consumptions of 250 g of red rare steak, raw vegetables and fruit, as opposed to a rate of 0% when the slides were not rehydrated.[302] As the consumption of red meat is far lower in the study area and even when it is consumed the meat is generally well cooked, the red meat factor is unlikely to have interfered significantly with the test despite rehydration of the slides. In the same study by Macrae *et al.* the positive rate in subjects who were on raw vegetables and fruits but not rare red meat increased from 0% to only 1.6% when rehydration was undertaken.[302] The amount of raw vegetables consumed per day in that study was in the order of 1 carrot, 1 small cucumber, and 2 servings of green salad;[302] quantities which would be several times in excess of the daily uncooked vegetable consumption of adults in North-eastern peninsular Malaysia, let alone children. In the large mass screening study by Mandel *et al.* the improved sensitivity in cancer detection gained by rehydrating the guaiac slides was at the cost of specificity decreasing from 97.7% to 90.45% and a decrease in the positive predictive value from 5.6% to 2.4%.[295] In screening programmes, even a relatively small decrease in the positive predictive value of the faecal occult blood test has a major impact as thousands of unnecessary and expensive follow-up investigations such as colonoscopy and barium studies would have to be performed. For the purpose of the current study increasing the sensitivity by rehydration was considered useful as it was anticipated that the rate of bleeding in the *Trichuris* infected population under study may be relatively low. Besides, any false positive effect would have been controlled for by the presence of a *Trichuris* negative group. Furthermore, post-treatment examination for faecal occult blood was planned to determine if any observed increase in the occult blood positive rate was in fact due to trichuriasis.

Intermittent bleeding of lesions or non-uniform mixing of blood in the stool could result in false negative results,[304,314,315] a problem which can at least partially be overcome by serial testing.[304] In screening for colorectal carcinoma, it is recommended that three slides are smeared with samples of stools on three different days.[301] Each slide generally has two windows which are smeared with samples from different areas of the stool.

A key question in the context of this study is the amount of colonic bleeding which would produce a positive guaiac slide test. In adressing this issue, it is pertinent to review the data on what constitutes normal gastrointestinal blood loss. Radiolabelled red cell studies have variously reported the upper limit of normal blood loss to be between 1 and 3 ml a day,[186,286,316] although some of this apparent blood loss may in fact represent excretion of ^{51}Cr in bile after elution from red cells.[317] The ideal test for this study should therefore be able to detect magnitudes of bleeding of the order of 2ml or more. Based on a daily stool weight of 150g in an adult and a haemoglobin concentration of 15g/dl, 1 to 3ml of blood loss a day broadly translates to a haemoglobin concentration of 1 to 3mg/g of stool. Similarly in a child with a stool weight of about 100g and a haemoglobin concentration of 11g/dl, detection of 1mg of haemoglobin per gram of stool represents a loss of 1ml of blood a day.

Earlier studies undertaken in the seventies using radiolabelled red cell studies as the gold standard reported low guaiac test sensitivities in the bleeding range of 4-10 ml of blood loss a day.[318,319] Strohlein *et al.* for example found that only 22% of specimens from patients with blood loss of 2-5 ml/day were occult blood positive by the guaiac slide test.[318] The earlier studies however included patients with upper gastrointestinal bleeding which as mentioned before is not reliably detected by the test.[318,319] Furthermore, rehydration of slides was not performed. Later studies conducted on patients with colonic lesions however showed improved sensitivities. Herzog *et al.* found that 52% of stool specimens from patients with colonic polyps with bleeding rates of as low as 1-2 ml/day were occult blood positive by the guaiac test; and this high positivity was observed without prior rehydration of the slides.[316] As mentioned previously, Macrae *et al.* increased the sensitivity of detecting 2-6 mg of blood per gram of faeces (as measured by radionuclide studies) from 40% to 70% simply by rehydrating the slides.[314] Doran and Hardcastle found that the chances of obtaining a positive guaiac slide test in colon cancer patients with a mean blood loss in the range of 0-5ml a day was only 17% while the sensitivity in the 5-10ml a day bleeding range was 53%.[304] However by testing on 3 consecutive days, the overall sensitivity of detecting cancer among patients in whom the median daily blood loss was a low 1.38 ml (range 0-38.9) was improved to 70%.[304] If stools were checked on 6 occasions the sensitivity rose to 90%. Rehydration of slides were not undertaken in that study.[304]

In addition to factors such as the amount of blood loss, intermittence of bleeding and non-uniform mixing of blood in stool, another factor which may have influenced the variability in the reported studies is the actual reading of the test slides. It has been shown that minimal training in the order of just an hour or so can improve the yield quite considerably.[320] This may explain why the guaiac test has been shown in at least one recent series to have a sensitivity of 89% in detecting symptomatic colonic carcinoma[321] even when slides were not rehydrated, while other studies done on similar patients have reported lower rates.[304,322] Training appeared to make a difference particularly in the interpretation of cases where the blue colour change was moderately positive.[320]

For the purpose of the present study, the most pertinent observation from studies comparing the guaiac test against radionuclide studies is that the test positive rate among patients with colonic lesions of even low bleeding rates were consistently higher than that of healthy controls.[304, 314, 316, 302] The positivity rate among controls was in fact virtually zero if slides were not rehydrated and even if they were rehydrated the rates were close to zero as long as the diet did not contain large amounts of rare red meat or substantial amounts of uncooked vegetables.[302] From the standpoint of the individual who is being screened for a life threatening condition such as colon cancer, a test which is only moderately sensitive may not be acceptable. This is less critical in the present study where the aim was simply to detect a difference in the propensity for colonic bleeding between the samples of two populations. Furthermore the sensitivity of the test can clearly be maximised by rehydration and serial testing. For reasons already alluded to, any trade off in terms of a loss in specificity is unlikely to significantly compromise the inferences. The evidence is quite clear that the guaiac slide test is a valid tool for the purpose of this study.

It has to be noted that much of the published data on the performance of the guaiac slide test has been undertaken on the Hemocult slide test a product of Smith Kline Diagnostics of Sunnyvale, California, USA. The kit used in the present study is the equivalent Coloscreen slide test which is a product of Helena Laboratories, Beaumont, Texas, USA. Direct *in vitro* comparisons between the the two kits indicate that they are of comparable sensitivities.[311] However there may have been some changes over the years in the composition of the guaiac impregnated paper. Furthermore differences in interpretation and storage conditions may influence the sensitivity and specificity of the test. It was for this reason that the *in vitro* performance of the kit was reassessed.

4.2.2 *In vitro* performance of the Coloscreen fecal occult blood slide test

The *in vitro* performance of the Coloscreen (Helena Laboratories, Beaumont, Texas, USA) guaiac slide test was tested by spiking test stool with varying concentrations of haemoglobin. Fresh stool passed within the preceding 24 hours was collected from healthy asymptomatic volunteers. The collected stool was mixed with distilled water and stirred with a glass rod into the consistency of a thick slurry. Anticoagulated whole blood from a healthy volunteer whose haemoglobin concentration was previously determined was added to aliquots of the homogenised stool slurry in volumes calculated to yield stool haemoglobin concentrations of 1, 2, 5, and 10mg/g of faeces. The final weight of each aliquot of stool with added blood was 100g. The blood was stirred well into the stool with a glass rod to attain uniform distribution. The Coloscreen slide (fig. 4.1) consists of a test card with a flap which when opened reveals two windows of guaiac impregnated paper. Within minutes of preparing the stool spiked with blood, a thin smear of the stool was made on each window and the flap closed. Another flap on the reverse side of the test card was then opened and two drops of the hydrogen peroxide containing developer solution were added to each window. A blue colour change within 30 seconds in either window was defined as a positive result. Ten test cards were prepared from samples at each faecal haemoglobin concentration and from an aliquot of stool with no added blood. The cards were developed and read by an observer who was blind to the faecal haemoglobin concentration. All the cards were read within a couple of hours of spiking the stool with blood. None of the control slides were positive while all the other slides were strongly positive (table 4.1).

It was anticipated however that in field studies the stool samples would be collected from the children, transported back to the institution and stored for up to 48 hours at -20°C before the faeces were smeared onto the slides and read. A delay of up to 48 hours between passage of the stool and the smearing of the faeces onto the card was anticipated. No delay however was anticipated between preparation of the slide and developing it. To simulate as closely as possible the maximum anticipated storage delay and conditions, the aliquots of stool were then stored at room temperature for 24 hours and at -20°C for a further 48 hours before undergoing repeat testing. The stool was allowed to thaw prior to repeat testing and a small quantity of stool was rehydrated on a glass slide with a couple of drops of distilled water before being smeared onto the slide. The procedure for developing the card was as before and was undertaken by the same observer who was again blind to the stool haemoglobin concentrations. At repeat testing, consistently positive results were obtained only at haemoglobin concentrations above 2mg/g of faeces (table 4.1). The reactions remained strong at concentrations of 5 and 10mg/g. The reactions at 2mg/g were weaker but unequivocally positive. Thirty percent of the slides at 1mg/g were very faint at repeat testing (table 4.1). Figure 4.2 illustrates examples of a strong reaction and a weak but unequivocally positive reaction.

Table 4.1 *In vitro* performance of the stool occult blood test kit.

	Rate of positive tests on:	
Haemoglobin concentration in spiked stool (mg/g)	Freshly prepared stool	Stool stored for 72 hours
0	0/10	0/10
1	9/10*	3/10**
2	10/10	9/10*
5	10/10	10/10
10	10/10	10/10

* The observed colour changes in the positive tests were unequivocal but generally weak.

** The observed colour changes in the positive tests were very faint.

4.2.3 Subjects and study protocol

This study was undertaken in September 1997 on a cohort of children attending the second grade at Tawang primary school. This was not the cohort which was subjected to the earlier investigation on intestinal permeability. Tawang school was selected because previous experience during the permeability studies had shown that the prevalence of intestinal helminthiasis is relatively high but there are sufficient numbers of uninfected children to serve as controls. Letters were sent out to the parents of all 117 children in the second grade of the school outlining the study and seeking consent for their children participating in the study. Although the parents of all but 2 children consented to the study, stool specimens were forthcoming in only 104 children who therefore constituted the study pool. These children were also the subject of a study on the effects of antihelminth treatment on short-term growth, the details of which are described in the next chapter. Each child was handed a small plastic container and spatula, with written instructions communicated to the parents to place a small amount of the child's stool in the container. Visits were made to the school on each morning for a week and stool specimens collected from whomever had brought it in on the day. The stool could have been passed at any time during the preceding 20 hours or so. The specimens were then transported back to the laboratory and examined on the same afternoon for helminth eggs or stored at -20°C and examined within the next 48 hours. After removing a small aliquot of stool for helminth egg examination the stool samples were stored in the refrigerator and occult blood testing generally done the next day. Examination for the presence of helminth eggs and quantification of the intensity of infection was as described in the previous chapter with the exception that concentration of stool samples prior to examination was dispensed with as previous experience had shown that the yield of hookworm infection was very low, and even when present it was almost never the sole infection. It was more economical in terms of time and effort to subject all stool samples to quantification for eggs with the Stoll's dilution method and to be particularly vigilant for hookworm eggs if the eggs of one or other of the two worms were observed. It is possible that this may have resulted in some underestimation of light hookworm infection but it is unlikely that moderate or heavy infection would have been missed. This is unlikely to have impacted on the study as the amount of bleeding in light hookworm infection has been shown conclusively to be low. Furthermore, the fact that the site of hookworm bleeding is in the upper gastrointestinal tract would make it unlikely to have been detected by the guaiac test. No specific dietary instructions were given because the dietary practices in the area as discussed earlier did not necessitate this.

Testing for faecal occult blood as described in the previous section was undertaken on all stool samples. It is emphasised that after thawing, a small quantity of stool was rehydrated with a couple of drops of distilled water before being smeared on the test slides and developed within minutes. The observer developing and reading the Coloscreen occult blood test result was blind to the infection status of the children. A result was defined as positive if a blue colour appeared in at least one of the windows of the slide.

If infection with *Trichuris trichiura* did cause occult colonic bleeding, the propensity to bleed would by reasonable extrapolation be highest in children with the highest intensity of infection. To maximise the sensitivity of detecting occult blood in this group, serial testing was undertaken in this subset and a control group. Children with heavy *Trichuris* infection as defined by an egg count of more than 10,000 eggs per gram (e.p.g.)[38] and an equivalent number of randomly selected uninfected children were asked to hand in three further samples of stool on three separate days. These samples were then examined for occult blood in the same way by an observer unaware of the infection status.

As any observed increase in the stool occult blood positive rate associated with trichuriasis would not necessarily equate to a cause and effect relationship between infection and occult bleeding, an interventional phase of the study was planned. In the original design, it was intended that stool occult blood and parasitological examinations would be repeated on all subjects 4 weeks after treatment with two doses of albendazole 400mg administered a week apart. Two doses as opposed to a single dose was chosen in view of the data showing that the efficacy of multiple doses in eliminating *Trichuris trichiura* was higher than with a single dose.[323] Although all infected children were in fact treated, the baseline results as discussed in the next section obviated the necessity for repeat occult blood examination.

