Published in final edited form as:
Adv Parasitol. 2004 ; 58: 197–288. doi:10.1016/S0065-308X(04)58004-1.

Human Hookworm Infection in the 21st Century

Simon Brooker[1], **Jeffrey Bethony**[2,3], and **Peter J. Hotez**[3]

[1]Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

[2]Cellular and Molecular Immunology Laboratory, "René Rachou" Research Centre FIOCRUZ, Barro Preto - CEP 30190-002, Belo Horizonte, Brazil

[3]Department of Microbiology and Tropical Medicine, The George Washington University, Washington DC 20037, USA

Abstract

The scientific study of human hookworm infection began at the dawn of the twentieth century. In recent years, there have been dramatic improvements in our understanding of many aspects of this globally widespread parasite. This article reviews recent advances in our understanding in the biology, immunology, epidemiology, public health significance and control of hookworm, and to look forward to the study of this important parasite in the 21st century. Advances in molecular biology has lead to the identification of a variety of new molecules from hookworms, which have importance either in the molecular pathogenesis of hookworm infection or in the host-parasite relationship; some are also promising vaccine targets. At present, relatively little is known about the immune responses to hookworm infection, although it has recently been speculated that hookworm and other helminths may modulate specific immune responses to other pathogens and vaccines. Our epidemiological understanding of hookworm has improved through the development of mathematical models of transmission dynamics, which coupled with decades of field research across mutliple epidemiological settings, have shown that certain population characteristics can now be recognised as common to the epidemiology, population biology and control of hookworm and other helminth species. Recent recognition of the subtle, but significant, impact of hookworm on health and education, together with the simplicity, safety, low cost, and efficacy of chemotherapy has spurred international efforts to control the morbidity due to infection. Large-scale treatment programmes are currently underway, supported by health education and integrated with the provision of improved water and sanitation. There are also efforts underway to develop novel anthelmintic drugs and anti-hookworm vaccines.

1. INTRODUCTION

The pathophysiology and epidemiology of human hookworm infection has been the subject of intensive study for over one hundred years, beginning with the work of Theodore Bilharz who linked intestinal helminths with tropical chlorosis in Egypt, and studies by Italian pathologists who identified *Ancylostoma duodenale* as the cause of anaemia among labourers constructing the Saint Gothard railway tunnel in the Swiss Alps (Peduzzi and Piffaretti, 1983; Nawalinski and Schad, 1991). At the beginning of the 20th century, the life cycle of *A. duodenale* was first elucidated by Looss (1901), and *Necator americanus* was discovered in the Western Hemisphere (Stiles, 1902; Dock and Bass, 1910; Chandler, 1929). In 1901, the public health importance of hookworm infection was revealed when it was found to be hyperendemic on the island of Puerto Rico and linked to the death of almost 12,000 of its inhabitants annually (Ashford, 1934). Subsequently, the Rockefeller Sanitary Commission was established to combat hookworm in the United States and the Internatonal Health Commission was formed to control hookworm overseas. In 1916, the first

Europe PMC Funders Author Manuscripts

Department of Helminthology in the United States was founded at the Johns Hopkins School of Hygiene and Public Health in order to begin applying modern science and quantitative methods to the study of hookworm (Fee & Acheson, 1987; Nawalinski and Schad, 1991). These early investigations conducted in the first four decades of the twentieth century provided an important framework for our current knowledge of the pathogenesis and population biology of hookworm infection.

Over 25 years ago, Miller (1979) reviewed the current understanding of the biology, pathology and morbidity of human hookworm infection in this series. At the time, the global recognition of the public health importance of this and other soil-transmitted helminths (STH) was not fully appreciated. Since then, numerous advances in several scientific fields have greatly added to our fundamental knowledge of these parasites. For instance, they have resulted in an improved understanding of the transmission dynamics of helminths within human communities (Anderson and May, 1991). Such analytical approaches recognize the dynamic interplay between parasites and their human hosts, and the role they have in influencing observed epidemiological patterns and the consequences of control interventions. It is also become increasingly clear that the effects of infection are worse than they were assumed to be in the past, and that helminths exert subtle, yet significant, insults on the growth, education and productivity of individuals, but that chemotherapy can cost-effectively reverse most of these effects on morbidity. Finally, our understanding of hookworm infection at the molecular, cellular and organismal level has also improved considerably. Taken together, these scientific advances have prompted renewed interest in the control of these infections, with a focus on providing regular population-based chemotherapy (Savioli *et al.*, 2002). They also facilitate the development of first generation anti-hookworm vaccines (Hotez *et al.*, 2003).