Each child underwent a brief clinical evaluation consisting of questions pertaining to current diarrhoea and rectal bleeding, clinical examination for pallor, angular stomatitis, glossitis and finger clubbing. The examination was conducted by the author and the intention was simply to determine if any of the children were suffering from the overt form of any of the colitides including the *Trichuris* Dysentery Syndrome. Clearly mild degrees of anaemia may have gone undetected. Height and weight measurements were also undertaken as part of the study on short term growth that will be discussed in the next chapter.

4.3 Results

Fifty-three of the 104 children were girls. The mean age was 8.2 years (s.d. 0.3). There was little variation in the age as the children were in the same grade at school. The overall helminth infection rate was 68.3% (71/104). Sixty-one children were infected with *Trichuris trichiura*. Among *Trichuris* infected children, the mean *Trichuris* intensity was 7,200 e.p.g. (range 400 – 84,600). Hookworm infection was not detected in the cohort. There was no difference in the sex ratio or mean age between children infected with *Trichuris* and those free of *Trichuris* (table 4.2). Not surprisingly there was an association between

Ascaris infection and trichuriasis. Eleven children had heavy *Trichuris* infection as defined by a stool egg count of more than 10,000 e.p.g. of faeces. The median *Trichuris* intensity of the heavily infected subset was 18,000 e.p.g. (range 10,400-84,600). None of the children demonstrated any overt clinical features of active colitis. This was corroborated by the fact that none of the stool samples were diarrhoeic in consistency or contained visible blood.

Five of the 11 children with heavy trichuriasis complied fully with the request to hand in three further stool samples on separate days while the rest submitted 9 further samples between them. Table 4.3 shows the worm intensities in relation to the number of stool samples tested for occult blood as well as the sex and age distribution of the subset with heavy trichuriaisis. The stool recovery rate from 8 uninfected children who were randomly selected to act as controls for the children with heavy trichuriasis was somewhat lower. Only 13 additional samples were obtained from the 8 controls. There may have been a bias on the part of the field worker towards more vigorous urging of the children with heavy *Trichuris* infection to submit stool samples for retesting.

At baseline, all *Trichuris* negative children were stool occult blood negative. Only one of the the 61 *Trichuris* infected children was occult blood positive. A weakly positive reaction was observed in this child who had a light *Trichuris* infection of only 800 e.p.g. and an *Ascaris* intensity of 5,200 e.p.g. Testing of serial stool samples from children with heavy trichuriasis did not yield a single positive faecal occult blood result, whereas there was a single weakly positive result among the additional 13 samples obtained from the uninfected control group.

These negative results at baseline made the intervention phase of the study clearly redundant. Stool occult blood was therefore not repeated after treatment.

Table 4.2. Sex, age and *Ascaris* distribution in relation to *Trichuris* infection status.

	Trichuris positive (n=61)	*Trichuris* negative (n=43)
Mean age (S.D.), years	8.1 (0.2)	8.2 (0.2)
Number of girls, (%).	31 (50.8)	22 (51.2)
Number of *Ascaris* infections, (%).	43 (70.5)	10 (23.3)
Mean *Ascaris* intensity (range), e.p.g.	53,400 (0 - 488,000)	3,500 (0 - 47,200)

S.D - standard deviation. e.p.g. eggs per gram of faeces.

Table 4.3 Age, sex, intensities and number of stool specimens examined for occult blood of the subset with heavy trichuriasis.

Case.	Age (years)	Sex	*Trichuris* intensity (e.p.g.)	*Ascaris* intensity (e.p.g.)	Number of stool specimens examined for occult blood.[a]
1.	8.0	F	10,400	50,000	4
2.	8.3	M	10,800	52,800	4
3.	8.3	F	11,200	44,000	4
4.	7.9	M	12,000	144,800	3
5.	7.9	M	16,200	62,400	2
6.	8.4	F	18,800	111,200	3
7.	7.8	F	19,600	94,000	4
8.	8.0	M	28,400	96,800	4
9.	8.2	M	32,200	169,600	3
10.	7.9	F	35,200	80,000	2
11.	7.8	M	84,600	112,200	2
					Total 35

a- Total number examined includes the initial baseline specimen.

e.p.g. eggs per gram of faeces.

4.4 Discussion

The main finding of this study was that the rate of occult bleeding in *Trichuris* infected children was no higher than among uninfected controls. Only 1.4% (2/141) of the total number of stool samples examined were positive for occult blood; a rate which is lower than the false positive rates among Australian adults on unrestricted diets as reported by Macrae *et al.*[302] In interpreting these results it is important to bear in mind that the sample studied represents a free living population of 7-8 year old children who were not suffering from the clinical features of a full blown *Trichuris* Dysentery syndrome. The findings do not negate previous observations that rectal bleeding and anaemia occur in the full blown syndrome. Although the number of children with heavy infection as defined by the criteria of a WHO expert committee[38] was rather small, it was nonetheless larger than the numbers of children with heavy trichuriasis in the ^{51}Cr labelled red cell studies reported previously.[186,188,189,190] The findings of this study are compatible with the radiolabelled red cell study of Lotero *et al.* who found that faecal blood loss was within the normal range in all 6 children with trichuriasis who were studied.[189] The findings are also in agreement with that of a larger study of 203 Zanzibari schoolchildren by Stoltzfus *et al.* who used a semiquantitative test that detects and quantifies faecal haem and other porphyrins; a test suitable for detecting blood loss from both the upper and lower gastrointestinal tract.[187] Studying a population with a high prevalence of hookworm infection, Stoltzfus *et al.* found a weak correlation between *Trichuris* egg counts and faecal blood (r^2=0.05) on simple regression analysis. However, even this weak correlation disappeared when adjustments were made for hookworm intensity.[187]

Two other radiolabelled red cell studies by Layrisse *et al.* [188] in Venezuela and Cho in Korea[190] however showed a strong correlation between *Trichuris* worm burden and faecal blood loss. Layrisse *et al.* [188] in calculating the correlation between worm burden and blood loss computed the number of worms recovered after pharmacological expulsion while Cho[190] used stool egg density as the index of intensity. The density of *Trichuris* eggs in the Korean study was comparable to that of the 11 subjects with heavy trichuriasis in the present study. In the study by Layrisse *et al.* who reported stool egg densities in addition to direct worm counts, the consistency of the stool was very variable rendering egg counts an unreliable index and hence preventing a comparison of intensity with the current study. Combining the results of the studies by Layrisse *et al.* and Cho, 69% (11/16) of the children with heavy trichuriasis recorded faecal blood losses in the range of 2-8.6 ml a day.[188,190] It is unlikely that the Coloscreen guaiac test used in the present study would have consistently missed detecting faecal losses of blood in the magnitude of 2-8 ml a day. The *in vitro* sensitivity of the Coloscreen test in detecting haemoglobin concentrations above 2mg/g of faeces approached 100% even after storage; the high sensitivity probably attributable to the rehydration of stool specimens prior to testing (table 1). It is acknowledged that the *in vivo* sensitivity for reasons already discussed in section 4.2.1 may be lower. Nonetheless, even if the test sensitivity is assumed for argument's sake to be a conservative 30%, at least 10 positive results would have been expected among the 35 specimens obtained in this study from children with heavy trichuriasis if faecal blood losses were indeed in the order of 2-8 ml/day.

As mentioned in chapter 1, close inspection of the data of Layrisse *et al.* reveals that 6 of the 9 children had watery diarrhoea and an iron deficiency anaemia.[188] The selection criteria of the study subjects was not specified and it is quite possible that the sample included children with the overt *Trichuris* Dysentery Syndrome. If so, the findings cannot be extrapolated to the majority of infected children in the community. In the Korean study, the correlation coefficient between *Trichuris* egg output per day and faecal blood loss was reported to be strong (r=0.67), but no details were given on the confidence interval or the level of statistical significance.[190] Reanalysis of the Korean data by this author using the SPSS statistical package revealed that by conventional terms the correlation was not statistically significant ($p=0.1$). While this does not entirely invalidate the conclusions of the study it does emphasise that caution has to be exercised in interpreting the results of small studies in which a change in one or two values can substantially alter the calculated correlation coefficient.

The small number of community based studies on anaemia and trichuriasis, have, on careful review, not consistently shown that trichuriasis is associated with iron deficiency. Although heavy trichuriasis is associated with mild anaemia,[136-138] none of the studies in which indices of body iron were measured showed conclusively that the anaemia was primarily due to iron deficiency.[136,138] In the study done almost two decades ago by Greenberg *et al.*, children with more than 10,000 *Trichuris* eggs per gram of faeces had significantly lower mean haemoglobin levels than uninfected or lightly infected children but there was no difference between the groups in serum iron or transferrin saturation.[136] A Jamaican study reported more recently by Ramdath *et al.* showed lower iron stores in children with heavy trichuriasis than in controls, but the difference did not quite reach conventional statistical significance.[138] The studies however had the limitation as in the present study of relatively small numbers of children with heavy trichuriasis. More importantly, the studies lacked an interventional phase to determine if removal of *Trichuris* infection alone reversed the albeit mild anaemia or had an effect on the iron indices. This is particularly important in communities where dietary iron intake may be marginal.

The results of this study do not provide any evidence to suggest that *Trichuris* induced blood loss is the mechanism of the anaemia observed in heavy trichuriasis. In this context however it is relevant to consider a number of possibilities. Firstly, the mild anaemia observed in the reported studies may represent an anaemia of chronic disease associated with chronic colonic inflammation induced by heavy trichuriasis rather than an iron deficiency anaemia. Secondly, the absence of stool occult blood does not necessarily exclude the possibility that minute amounts of blood are consumed directly by the worm itself. In the case of hookworm infection it has been shown that the worm draws in a mucosal plug of the intestinal villus into the buccal cavity which results in capillary bleeding.[35] Some of this blood is consumed by the worm but bleeding continues after the hookworm has moved on to another site. In the case of the *Trichuris* worm the narrow anterior end where the mouth is located is embedded within an epithelial syncitium. Burrows and Lillis using the benzidine chemical test found traces of blood in the intestine of almost all *Trichuris vulpis* worms recoverd from dogs.[324] The negative results of the present study do not discount the possibility that heavy *Trichuris* infections induce loss of minute amounts of blood into the mucosa which is almost entirely consumed by the parasite and does not spill into the lumen. It is conceivable that the net amount of blood ingested would be considerable if the worm burden is in the order of several hundred worms. A cumulative loss of even 1 ml a day in a growing child may produce iron deficiency if the dietary iron intake is marginal. It is also possible that if the magnitude of blood loss in children with heavy infection resulted in a stool haemoglobin concentration in the order of 0.5-1mg/g of faeces, the sensitivity of the guaiac test and the relatively small number of children with heavy trichuriasis in the present study may not have resulted in detection of the effect.

The full blown *Trichuris* Dysentery Syndrome results in growth retardation, anaemia and dysentery. It is still unclear whether *Trichuris* induced disease is a continuum at the extreme end of which is the *Trichuris* Dysentery Syndrome or whether there is a threshold or multifactorial setting at which point the disease process is abruptly triggered resulting in damage to the colonic mucosa, bleeding, anaemia and the ensuing systemic manifestations. It is not unreasonable to presume that if the former were the case, subclinical colonic mucosal injury may be present in a substantial proportion of children with moderate to heavy intensities of infection who do not manifest the full blown syndrome. This study was to a large extent an effort to look for evidence of subclinical colonic mucosal injury based on the presumption that if the full blown syndrome caused overt bleeding then occult bleeding may be a consequence of a milder form of the disease. While the results do not provide any evidence for subclinical colonic injury, the possibility cannot yet be excluded. Occult bleeding may simply not be a sensitive enough method of detecting mild mucosal injury.

In summary, the results of this study suggest that in the absence of the overt *Trichuris* Dysentery Syndrome, occult colonic bleeding is unlikely to be a high risk event in *Trichuris* infected children of early primary school going age in North-eastern peninsular Malaysia. The possibility that it is a low risk event can only be excluded by testing even larger numbers of children with heavy infections.

Chapter 5 Intestinal helminthiasis and growth.

Intestinal helminthiasis and growth.

5.1 Aim and summary of study design:

The aim of this study was to determine the effect of intestinal geohelminth infection on statural and ponderal growth among early primary school children in Northeastern Peninsular Malaysia. To this end an interventional study was undertaken between September 1997 and January 1998 on the same cohort of children at Tawang primary school involved in the study on trichuriasis and occult colonic bleeding (chapter 4). Baseline evaluation included stool examination for helminth eggs, and measurement of height and weight. Infected children were treated with an antihelminthic drug and both infected and uninfected children were reevaluated 14 weeks later. The main outcome measurements were changes in weight, height, age adjusted height and weight, as well as weight for height. The study was based on the assumption that if helminthiasis adversely affected growth and nutritional status, removal or reduction of the worm burden would result in a degree of accelerated growth and improved nutritional state relative to the control group of uninfected children. Given the duration of follow-up it was hypothesised that treatment of infection would at least be associated with gains in weight for age and weight for height if not height for age.

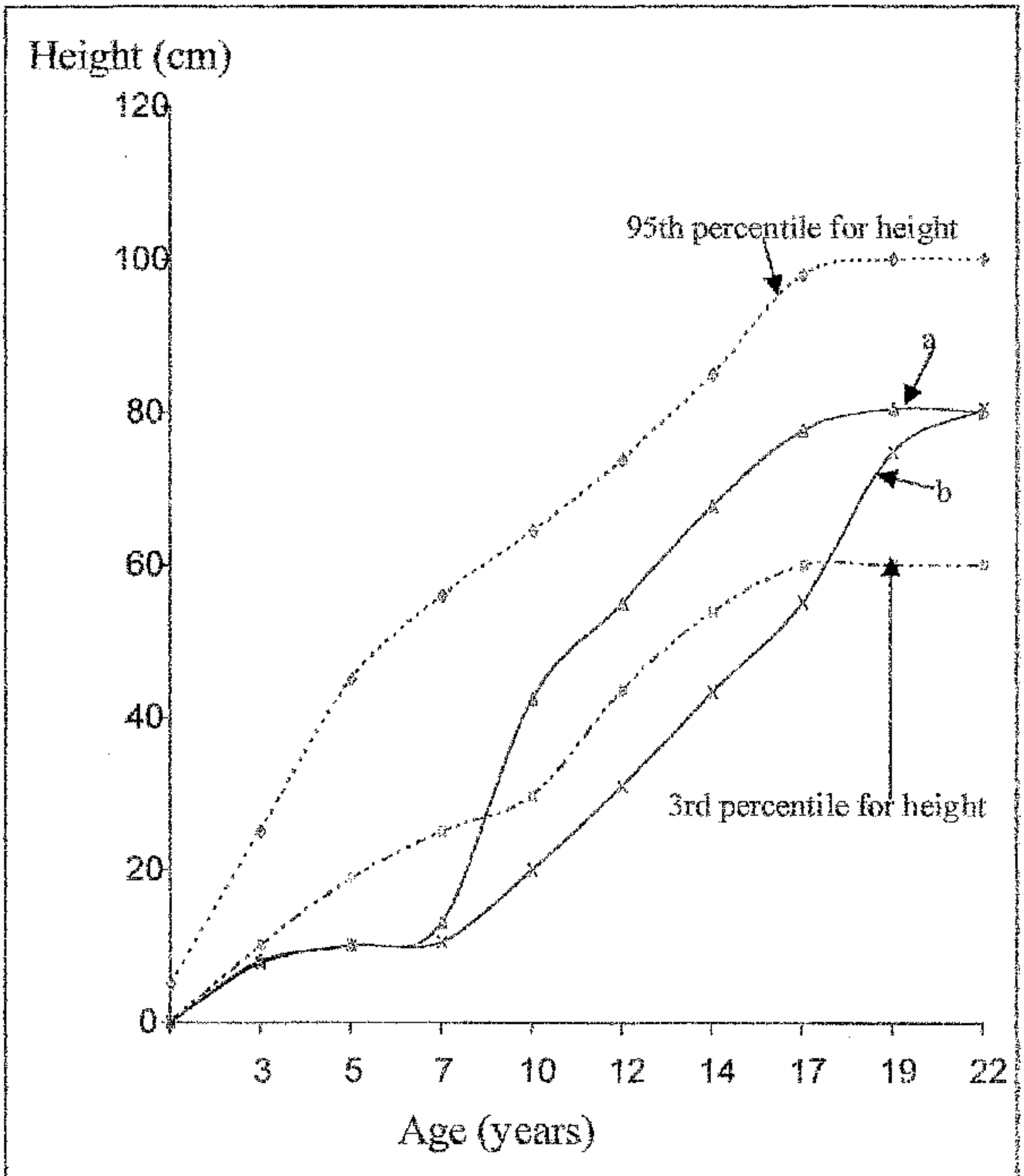
5.2 Growth faltering and the concept of catch-up growth.

Growth impairment in children has many causes including malnutrition due to poverty, chronic illness, and even emotional deprivation. In the third world, lack of food and recurrent infections such as diarrhoeal diseases are thought to be the important causes of failure to attain maximum growth potential.[325-327] While there is good evidence that food availability is an important determinant of growth in the third world,[327,328] controversy surrounds the importance of infections such as diarrhoeal diseases in the genesis of chronic growth faltering.[327,329,330] Evidence from studies on migrant populations,[331,332] adopted children[333-336] and the secular trends observed in societies which have experienced significant improvements in socioeconomic conditions suggest that a major part of the failure to attain maximum height potential is attributable to environmental factors.[337,338]

A child exposed to ill health or malnutrition brought about by socioeconomic factors may be stunted or wasted.[129] Stunting is the result of slowing of linear growth over a period of time resulting in a low height for age.[129,339] Wasting on the other hand reflects lowered fat and tissue mass and is reflected by a low weight for height. The indices of height for age and weight for age are derived by relating the height and weight of the child to the average values of a reference population for a given age.[129]. Similarly weight for height is also derived by relating the weight of the child to the average weight of a reference population for a given height.[129] Malnourished children may be stunted or wasted to varying degrees.[129] While stunting is almost invariably a chronic process which occurs when the growth restricting factor continues over a prolonged period of time, wasting may occur rapidly in the course of an acute insult such as acute shortage of food or an acute illness.[129,340] Wasting which occurs acutely may just as rapidly be reversed if the causative factor is short lived and there is access to adequate nutrition.[341]

The design of the present study is based on the assumption that removal of a growth restricting factor results in a period of accelerated growth which acts to return the child to the normal growth trajectory. This is one of the responses which constitutes the phenomenon of catch-up growth.[325,342-344] Figure 5.1 illustrates the two ways in which catch up growth occurs.[342,344] During the period of growth faltering, the height velocity falls and the growth curve is deflected to the right. If the growth restricting factor is terminated, height velocity may increase to supranormal levels as illustrated in figure 5.1(curve a). The child tends to be restored towards its original height centile and growth then proceeds normally until maturation. Alternatively, the height velocity improves towards normal but maturation is delayed permitting a more prolonged period of growth which also results ultimately in normal adult height. (figure 5.1, curve b). In reality a mixture of these two mechanisms seem to operate.[342-344]

Figure 5.1 Catch-up growth due to (a) accelerated growth and (b) maturational delay.



The potential for catch up growth to some extent depends on the maturation age as reflected by bone age.[340,344] Stunted children are not only small for age but are also generally retarded in bone age. If bone age is markedly delayed, more time is available for catch up growth before maturation and the final adult height is attained. Catch up growth may be complete or incomplete and seems to depend on a number of factors such as the age at which the restricting factor is terminated, the cause, severity and duration of the offending stimulus and the adequacy of nutrition.[325,340,342,345]

The literature is replete with examples of catch up growth. The term catch-up growth was first introduced into the literature by Prader *et al* who described 5 children stunted by illness who demonstrated accelerated growth following treatment.[346] Children with coeliac disease who are often growth retarded at diagnosis, experience gratifying growth acceleration after institution of a gluten free diet; the majority of chidren attaining normal height for age within 1 to 3 years.[347-351] The degree of wasting seems to correct even more rapidly. Normal weight for height is often restored within 3-6 months and may even overshoot by the end of the first year after initiation of a gluten free diet.[350] There is evidence to suggest that catch up growth in coeliacs occurs in bursts of growth acceleration and that the frequency and widths of these bursts are higher during the early period after institution of the appropriate diet.[352] Although most coeliac children are diagnosed during the first three years of life, most children who are diagnosed later in the pre-pubertal period also seem to complete catch up growth.[349-351]

Catch-up growth has been observed in subsets of children and adolescents treated for inflammatory bowel disease. Aggressive nutritional supplementation in children and adolescents with Crohn's disease has been shown to reverse growth failure. A significant proportion of these cases showed accelerated growth.[353-359] Similar trends have been demonstrated in children who have undergone resection of diseased segments of bowel.[360,361] There is also a suggestion that colectomy results in reversal of growth failure in ulcerative colitis.[362-364] In one study, height velocities increased dramatically following colectomy in almost half the children while in the other half the declining trend in height velocity was halted.[364]

Significant catch-up growth has been demonstrated after hormonal replacement in endocrine deficiency syndromes. In growth hormone deficiency states, accelerated height velocities were observed soon after the initiation of hormone replacement therapy.[365-369] Although catch up growth proceeds into the pubertal period, the velocity of growth is somewhat attenuated in later years.[367,369] Complete catch up is often attained if growth hormone treatment is started early. Similarly, dramatic growth acceleration is often observed after thyroxine replacement in congenital hypothyroidism, although catch-up may be incomplete.[370-372]

It was hoped that growth failure in chronic renal failure would be reversed by renal transplantation. Unfortunately the degree of catch-up growth is modest and transplantation has a significant influence on growth only in children under 6 years of age.[373-376] Failure to achieve better catch-up growth may be partially related to the post-transplantation immunosuppressive regime which includes steroids. Recent evidence suggests that growth hormone administration[378,379] and aggressive enteral nutrition[379] may enhance growth in these patients. A number of studies undertaken on children treated for acute lymboblastic leukaemia have shown that growth faltering occurs during treatment.[380-382] There is evidence to suggest that a degree of accelerated growth occurs after cessation of chemotherapy but intracranial radiotherapy significantly blunts this response perhaps by affecting the growth regulatory centre in the brain.[381,383]

Acute diarrhoeal illness in infants and young children may cause acute weight loss. Recovery from the acute diarrhoea is accompanied by accelerated weight gain.[329,384] The rate of weight gain may be increased by early and vigorous nutritional intervention.[384]

Increased growth velocity has been demonstrated among immigrants or refugee children who have been translocated to more affluent countries.[331,332,335,336] In one particular study however, rapid pre-pubertal catch-up growth occurred at the expense of early puberty and lower final adult heights than the average of the local population.[335] Rapid catch-up growth has also been demonstrated in children from deprived homes who have been adopted into affluent homes within the same country.[334,385]

Catch-up growth has been recorded in a number of other groups including children who have undergone surgical correction of vesicoureteric reflux,[386] institutionalised children with severe neurological disability following nutritional intervention,[387] and in children on systemic steroids following substitution with an alternative immunosuppressant such as cyclosporin.[388]

As discussed in chapter 1, dramatic growth spurts have been shown to occur following treatment of the *Trichuris* Dysentery Syndrome.[87-89] It was hoped that the present study would shed some light on whether treatment results in a measure of accelerated growth in children with modest burdens of *Trichuris trichiura* who do not manifest the full blown *Trichuris* dysentery syndrome. This group in fact constitutes the vast majority of children infected with trichuriasis.

It does not seem unreasonable therefore to assume that if helminth infection is an important determinant of growth faltering, treatment would result in accelerated growth at least in comparison with a local control group. It is acknowledged however that accelerated growth following removal of a growth resticting factor is not invariable. Failure to detect accelerated growth following antihelminth treatment would therefore not entirely exclude the possibility that helminthiasis impairs growth. This caveat notwithstanding, the detection of a growth spurt following treatment would certainly be strong evidence that helminthiasis does impair growth.

Catch-up growth has been measured in terms of increase in height for age, weight for age or weight for height over a period of time. The aforementioned parameters are computed from growth curves of a reference population. One such set of reference growth curves were developed by the National Center for Health Statistics and the Centers for Disease Control based on measurement data obtained from a North American population.[389] This has been widely accepted as an international reference dataset for the purpose of monitoring the growth of individual children and assesing the nutritional status of populations. Weight for age and height for age may be expressed as a percentage of the median of the reference population for a particular age or in terms of a Z-score, which is essentially the standard deviation units from the reference median for a particular age. A child whose height is one standard deviation below the median of the reference population for his age would therefore have a height for age Z-score of -1.0 while a child whose height is one standard deviation above the median of the reference population would have a Z-score of +1.0.

The ideal control group would have been an infected group randomly allocated to receiving a placebo. However the same reservations expressed in the context of the permeability studies regarding the acceptability to the community of placebo treatment precluded the ideal design. Uninfected children therefore served as a compromise control group. Socioeconomic status was to some extent adjusted for by obtaining information at the start of the study.

5.3 Background cross-sectional and longitudinal data on the relationship between helminthisis, nutritional status and growth in North-eastern Peninsular Malaysia.

Prior to this interventional study, preliminary cross-sectional and longitudinal observations had been undertaken on the relationship between the helminth infection and anthropometric parameters on the children from Tapang and Tawang schools who had participated in the earlier intestinal permeability studies (chapter 3). The baseline infection prevalence rates and intensities of those cohorts have been summarised in tables 3.1a and 3.1b of chapter 3.

Examination of the preliminary observational data by this author revealed that helminth infection *per se* was not associated with a lower weight for age, height for age or weight for height.[390] Ascariasis was associated with stunting at Tapang school but not at Tawang school. Heavy ascariasis (>50,000 e.p.g) on the other hand was consistently associated with stunting but not wasting. No significant association could be detected between *Trichuris* infection and stunting or wasting at either school. There was a weak and inconsistent negative correlation between *Ascaris* intensity and height for age. Regression analysis after log transformation of the egg counts made no appreciable difference to these trends. These preliminary observations at first glance therefore suggested that ascariasis, and in particular heavy ascariasis, was associated with stunting. Needless to say there are obvious limitations in interpreting the results of observational data.

As mentioned above, the earlier Tawang cohort first underwent height and weight measurements during the course of the permeability study in 1995. This cohort was followed-up 16 months later, the infection status re-evaluated and measurements repeated.[391] Infected members of the cohort were then treated at periodic intervals over the subsequent year, at the end of which they were evaluated once more. During the year of intervention, there was no difference in growth rates between the infected group who received periodic treatment and uninfected controls.[391] In particular, children with heavy ascariasis at the beginning of the periodic intervention did not demonstrate any evidence of accelerated growth. Subset analysis however revealed growth gains among treated girls but not boys. There was also a suggestion that infected children who were rendered worm free at the final evaluation may have benefitted.[391]

However interpretability of the aforementioned data was limited for a number of reasons. Firstly, the previous intervention had altered the natural intensity patterns of helminthiasis. At the beginning of the one year intervention period, the intensities were considerably lower than at the first encounter 16 months before. These somewhat inconclusive findings justified another study on a fresh cohort who had not been subjected to any previous systematic antihelminth treatment programme. The experience from previous studies also confirmed the limited efficacy of single 400mg doses of albendazole in treating trichuriasis. This influenced the choice of dosage in the subsequent study.

5.4 Materials and methods

5.4.1 Subjects and study protocol.

The subjects of this study cosisted of the same cohort of children who participated in the investigation of occult colonic bleeding (chapter 4). As described in the previous chapter, all 117 children in the second grade of Tawang school in September 1997 were targetted for the study but only 104 children handed in stool samples for parasitological exmaination. One of the 104 children moved to another area soon after collection of the baseline stool sample and was excluded from analysis. Stool examination for helminth eggs was undertaken as described in the methodology section of Chapter 4.

The heights and weights of all children were measured at baseline in September 1997. Vertical height and weight were measured with the children in school uniform but without shoes. Weight was measured using a portable weighing machine (Tanita, model 1567, Japan) which electronically displays a reading to the nearest 0.2kg (fig.5.2a). The weighing machine was calibrated periodically with standard weights. Standing height was measured to the nearest 0.1 cm with a vertical stadiometer (fig. 5.2b). The stadiometer was placed at the same site for all measurements and the measurements were undertaken by a single observer who was blind to the results of the stool parasiological examination. The accuracy of the stadiometer was checked before measuring each batch of students for the day with standard lengths. The intra-observer coefficient of variation of the weight and height measurements was based on two sets of measurements of 30 children. The repeat measurements were done in random order to avoid bias. There was no variation in the weight measurements. The coefficient of variation of height measurements was 0.25%.

Baseline height and weight measurements were undertaken on a single morning to minimise day to day variation. There were however a few absentees who were measured on the subsequent 2 days. All children infected with one or more helminths were treated with two 400 mg doses of albendazole syrup administered under supervision. The two doses were administered a week apart.

Repeat height and weight measurements were undertaken on all children 14 weeks after the second dose of albendazole. As at baseline all measurements were undertaken on a single morning except in the case of a handful of absentees who were measured over the subsequent days. Repeat stool examination for helminth eggs was undertaken over a two week period after the follow-up height and weight measurement.

Height for age Z-score (HAZ), weight for age Z-score (WAZ) and weight for height Z-score (WHZ) were computed with the aid of the anthropometry programme in Epiinfo version 6 (World Health Organization, Geneva, Switzerland and the Centers for Disease Control, Atlanta, U.S.A.). The programme uses normalised growth curves constructed by the National Center for Health Statistics (NCHS) and Centers for Disease Control task force based on data obtained from the Fels Research Institute and the US Health Examination Surveys of the NCHS. The dates of birth of the study subjects were obtained from the school records. The date of birth in the school records is transcibed directly from the birth certificates when the child is first admitted to school and is generally considered to be reliable.

A composite socioeconomic score for each child was computed based on household income, father's occupation, number of dependents in the household, type of housing, facilities within the house and the type of vehicle owned by the family; information which was volunteered by the parents and guardians of the children (appendix). Based on the score, each child was categorised as belonging to one of three socioeconomic groups; group one representing the most advantaged and group three the least. The categorisation was arbitrary and less than perfect beacause verification of the volunteered information was logistically difficult and the validation of any socioeconomic scoring system is in itself notoriously difficult. Practical considerations in a given area often dictate the system used.[392] In this context, it should be noted that the system of socioeconomic categorisation used in the earlier study on intestinal permeability (chapter 3) was different and direct comparison with the earlier study in terms of the frequency distribution of the socioeconomic categories is not valid. Informed consent was obtained from the parents and guardians of all children and the study was approved by the research and ethics committee of the School of Medical Sciences, Universiti Sains Malaysia.

5.4.2 Statistical analysis.

As distribution of some of the anthropometric variables was slightly skewed, medians and quartile deviations (Q.D.) were the summary statistics generally used to describe the data sets.

Comparisons were made between helminth infected and helminth free children with respect to the various anthropometric parameters at baseline. Comparisons between the two groups were also made with respect to the change in the various anthropometric parameters over the 14 week follow-up period. As the assumptions required for the use of parametric tests were sometimes not satisfied, the non-parametric Mann Whitney U test was generally used for the purpose of comparing groups in terms of anthropometric parameters. The Students t-test was used only to compare the groups in term of age. The χ^2 test was employed to detect an association between socioeconomic status and helminth infection status.

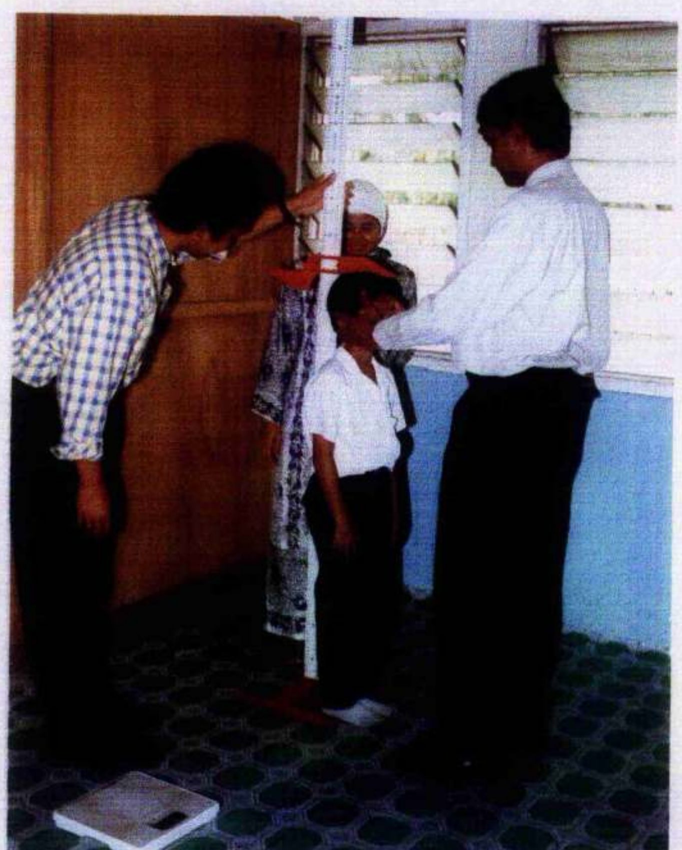
The relationship between intensity of infection as defined by log transformed egg counts (ie log egg count +1) and growth parameters was initially assessed by simple linear regression analysis. Stepwise multiple regression analysis was then used to examine the influence of log transformed intensity and a number of other factors such as age, sex, socioeconomic status, and baseline measurements on growth.

Mantel-Haenszel stratified analysis was used to crudely assess the independent effect of treatment on growth. The approach taken was to collapse the growth parameters which were continuous varables into dichotomous variables by arbitrarily dividing at the 50th percentile and then assessing the association between worm infection and the upper 50% of values after adjusting for sex and socioeconomic status. Statistical computations were undertaken with the aid of the Epiinfo version 6 (World Health Organisation, Geneva, Switzerland and the Centre for Disease Control, Atlanta, U.S.A) and SPSS for Windows statistical software programmes. Two tailed significance values were quoted at all times.

Figure 5.2 Measurement of a) weight and b) height.

(a)

(b)



5.5 Results

5.5.1 Demographic and worm infection data.

Of the 103 children in the study cohort, 71 were infected with *Ascaris lumbricoides* and/or *Trichuris trichiura*. No hookworm infection was detected. The children were all Malays and just over half of them (52/103) were girls. The variation in age was small as reflected by a standard deviation of only 0.2 years in the cohort as a whole. One child was categorised into socioeconomic group one, 71 to socioeconomic group 2 and the rest to socioeconomic group 3. The socioeconomic categorisation was arbitrary and discriminated within the cohort but is unsuitable for comparisons with children of other communities. To put the picture into perspective, children in socioeconomic group 2 came from homes with a mean monthly income of RM275 (US74).

Stunting as defined by an HAZ of less than -2.0 was present in 31.1% (32/103) of subjects. However none of the subjects were wasted as defined by an WHZ of less than -2.0. As expected, baseline HAZ was significantly lower in children of socioeconomic group 3 (median -1.98; QD 0.48) than in children of socioeconomic groups 1 and 2 combined (median -1.46; QD 0.56; p=0.01). There was however no difference in baseline WHZ between the groups (median 0.23; QD 0.45 vs median 0.13; QD 0.55; p=0.41).

As shown in table 5.1, infected and uninfected groups were comparable in terms of sex and age distribution but children of socioeconomic group 3 were overrepresented in the infected group (table 5.1).

Forty-three children harboured mixed infections of *Ascaris lumbricoides* and *Trichuris trichiura*. Twenty-two children had heavy ascariasis (>50,000 e.p.g.), 11 children had heavy trichuriasis (>10,000 e.p.g.) and 9 were heavily infected with both species. Fifteen children were only lightly infected as defined by an *Ascaris* intensity of less than 5,000 e.p.g. and a *Trichuris* intensity of less than 1,000 epg. The *Ascaris* and *Trichuris* infection intensities in the infected group as a whole are shown in table 5.1. The mean *Ascaris* intensity among children positive for the worm (n=53) was 64,300 e.p.g. (range 400-488,000) while the mean *Trichuris* intensity among *Trichuris* positive children (n=61) was 7,200 e.p.g. (range 400-84,600).

At follow up, the *Ascaris* prevalence rate in the infected group was found to have halved and the intensity of infection had decreased considerably (table 5.1). Although the *Trichuris* prevalence rate in comparison had fallen by only 7.3%, the mean intensity had decreased by almost 40% indicating that treatment had substantially reduced the *Trichuris* worm burden (table 5.1). At follow-up, 8 of the treated children had heavy *Ascaris* infection and 10 had heavy trichuriasis. Thirteen children who were uninfected at baseline were found to harbour worms at re-examination but none of them were heavily infected (table 5.1).

5.5.2 Anthropometric data:

Comparison between infected group and uninfected control group.

There was no significant difference between the infected and uninfected groups with respect to baseline HAZ, WAZ or WHZ (table 5.2). Over the follow-up period however, infected children all of whom received antihelminthic treatment registered higher growth rates in terms of increase in raw weight and raw height (fig. 5.3 and 5.4). This trend was also reflected in terms of changes in HAZ, WAZ, WHZ.(Table 5.2). Although the median WAZ and WHZ fell in both infected and uninfected groups, the fall was significantly greater in the uninfected control group (table 5.2).

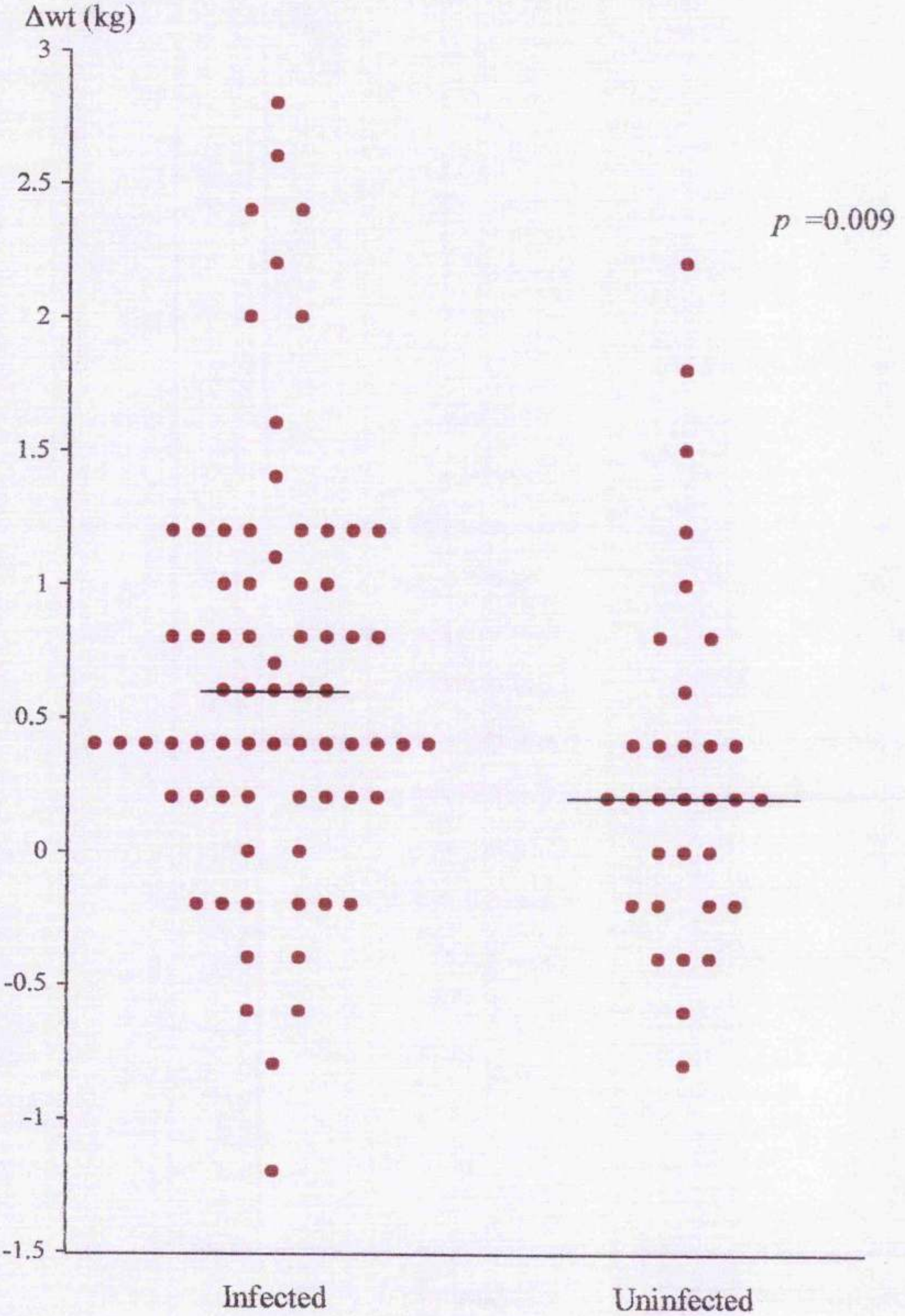
Table 5.1 Demographic data, infection prevalence and intensity.

	Uninfected (n=32)	Infected (n=71)
Mean age (S.D.), years	8.2 (0.2)	8.2 (0.2)
Number of girls (%)	17 (53.1%)	35 (49.3%)
Number in socioeconomic group 3 (%)	3 (9.4%)	28 (39.4%)[a]
Ascaris prevalence rate, %:		
baseline	-	53/71 (74.6%)
follow-up	2/29 (6.9%)	26/66 (39.4%)
Trichuris prevalence rate, %		
baseline	-	61/71(85.9%)
follow-up	11/29 (37.9%)	52/66 (78.8%)
Mean *Ascaris* intensity (range), epg X 10^3:		
baseline	-	48.0 (0.0-488.0)
follow-up	0.1 (0-34.2)	15.7 (0.0-226.4)
Mean *Trichuris* intensity (range), epg X 10^3:		
baseline	-	6.2 (0.0-84.6)
follow-up	0.7 (0-6.4)	3.8 (0.0-20.4)

epg - eggs per gram of faeces.

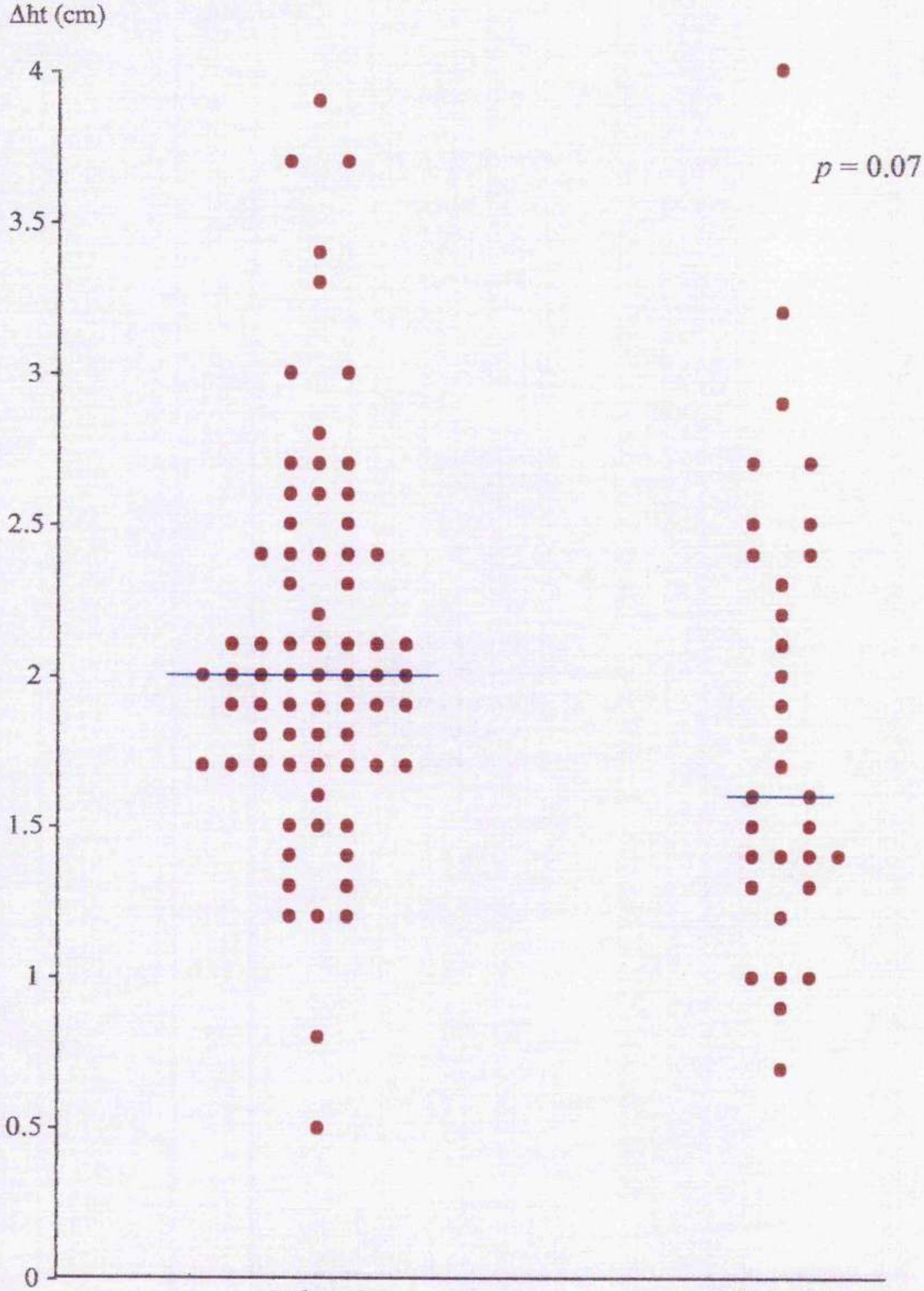
a - Significant difference; p=0.005 (χ^2 test)

Figure 5.3 Change in weight (Δweight) over follow-up period.



* Horizontal line represents median

Figure 5.4 Change in height (Δheight) over follow-up period.



* Horizontal line represents median

Table 5.2 Baseline nutritional status and growth over 14 weeks

Group	Uninfected (n=32)	Infected (n=71)	*p value* [b]
Baseline HAZ[a]	-1.60 (0.56)	-1.56 (0.54)	*0.37*
Baseline WAZ[a]	-1.19 (0.51)	-1.25 (0.46)	*0.47*
Baseline WHZ[a]	0.16 (0.49)	0.22 (0.54)	*0.66*
ΔHAZ[a]	0.02 (0.08)	0.10 (0.10)	*0.03*
ΔWAZ[a]	-0.14 (0.09)	-0.04 (0.11)	*0.003*
ΔWHZ[a]	-0.20 (0.19)	-0.15 (0.20)	*0.03*
Δheight, cm[a]	1.7 (0.5)	2.0 (0.4)	*0.07*
Δweight, kg[a]	0.2 (0.4)	0.6 (0.5)	*0.008*

HAZ- Height for age Z-score
WAZ- Weight for age Z-score
WHZ- Weight for height Z-score
Δ= Change in parameter over follow-up period, (parameter at follow-up – parameter at baseline).
a=median (quartile deviation in parentheses) b=Mann-Whitney U test

Mantel-Haenszel stratified analysis to examine the effect of treating infection independent of sex and socioeconomic status.

After collapsing the growth parameters into dichotomous string variables as outlined earlier (page 158), stratified analysis was used to examine the association between treating worm infection and the upper 50% of values of change in HAZ, WAZ, WHZ raw height and weight respectively. The summary odds ratios defining the association are shown in table 5.3. Treatment of infection was associated with gains in WAZ, WHZ and raw weight independent of sex and socioeconomic status. The association between worm infection and the upper values of changes in WAZ, WHZ and raw weight was strongest in the stratum of female children within socioeconomic group 2, the odds ratios being 8.7 (p=0.003), 26.3 (p=0.0004) and 6.0 (p=0.01) respectively. In contrast, within the stratum of male children drawn from socioeconomic group 2, no association between infection and growth gains were detected. There were too few non-infected children in socioeconomic group 3 to permit meaningful inferences about the association between worm infection and growth within that stratum.

When sex and socioeconomic status were each in turn defined as the main independent variables, and infection maintained as a confounder, female sex was found to be independently associated with the upper 50% of values of raw height (odds ratio 2.8; 95% C.I. 1.2-6.6). Socioeconomic status did not have an independent effect on any of the growth parameters.

Table 5.3 Association between baseline infection status and improved growth after adjusting for sex and socioeconomic status

	Odds ratio[b]	95% C.I.	*p value*
ΔHAZ[a]	2.4	0.9-6.3	*0.11*
ΔWAZ[a]	4.0	1.5-10.5	*0.006*
ΔWHZ[a]	3.9	1.4-10.5	*0.01*
ΔHeight[a]	1.7	0.6-4.0	*0.45*
ΔWeight[a]	3.8	1.4-10.7	*0.01*

HAZ- Height for age Z-score
WAZ- Weight for age Z-score
WHZ- Weight for height Z-score
Δ= Change in parameter over follow-up period, (parameter at follow-up – parameter at baseline).
95% C.I= 95% Confidence interval.
a- Parameters converted into dichotomous variables by arbitrarily dividing at 50th percentile.
b- Mantel-Haenszel weighted odds ratio defining the association between worm infection and the upper 50% of values of increase in growth parameters.
c- significance value corresponding to the Mantel-Haenszel summary chi square.

Simple regression analyses between intensities of infection and anthropometric parameters.

Simple linear regression analysis was conducted to examine the extent to which log transformed egg counts (log e.p.g.+1) at baseline and the subsequent post-treatment decrease in log transformed egg counts predicted growth. Table 5.4 is a matrix of the calculated correlation coefficients which shows that decrease in *Trichuris* intensity significantly predicted an increase in raw weight (p=0.02) as well as increases in WAZ and WHZ which approached conventional statistical significance (p=0.06). Baseline *Trichuris* intensity predicted not only a rise in HAZ (p=0.04) but also increases in WAZ (p=0.07) and raw height (p=0.06) which approached statistical significance. The coefficients of determination however were uniformly low and never accounted for more than 4% of the variation in growth in the follow-up period. As shown in table 5.4 there was a weak correlation between baseline *Ascaris* intensity and baseline HAZ which approached significance (p=0.06). However neither the correlation between baseline *Ascaris* intensity and growth nor the correlation between the post-treatment fall in *Ascaris* intensity and growth approached statistical significance.

Stepwise multiple regression analyses on the effects of worm intensity, age, sex, socioeconomic status and baseline anthropometry on growth parameters.

Stepwise multiple regression analyses were performed with increases in WAZ, HAZ, WHZ, raw weight and raw height each in turn specified as the dependent variables in five equations. Sex, age socioeconomic status, baseline HAZ, baseline WAZ, baseline WHZ, log transformed baseline intensities and decrease in log intensities were specified as independent variables.

Increase in HAZ was significantly predicted by baseline *Trichuris* intensity. Baseline WAZ and decrease in *Trichuris* intensity were the only two variables which predicted an increase in raw weight while baseline WHZ was the only variable which predicted the increase in raw height. None of the independent variables managed to enter the equations predicting change in WAZ or WHZ. Table 5.5 summarises the data pertaining to the multiple correlation coefficients and the regression coefficients where appropriate.

Table 5.4 Matrix of correlation coefficients derived from simple regression analyses, relating intensities of infection to growth parameters.

	ΔHAZ	ΔWAZ	ΔWHZ	Δheight	Δweight
Baseline *Trichuris* intensity	0.20**	0.18*	ns	0.19*	ns
Baseline *Ascaris* intensity	ns	ns	ns	ns	ns
Decrease in *Trichuris* intensity	ns	0.20*	ns	ns	0.24**
Decrease in *Ascaris* intensity	ns	ns	ns	ns	ns

HAZ - Height for age Z-score.
WAZ -Weight for age Z-score.
WHZ -Weight for height Z-score.
Δ - Change in parameter over follow-up period, (parameter at follow-up - parameter at baseline).
ns = No significant correlation (p>0.1)
* correlation significant at 0.1 < p< 0.05
** correlation significant at p<0.05

Table 5.5. Multiple regression analyses predicting change in HAZ, raw weight and raw height.

Independent variables: Baseline WAZ, HAZ, WHZ, age, baseline log *Trichuris* and *Ascaris* egg counts, decrease in log egg counts, sex and socioeconomic group.

Dependent variable : Increase in HAZ: R=0.20

Variables entered:	*Partial regression coefficient:*	*p value*
Baseline *Trichuris intensity*	0.01	0.05

Dependent variable : Increase in raw weight R=0.40

Variables entered:	*Partial regression coefficient:*	*p value*
Baseline WAZ	0.28	0.002
Decrease in *Trichuris* intensity	0.09	0.04

Dependent variable : Increase in raw height R=0.23

Variables entered:	*Partial regression coefficient:*	*p value*
Baseline WHZ	0.18	0.03

HAZ =Height for age Z-score
WAZ= Weight for age Z-score
WHZ= Weight for height Z-score
R= Multiple correlation coefficient.

Post hoc analysis: Comparison between subsets.

A *post hoc* comparison was made between children with *Trichuris* infection alone (n=18) and the uninfected control group (table 5.6). Infection in the former group was relatively light (median intensity 1,400 epg; range 600-4000 epg). The mean age of 8.1 years (s.d. 0.3 years) in the subset was comparable to that of the control group (p=0.97). There was a female preponderance of 66.7% (12/18) in the the subset but the sex ratio was not significantly different from that of the control group (p=0.36). Eight children in the "*Trichuris* only" subset were drawn from socioeconomic group 3, a proportion which was higher than in the control group (p=0.01). Children with *Trichuris* infection only appeared to have experienced benefits in terms of gains in WAZ, WHZ, and raw weight.(Table 5.6) As there were only 10 children with *Ascaris* infection alone, it was not considered meaningful to similarly compare this subset with the control group.

Table 5.6 Baseline nutritional status and growth over 14 weeks: Comparison between children with *Trichuris* infection alone and the uninfected control group.

Group	Uninfected (n=32)	*Trichuris* only (n=18)	*p value* [b]
ΔHAZ[a]	0.02(0.08)	0.07(0.09)	*0.16*
ΔWAZ[a]	-0.14(0.09)	-0.02(0.09)	*0.01*
ΔWHZ[a]	-0.20(0.19)	-0.07(0.18)	*0.03*
Δheight, cm[a]	1.7 (0.5)	2.1 (0.5)	*0.11*
Δweight, kg[a]	0.2 (0.4)	0.6 (0.4)	*0.02*

HAZ =Height for age Z-score
WAZ= Weight for age Z-score
WHZ= Weight for height Z-score
Δ= Increase in parameter over follow-up period
a=median (quartile deviation in parentheses) b=Mann-Whitney U test

5.6 Discussion

As has been acknowledged at the outset, the ideal design for such a study would have been a randomised double blind placebo controlled study. Reservations about the acceptance by the community of the concept of placebo treatment precluded such a design. The design limitation notwithstanding, a number of inferences are possible.

Baseline HAZ was less than 2 standard deviations of the reference median in 31% of the study sample indicating a relatively high prevalence of stunting. In contrast none of the children had a weight for height Z-score of less than -2 indicating that wasting was less of a problem. The absence at baseline of any difference between the infected cohort and the uninfected control group in terms of the anthropometric parameters WAZ, HAZ and WHZ is not entirely surprising. As has been pointed out earlier, the children in any given endemic area may be infected at different times.[131] This is demonstrated to some extent by the fact that 45%(13/29) of initially uninfected children were found to be infected at follow-up. As growth stunting is a chronic process, a cross-sectional examination may therefore not capture an association between stunting and infection.

The main outcome measured in this study was growth over the follow up period as determined by increases in the parameters WAZ, HAZ, WHZ, raw weight and raw height. The hypothesis tested was that treatment of helminth infection would result in increased growth rates relative to the initially uninfected control group. This was based on the assumption that if infection adversely affected growth, then removal of infection would induce a compensatory growth spurt at least in comparison to the local control group. In this respect, the results indicate that treatment of helminthiasis was associated with modest gains in growth. The improvement was observed in all of the indices; WAZ, HAZ, WHZ, raw height and weight, indicating that treated children gained in terms of both ponderal and statural growth. In general, the gains in raw weight, WAZ and WHZ were more easily detectable than gains in height and HAZ.(Tables 5.2-5.4) This is compatible with the results of other short term treatment studies which have detected weight gains but not height gains over periods of between 3 and 16 weeks.[120,127] It has been suggested that malnourished children when refed tend to "fill out" first before statural growth occurs.[393] It cannot be discounted however that part of the difficulty in detecting height gains especially in the short term relates to the fact that the precision of height mesurement is somewhat lower than that of weight measurement. This is reflected by the higher coefficient of variation of height measurements (0.25%) than weight measurements (0.0%) as documented in this study. It is therefore all the more notable that improvements in linear growth were detected in the present study over the relatively short follow up period of only 14 weeks.

Antihelminthic treatment was the only intervention undertaken and it is not unreasonble to attribute the growth gains to reduction of the worm burden. Investigation in the area did not reveal any evidence that the observed growth improvements among treated chidren represented the recovery phase from some other recent insult such as an epidemic of diarrhoea or acute food shortage due to a poor harvest.

The children drawn from socioeconomic group 3 were found to have a lower baseline HAZ than the children from relatively better off backgrounds. This is consistent with the widely held view that lower socioeconomic status is associated with chronic stunting. It is therefore noteworthy that growth gains were detected after treatment despite the fact that children from socioeconomic group 3 were overrepresented in the treated group. It is equally significant that the children of lower socioeconomic status were not wasted relative to the children from relatively better off backgrounds. This is corroborative evidence that there was unlikely to have been any recent epidemic or unusual acute food shortage which may have caused acute wasting in the infected group as a whole.

Stratified analysis showed that antihelminthic treatment was associated with gains in raw weight, WAZ and WHZ independent of sex and socioeconomic status (table 5.3). The gains were detectable despite the fact that in undertaking the stratified analyses, the anthropometric data which were in the form of continuous variables were converted to dichotomous variables and would invariably have resulted in some loss of statistical power. Indeed this may explain the failure to detect an independent effect of treatment on height and HAZ. The failure to detect a main independent effect of socioeconomic status on growth is perhaps not surprising given the relatively short time frame.

Another striking observation which emerged from the stratified analyses was that the major benefit of antihelminthic treatment on growth was observed among girls drawn from socioeconomic group 2. Indeed the odds ratios defining the association between treating the infection and growth were statistically significant only within that stratum (n=40) and the magnitude of the odds ratios within that stratum were generally higher than the summary odds ratios for the cohort as a whole. The absence of a detectable effect within socioeconomic group 3 is likely to be due to the fact that there were too few uninfected children within that group to enable a meaningful comparison. The same argument however cannot be used to explain the failure of detecting a beneficial effect among boys within socioeconomic group 2.

As mentioned in section 5.3, a preliminary study on the earlier Tawang cohort revealed that girls but not boys derived some growth benefits from periodic antihelminthic treatment delivered over a year. The consistency of the gender difference in the growth response to antihelminthic treatment suggests that it is a true effect at least among pre-adolescent children in this locality. It is conceivable that girls in this age group are closer to puberty than boys and are therefore physiologically in a more ready state to mount a growth spurt in response to removal of a deleterious factor. The difference may equally lie in sociocultural influences relating to gender. The explanations for the observation however will for the moment remain speculative.

In the presence of a high prevalence of mixed infections of *Ascaris lumbricoides* and *Trichuris trichiura* it is difficult to determine the relative effects of the two species of worms on growth. Any analytical attempt to unravel what is potentially a complex relationship would inevitably be simplistic. Regression analyses (tables 5.5-5.6) showed the intensity effect relationship more clearly in the case of *Trichuris* infection than in *Ascaris* infection, even if the magnitude of the coefficents of determination (R^2) were small. Indeed, the baseline *Trichuris* intensity or fall in intensity explained less than 5% of the variation in the growth parameters. Regression analysis however has its limitations. The relationship between intensity (even after log transformation) and growth may well be too complex to be defined by a simple linear equation. Furthermore egg counts are an imperfect index of worm intensity and are subject to day to day variability.

Another possible reason for *Trichuris* intensity being a better predictor of post treatment growth than *Ascaris* intensity, relates to the consistent finding that *Ascaris* infection is easier to treat than trichuriasis. In the studies undertaken for this thesis, the decrease in post-treatment *Ascaris* prevalence rates and intensities have consistently been more impressive than the corresponding decreases in the *Trichuris* burdens. It is tempting to postulate that even if ascariasis is successfully treated, the persistence of modest *Trichuris* burdens dampens the potential growth response. In other words in the presence of mixed infections, it is plausible that the degree to which *Trichuris* is successfully eliminated is the rate limiting step which determines the magnitude of the growth response. In the earlier Tawang study which has been referred to, periodic treatment had no beneficial overall effect on growth.[391] There are however two notable differences between the earlier study and the present one. Firstly, the average intensities of infection at the start of the earlier study were lower because of previous interventions with antihelminthic drugs during the course of the intestinal permeability studies. In particular, the median baseline *Trichuris* intensity in the earlier study was less than 50% of the median

Trichuris intensity of the present study. Secondly, in the earlier study single 400mg doses of Albendazole were used as opposed to the dual doses used in the the present study. It has certainly been demonstrated that multiple doses of albendazole are more efficacious in eliminating *Trichuris* infection than single doses.[323] Whether differences in the drug dosage account for the differing results and whether the the degree to which *Trichuris* is eliminated is indeed the critical factor, are questions which are intriguing but not answered by the data from the present study. Only further carefully designed studies on the same population can resolve these issues.

While the results of *post hoc* analyses should always be regarded with extreme caution particularly when the numbers become smaller, they often provide clues which at the very least justify further investigation. For this reason it would be imprudent to entirely ignore the findings relating to the subsets of children with *Trichuris* infection alone. Children infected with *Trichuris* alone (n=18) demonstrated significant improvements in nutritional status as reflected by gains in WAZ and WHZ. This is noteworthy not only because the benefits were observed in a reasonably short period but also because the average intensity of infection in the group was relatively modest. In fact half of the children in the subset were lightly infected (ie <1000 epg). Furthermore the average socioeconomic status of this subset was lower than the control group. The obvious implication is that even modest burdens of *Trichuris* infection may independently affect growth.

There is little doubt that the most impressive published results thus far on the effect of antihelminthic treatment on growth of primary school children have emerged from a series of placebo controlled treatment studies undertaken in a single district in the coast province of Kenya.[119-122] Gains in weight of the order of 150% (1kg) in comparison to placebo treated controls were recorded between 4 and 6 months after treatment as were height gains of the order of

30-40% (0.6cm) over the same follow up period.[119,120] Indeed weight gains of the same magnitude were detected as early as 9 weeks after treatment in the Kenyan studies.[122] The results of the Kenyan studies however cannot be extrapolated to North-eastern peninsular Malaysia for a number of reasons, the most important of which is that almost 100% of the Kenyan schoolchildren were infected with hookworm in sharp contrast to the situation in Tawang. Fall in hookworm intensity was consistently shown in the Kenyan studies to be a better predictor of post-treatment growth than *Trichuris* or *Ascaris* intensities. Nonetheless it is interesting to note that the median post treatment weight gain of infected children in the present study was also 200% greater than the weight gain among uninfected controls while the median height gain was 18% higher (table 5.2).

In contrast, to the Kenyan studies, the results of other studies conducted in areas with high prevalence rates of *Ascaris lumbricoides* and *Trichuris trichiura* but low hookworm rates have in the main been rather disappointing. In this context, the gains detected in the present study are in fact in comparison somewhat more encouraging. In the large study reported from Myanmar which included 6-10 year old schoolchildren with almost 100% prevalence rates of *Ascaris* but virtually no hookworm or *Trichuris* infection, height gains of the order of 8% were detected only 18 months after 3-monthly chemotherapy while weight gains of the order of 25% were detectable only after 2 years of treatment.[116] Curiously in that study, treated children lost weight relative to controls during the first year and a half of periodic chemotherapy. The findings of the study from Myanmar is often quoted as evidence that treatment of ascariasis is beneficial. However close examination of the magnitude of benefit detected in that study would suggest that the impact of ascariasis upon growth is either quite small or else the effect is irreversible.

In the Guatemalan study of primary schoolchildren with high rates of *Ascaris* and *Trichuris* infection, only marginal weight gain was observed in children treated twice over the course of 6 months.[126] An important observation in the Guatemalan study was that while the *Ascaris* prevalence and mean intensity in the treated arm had decreased substantially by the end of the follow-up period there was little change change in the total *Trichuris* burden. Clearly the single 400mg doses of albendazole which were employed in the Guatemalan study had very little efficacy in treating trichuriasis. This study also illustrates the limited benefits on growth of removing *Ascaris* infection without at the same time substantially reducing *Trichuris* burdens.

If ineffective eradication of trichuriasis can be blamed for the negative results of the Guatemalan study, the same cannot be said of a Jamaican study, in which periodic 800 mg doses of albendazole resulted in *Trichuris* clearance rates of 50% despite which no main effect of treatment on growth was detected.[123] Multivariate analyses however revealed that children with modest *Trichuris* burdens at baseline (<7000 epg) did derive some benefit in terms of improved body mass index. The results of an earlier Malaysian study undertaken on primary schoolchildren of Indian ethnic origin in Western Peninsular Malaysia with a pattern of infection similar to the cohort of the present study was even more disappointing.[124] The investigators encountered some difficulties with parasitological surveillance due to poor compliance during the course of that study which makes assessment of the true efficacy of the treatment regime which consisted of mebendazole and pyrantel oxantel pamoate rather difficult. Furthermore, the outcome measurements in the study were the proportions of children below 2 standard deviations of the reference medians of WAZ, HAZ and WHZ rather than the absolute values themselves. The use of nominal outcome variables rather than the continuous anthropometric data would to some extent have reduced the sensitivity of the study. As discussed in chapter

1, the two studies on Indonesian schoolchildren who would ethnically be similar to the Malay children in Tawang yielded somewhat divergent results.[127,128] The variability observed in the studies may well be due not only to methodological factors such as the treatment regime used and the outcome measured but also to a multitude of other factors such as genetic potential for growth, availability of nutrition and other local factors which may vary with time.

Although the post-treatment growth gains detected in the present study are modest in magnitude, the findings if found to be reproducible are of some significance given the scale of infection in the area. Furthermore, the significance of the results lie not only on the potential impact of infection on growth, as it would seem likely that any infection which is noxious enough to affect growth may well have other as yet unmeasurable consequences on general health and wellbeing.

In conclusion, the results of this study suggest that antihelminthic treatment exerts a beneficial effect of modest proportions on the growth of early primary school children at least in the short term. Further controlled studies on the same population are clearly needed to assess the reproducibility of these results.

Chapter 6 The effect of intestinal helminthiasis on school attendance.

The effect of intestinal helminthiasis on school attendance.

6.1 Aim and summary of study design

The aim of this study was to determine the effect of intestinal helminthiasis on school attendance rates of children attending early primary school in North-eastern peninsular Malaysia. The study tested the hypothesis that helminthiasis is associated with lower attendance rates which are improved by antihelminthic treatment. The design which was similar to that of the other studies described in this thesis essentially involved the comparison of school attendance rates between helminth infected children and uninfected controls before and after treatment. The importance of studying the effects of intestinal helminthiasis on school attendance lies not only in the obvious implications on learning achievements of children in their crucial formative years but also in the fact that school attendance may be an albeit crude and indirect indicator of the general health and well being of the children in question.

6.2 Materials and methods.

6.2.1 Subjects and study protocol.

The study cohort consisted of the same children at Tapang and Tawang schools who were the subjects of the study on intestinal permeability (Chapter 3). While the primary intention was to determine the effects of intestinal helminthiasis on intestinal permeability, the presence of accurate records on school attendance was exploited to study the effects of infection on school attendance rates. The class teacher customarily takes attendance in the class at the start of each day, a record of which is entered in the attendance register. Baseline stool examination was undertaken in August 1994 at Tapang and in March 1995 at Tawang. As stated in chapter 3, children infected at baseline were treated with 400mg of albendazole and stool examination repeated 6-8 weeks later. Also as mentioned in chapter 3, repeat stool examination at

Tapang was done on infected children and 26 randomly selected controls; whereas at Tawang repeat stool examination was undertaken on all children irrespective of baseline infection status. Any child found to be infected at the second examination received another supervised 400 mg dose of albendazole. The class registers were examined in mid-1996 and the attendance of each child was noted over three specific time periods, each consisting of 60 consecutive school days. The first 60 day period was that immediately prior to the first treatment; the second started a week after the first treatment and the third followed on immediately after the second. As outlined in chapter 3, a crude socioeconomic categorisation was devised based on the father's occupation, source of water for domestic use and the number of siblings in the family.

6.2.2 Statistical analysis

Comparisons in terms of days of absence from school were made between infected and uninfected children during each of the three 60 day periods using the non-parametric Mann-U Whitney test. Simple regression analysis was used to assess the correlation between log transformed egg counts and the number of schooldays lost as well as the correlation between each of three baseline anthropometric parameters (height for age, weight for age and weight for height) and schooldays lost. Two tailed statistical values were quoted at all times. Statistical computations were performed with the aid of the EpiInfo (World Health Organization, Geneva and the Centers for Disease Control, Atlanta, Georgia, USA) and SPSS statistical software packages.

6.3 Results

6.3.1 Demographic data, infection prevalence and intensities

The age and sex distribution of the study cohorts at both schools as well as the baseline infection prevalence rates and intensities have been summarised in tables 3.1a and b of chapter 3 (pages 109 and110). Two points merit repetition. The first is that the children at Tawang came from relatively poorer backgrounds than the children at Tapang, and the second is that the initial treatment was more effective in removing *Ascaris* than *Trichuris* infection (tables 3.1a and b; chapter 3).

6.3.2 School attendance in relation to worm infection status.

The number of school days lost in relation to the worm infection status and school attended is summarised in table 6.1. During the pre-treatment period, there was no significant difference in the frequency of lost schooldays between infected and uninfected children at either Tapang or Tawang. At Tawang, the rate of absenteeism in the infected group ironically worsened relative to the uninfected controls during the first post-treatment 60 day period (table 6.1).

At Tapang, comparisons between *Ascaris* positive and negative groups showed that baseline ascariasis was consistently associated with a higher rate of absenteeism in the pre- and post-intervention periods (table 6.1). The trends were quite clear that treatment of ascariasis did not result in any improvement in school attendance at Tapang (table 6.1). At Tawang, ascariasis was not associated with increased absenteeism at baseline but the attendance rate deteriorated relative to the *Ascaris* negative group during the first post-treatment 60 day period (table 6.1).

Trichuris infection *per se* was not associated with absenteeism in either school prior to treatment (table 6.1). Treatment of trichuriasis was not associated with an improvement in attendance rate at either school (table 6.1)

Table 6.1 Numbers of school days lost in relation to helminth infection.

	Median number of school days lost[a]					
	Tapang			Tawang		
	Infected	Uninfected	*p*	Infected	Uninfected	*p*
<u>All infections</u>						
No. of children	49	95		59	46	
Period[b]						
1	2.0 (2.5)	1.0 (1.5)	*0.14*	2.0 (2.0)	2.0 (2.0)	*0.15*
2	2.0 (2.5)	1.0 (1.0)	*0.13*	2.0 (2.5)	1.0 (1.0)	*0.02*
3	2.0 (2.5)	1.0 (1.5)	*0.10*	2.0 (1.5)	2.0 (1.5)	*0.17*
<u>*Ascaris*</u>						
No. of children	23	121	-	50	55	-
Period[b]						
1	3.0 (4.5)	2.0 (1.5)	*0.01*	2.0 (1.5)	2.0 (2.5)	*0.20*
2	3.0 (3.0)	1.0 (1.0)	*0.0001*	2.0 (2.5)	1.0 (1.0)	*0.02*
3	3.0 (2.5)	1.0 (1.5)	*0.006*	2.0 (1.5)	1.0 (1.5)	*0.10*
<u>*Trichuris*</u>						
No. of children	48	96	-	55	50	-
Period[b]						
1	2.0 (2.5)	1.0 (1.5)	*0.09*	2.0 (2.0)	1.5 (1.5)	*0.14*
2	2.0 (2.8)	1.0 (1.0)	*0.12*	2.0 (2.5)	1.0 (1.0)	*0.02*
3	2.0 (2.5)	1.0 (1.5)	*0.18*	2.0 (2.0)	2.0 (1.5)	*0.35*

a Quartile deviation in parentheses

b Period1: 60 consecutive school days immediately prior to treatment.
Period 2: 60 consecutive school days starting a week after treatment.
Period 3: 60 consecutive school days following on from period 2.

<u>*Simple regression analyses to determine the effects of worm intensity and baseline anthropometric parameters on the number of lost school days.*</u>

From the series of simple regression analyses undertaken to determine the extent to which intensity of infection and baseline anthropometric parameters predicted the number of school days lost, three statistically significant correlations were observed at Tapang school. The three statistically significant correlations are presented in table 6.2. A weak but significant correlation was observed between baseline log transformed *Ascaris* egg count and the number of pre-treatment school days lost at Tapang (table 6.2) However the magnitude of the correlation coefficient between baseline *Ascaris* intensity and lost schooldays at Tapang remained stable in both post-treatment periods (table 6.2), strengthening the argument that the relationship between *Ascaris* intensity and absenteeism was likely to be merely an association rather than cause and effect. Furthermore the fall in *Ascaris* intensity between examinations at Tapang did not predict a decrease in the number of schooldays lost from the pre-treatment to the post treatment periods (data not shown). There was no significant correlation at Tapang between baseline *Trichuris* intensity and school days lost in the pre-treatment period nor between the fall in *Trichuris* intensity and decrease in school days lost after treatment (data not shown).

A significant negative correlation was observed at Tapang between height for age and pre-treatment absenteeism (table 6.2). Weight for age and weight for height however were not significant predictors of the number of school days lost.

In contrast to the trends observed at Tapang, none of the independent variables explored were statistically significant predictors of the number of school days lost during the pre-treatment period at Tawang; although the correlation between log transformed baseline *Trichuris* intensity and pre-treatment absenteeism approached significance (r=0.19; 95% C.I. 0.00 - 0.37). The decrease in log transformed worm intensities at Tawang did not predict a decrease in the number of school days lost in the post-treatment periods.

Table 6.2 Simple regression analysis of the influence of intensity of infection and baseline abthropometric parameters on the number of school days lost at Tapang school.

	Correlation coefficient between independent variables and number of school days lost in: [a]		
	Period 1	Period 2	Period 3
Independent variables:			
Baseline *Ascaris* intensity[b]	0.26	0.25	0.35
Height for age[c]	-0.19	-	-

a Only correlation coefficients which were statistically significant ($p<0.05$) are quoted.
b Intensity expressed as log transformed baseline egg counts ie log (e.p.g.+1)
c Height for age expressed as z-score
Period1: 60 consecutive school days immediately prior to treatment.
Period 2: 60 consecutive school days starting a week after treatment.
Period 3: 60 consecutive school days following on from period 2.

6.4 Discussion

This study like the other studies which form the body of this thesis, was an interventional one which used uninfected children as controls. The reason for not being able to follow the optimum design of a randomised double blind placebo controlled study has already been alluded to. A number of clear trends emerged from the results. Most obviously, ascariasis was associated with absenteeism; although the magnitude of excess schooldays lost was relatively small when compared to controls. This trend was most clearly observed at Tapang school where an albeit weak negative correlation between *Ascaris* intensity and the number of lost school days pre-treatment was detected. The weight of the evidence from this study however suggests that the relationship between ascariasis and school absenteeism is merely an association rather than one of cause and effect. Despite the fact that there was a high degree of *Ascaris* eradication in the treated cohort, there was no improvement in the school attendance rates. It is also illuminating that the strength of the correlation between the baseline *Ascaris* intensity and the number of lost schooldays remained remarkably stable during the three 60 day periods of study. Clearly ascariasis is a fairly strong marker for other factors which do predispose to absenteeism from school. The absence of evidence from this study that ascariasis causes school absenteeism has particular relevance in the context of the prevailing belief even among medical practitioners in North-eastern Peninsular Malaysia that recurrent abdominal pain in school children is often due to "worm colic". The present study does not provide any corroborative evidence that ascariasis among early primary school children causes recurrent abdominal pain, a condition which might not unreasonably be expected to cause absenteeism. However it is conceded that this study does not entirely exclude the possibility as it was not primarily designed to do so.

Trichuris infection *per se* was not significantly associated with absenteeism in the pre-treatment period and there was no statistically significant correlation between *Trichuris* intensity at baseline and the number of school days lost prior to treatment. There was no convincing evidence that treatment of trichuriasis improved attendance rates. Although the *Trichuris* prevalence rates in the worm infected subsets had fallen by a smaller quantum in comparison with the decrease in *Ascaris* prevalence rates, there was a significant reduction in the intensity of infection (tables 3.1a and b; chapter 3). Furthermore it should be noted that children who were found to be infected at the repeat examination 6-8 weeks after the first dose received a second dose at that point.

Despite the ubiquity of intestinal helminthiasis, data on the effect of the infection on school attendance rates is surprisingly scarce. The little available data is however broadly concordant with that of the present study. An association between *Trichuris* infection and absenteeism in Jamaica has been documented and reported.[133] The absenteeism rate in that report correlated well with intensity. In an interventional study in Guatemala however, antihelminthic treatment with albendazole did not improve school attendance rates.[143] The investigators were unable to arrive at any definite conclusions on the effect of treating trichuriasis on school attendance as the efficacy of eliminating *Trichuris* infection was very poor.[143] In another interventional study conducted in Jamaica, treatment of *Trichuris* infected children did not improve school attendance except in the presence of stunting.[123]

In summary, the results of this study provide no evidence that intestinal helminthiasis adversely affects school attendance among early primary school children in North-eastern Peninsular Malaysia.

Chapter 7 Conclusions and recommendations for future research.

Conclusions and recommendations for future research

The cause and effect relationship between ascariasis and acute complications such as intestinal obstruction and cholangitis is obvious as is the relationship between intense *Trichuris* infection and the *Trichuris* Dysentery syndrome. These dramatic complications are however relatively rare events in relation to the fact that a quarter of the world's population is infected by one or other of these helminths. In contrast, there is a dearth of data on the chronic impact of these infections on the vast majority of infected individuals, a large proportion of whom are children of primary school going age. The work outlined in this thesis represented an effort towards adding to that existing body of data; and consisted of studies examining the effects of the two geohelminth infections on small intestinal permeability, occult colonic bleeding, short term growth and school attendance. Each of the four studies was an attempt at looking for corroborative evidence that helminth infection in North-eastern peninsular Malaysia is deleterious.

The design of the studies were interventional and essentially similar. The approach taken was to compare infected and uninfected children at baseline with respect to the outcome variable of interest and then to measure the degree of change after treating infected children. As has been periodically acknowledged throughout the thesis thus far, logistic considerations relating particularly to the acceptability to the community of the concept of placebo teatment, precluded a double blind placebo controlled design which would have been optimum. This limitation notwithstanding, a number of inferences were nonetheless possible.

It has to be emphasised that the studies investigated the effect of removing or reducing the burden of adult worms, and do not permit conclusions on the effect of the larval stages of the worms which were largely unaffected by the intervention undertaken. This is a point of some importance as the process of infection in the communities studied is a continual one and at any one point in time, children may be harbouring larvae in various phases of development which are unaffected by antihelminthic treatment. It should also be appreciated that variables such as growth, school attendance and small intestinal permeability may be influenced by a myriad of factors and as such caution has to be exercised in attempting to extrapolate the findings to other age groups or even to children of similar age in other communities.

The study on the effects of intestinal helminthiasis revealed two main findings. Firstly it confirmed the existence in Northeatern Peninsular Malaysia of a tropical enteropathy. The lactulose mannitol ratios were generally higher than in European controls when equivalent doses of lactulose and mannitol were used.[212,214] Indeed the median lactulose mannitol ratios at Tawang of both helminth infected and uninfected groups were of the same magnitude as coeliac patients detected during screening in Italy. Secondly, and more directly relevant to this thesis, the contribution of ascariasis to this enteropathy is marginal. In children drawn from lower socioeconomic backgrounds, the effect of *Ascaris* infection on small intestinal mucosal damage is negligible in comparison to the cummulative effects of other factors. It seems unlikely that ascariasis causes mucosal damage of a sufficient magnitude to independently account for malabsorption or a significant breakdown of the intestinal protective barrier. The absence of a consistent relationship between trichuriasis and small intestinal permeability is not entirely surprising as the adult *Trichuris trichiura* inhabits the colon.

The investigation of occult colonic bleeding in *Trichuris* infected children was conducted not only because of the obvious implications of blood loss on the genesis of chronic iron deficiency anaemia, but also because occult bleeding may be regarded as a surrogate marker of colonic mucosal damage. Since very heavy *Trichuris* infection reportedly causes a dysenteric illness, it was not unreasonable to hypothesise that lesser burdens of infection may cause colonic damage of a sufficient degree to result in occult bleeding. The results however were clear that trichuriasis does not predispose to occult colonic bleeding at least in the intensity range observed in the sample of schoolchildren in Northeastern Peninsular Malaysia. The issue as to whether heavier worm burdens may cause occult bleeding, albeit uncommonly can only be resolved by testing even larger numbers of children with heavy trichuriasis. The possibility remains of course that modest burdens of *Trichuris* infection cause damage to the colonic mucosa that result in effects other than microscopic bleeding.

The study on the relationship between helminthiasis and growth revealed modest gains in ponderal and statural growth which were detectable 14 weeks after antihelminthic treatment. The magnitude of the gains relative to the North American reference population[389] upon which calculation of weight for age, height for age and weight for height was based may not have been impressive. It is nonetheless notable that gains were detected relative to the local control group in a relatively short period of time after antihelminthic treatment, adding to the existing evidence that helminthiasis does indeed impair growth. It is difficult to be sure if the gains were a result of reductions in the burdens of *Ascaris, Trichuris* or both. While remaining cognisant of the limitations of interpreting the results of regression analyses, the fact that baseline *Trichuris* intensity and decrease in *Trichuris* intensities were better predictors of anthropometric gains than the corresponding *Ascaris* intensities does suggest that reduction of *Trichuris* levels may be a more important determinant.

There are a number of potential mechanisms by which ascariasis and trichuriasis may affect growth. Based on the data on helminthiasis and small intestinal permeability derived from the work of this thesis, malabsorption is unlikely to be one of them. Objective improvements in appetite following antihelminthic treatment have been demonstrated in two studies on schoolchildren conducted in Kenya[120] and Indonesia[127] repectively. In the Kenyan study, although the decrease in hookworm intensity was the best predictor of growth, decrease in *Trichuris* intensity was interestingly the best predictor of improvements in appetite and weight gain.[120] In the Indonesian study, improved appetite was attributed to treatment of ascariasis as the drug used was effective against *Ascaris* but not *Trichuris*.[127] The post-treatment *Trichuris* levels were however not reported in that study. It has been suggested that reduction in appetite induced by helminthiasis may be mediated by increased production of cytokines such as tumour necrosis factor a (TNFα).[127] Children with the *Trichuris* dysentery syndrome have certainly been shown to have increased circulating levels of TNFα and large numbers of cells containing TNFα have been demonstrated on immunohistochemistry of colonic mucosal biopsies.[93] It is conceivable that cytokine levels may also be increased in children with lower burdens of *Trichuris* infection, albeit to a lesser degree than in the full blown *Trichuris* Dysentery Syndrome.

Studying the effect of helminthiasis on school attendance was relevant not only because the latter is a determinant of scholastic achievement but also because it reflects to some extent the state of general health and well being. In this respect the results were quite clear that helminthiasis in Northeastern peninsular Malaysia is unlikely to be deleterious enough to independently cause school absenteeism among early primary schoolchildren. This does not of course exclude the possibility that the infections may have subtle effects on other determinants of scholastic achievement such as cognitive function although the evidence for this as discussed in the introductory chapter is limited.

Based on the findings described in chapter 5 on the relationship between growth and antihelminthic treatment, it is certainly tempting to hypothesise that in North-eastern Peninsular Malaysia at least, effective treatment of trichuriasis is the key determinant of reversing the deleterious effects of chronic intestinal helminth infection. This dose not imply that ascariasis is harmless. It is just that the drugs used are generally more effective against *Ascaris* than *Trichuris* and removal of the former without significantly reducing the latter is insufficient to reverse the ill effects of helminthiasis. It cannot be emphasised enough that considerably more work is required to test this hypothesis. One of the first steps towards testing this hypothesis further would be to assess the reproducibility of the results relating to the effects of treatment on growth (chapter 5). It is critical that in future studies, regimes are used which have greater efficacy against trichuriasis. In studies with short follow-up periods of 4 months or so, it may be necessary to use three to five 400 mg doses of albendazole on consecutive days[323] with an interval of two months between treatments. Comparison against a group treated with a regime which is very effective against ascariasis but less effective against trichuriasis such as the usual 400 mg single doses of albendazole may help shed some light on the relative importance of the two worms. Ideally the design should be randomised, double blind and placebo controlled but if similar logistic considerations as before are encountered, uninfected controls may have to suffice with blinding of the observer.

Using a similar design it may be enlightening to further study the effects of modest burdens of *Trichuris* infection on colonic mucosa. Direct histological examination requires an invasive procedure such as colonoscopy which is ethically questionable in the vast majority of *Trichuris* infected children who do not present with overt symptoms and logistically impracticable. Assessing colonic permeability in a manner similar to that undertaken in the work for this thesis is an option. Assessment of colonic permeability using ^{51}Cr labelled EDTA (ethylene diamine tetra-acetic acid) as the probe molecule has been described[394,395] but the administration of radiolabelled substances to free living children who have not actually sought medical attention would inevitably raise ethical questions. In an interesting report on AIDS patients published recently, urinary butyrate levels were measured as an indicator of colonic integrity.[396] This was based on the principle that butyrate, a product of the breakdown of complex carbohydrate in the colon under anaerobic conditions, is usually found in very low concentrations in plasma and urine and is not endogenously formed by the human body. As the colonic lumen is the sole source of butyrate, alteration of colonic permeability may result in elevated levels in urine. This was certainly the case in AIDS patients. The technique may require further validation but is potentially a useful tool to assess colonic permeability in *Trichuris* infected children.

It may be argued that the use of the higher and more frequent drug dosing regime in future studies is illogical as even if it were shown to have benefits on growth and other variables such as cognitive function, cost considerations would prevent these regimes being used in mass chemotherapy programmes. However the effects of helminthiasis and especially trichuriasis can only be quantified adequately in interventional studies if elimination of the worms is complete or at least near complete. Acquiring such empirical data is important in the planning and allocation of resources for health programmes. Furthermore the annual drug cost to the Ministry of Health of Malaysia of administering three 400 mg doses of albendazole on a three monthly basis would be approximately RM12.00 (US3.00) per child per year. This may not be prohibitive to a rapidly developing country like Malaysia considering that the organisational infrastructure to administer such a programme is already in place. If the morbidity due to helminthiasis is shown by well designed studies to be substantial enough, US3.00 a year per child may well be a justified interim investment in communities where helminthiasis is endemic. The long term solution not only in parts of Malaysia which are endemic for helminthiasis but also in much of the third world indisputably lies in improving the overall economic wellbeing of the communities concerned which is inextricably linked to better education, improved income opportunities, the provision of treated water for domestic use and adequate sanitary facilities. The likelihood of that goal being attained in the foreseeable future for much of the developing world is unfortunately minute and continued efforts to elucidate and quantify the morbidity attributable to intestinal helminth infection would remain important and relevant for many years to come.

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Appendix.

Socioeconomic scoring system used in the growth study (chapter 5).

	Score:
Occupation:	
Casual labourer	1
Small scale farmer	1
Trishaw rider	1
Driver	2
Manual worker with a regular salary	2
Small scale business	2
Shopkeper	2
Businessman	3
Teacher/Clerk/Professional	3
Gross monthly income:	
<RM200	1
RM200 -RM400	2
>RM400	3
Type of House:	
Shed	1
Wooden house	2
Brick and concrete house	3
Type of vehicle owned:	
Bicycle/trishaw	1
Motorcycle	2
Car/van	3
Number of dependents in the home:	
>6	1
4 - 6	2
0 - 3	3

Composite score	**Socioeconomic group:**
>12	1
9 - 12	2
< 9	3