On-going scientific efforts to study hookworm are vital since it remains one of the most common chronic infections of humans, with an estimated 740 million cases in areas of rural poverty in the tropics and subtropics (de Silva *et al.*, 2003). Many individuals also harbour multiple helminth infections, including *Ascaris lumbricoides, Trichuris trichiura* and schistosome species, which together with hookworm, infect a quarter of the world's population. When measured in terms of disability adjusted life years (DALYs), which incorporates the effects of both morbidity and mortality, the burden of disease caused by helminths exceeded the burden from a number of important tropical diseases including African trypanosomiasis, dengue, and leprosy (WHO, 2002). Against this background, the 54th World Health Assembly, held in 2001, passed a resolution urging member states to provide regular drug treatment of high-risk groups. In support of this important milestone in helminth control, WHO has set the global target of treating on a regular basis at least 75% of all school aged children. This goal has been given added impetus with the recent influx of major new sources of funding, notably from the Bill and Melinda Gates Foundation and the World Bank. Together with the recent scientific advances that have been made, such political developments provide hope for the global control of morbidity due to hookworm and other helminth species.

The aim of this review is to summarize the biology and molecular biology, immunology, epidemiology, public health significance, and control of hookworm in light of advances in understanding that have been made in recent years, and to look forward to the study of this important parasite in the 21st century. In doing so, it provides complementary information in our understanding of STH to recent excellent reviews of *Trichuris* and trichuriasis (Bundy and Cooper, 1989) and *Ascaris* and ascariasis (Crompton, 2001).

2. BIOLOGY

2.1. Systematics

Hookworms are nematodes belonging to the family Ancylostomatidae, a part of the superfamily Strongyloidea. The two major genera that affect humans, *Necator* and *Ancylostoma*, are characterized by the presence of either teeth or cutting plates that line the adult parasite buccal capsule (Hotez, 1995). *Necator americanus* is generally considered the only member of its genus to infect humans. This species has also been recovered on occasion from non-human primates (Orihel, 1971; Michaud *et al.*, 2003). It has been further suggested that the pig may serve as a transport host for *N. americanus* (Steenhard *et al.*, 2000). *Ancylostoma duodenale* is the only signficiant human (anthrophilic) hookworm of the genus Ancylostoma. Parasitic to cats and dogs, *A. ceylanicum*, is infective to humans as a zoonosis in some regions of Asia, but it is not associated with host blood loss in humans (Carroll and Grove, 1986), and therefore is not considered a major pathogen (Hotez, 1995). In northeastern Australia, the dog hookworm *A. caninum* has been reported to cause both eosinophilic enteritis and aphthous ileitis syndromes (Prociv and Croese, 1996; Landmann and Prociv, 2003). The natural history of zoonotic *A. caninum* infection has been extensively reviewed recently and will not be considered further (Prociv and Croese, 1996; Prociv, 1997). Yet another canine and feline hookworm, *A. braziliense* is the major cause of cutaneous larva migrans.

2.2. Life History and transmission

The life cycle of hookworms is direct (Hoagland and Schad, 1978; Schad and Banwell, 1984). Humans acquire hookworm when the infective larval stages (known as third-stage larvae or L3) living in the soil either penetrate through the skin (both *N. americanus* and *A. duodenale*) or when they are ingested (for *A. duodenale)*. It has also been reported that *N. americanus* L3 will invade the buccal epithelium if they enter through the mouth (Nagahana *et al.*, 1963). The L3 stages, approximately 600 microns in length, developmentally arrested and can live in the soil for weeks if there is appropriate warmth, shade, and moisture. Following host entry, the L3 receive a signal present in mammalian serum and tissue that causes them to resume development and secrete bioactive polypeptides (Hotez *et al.*, 1993; Hawdon and Hotez, 1996). Resumption of development is cGMP-dependent and involves a muscurinic neuronal pathway, which is similar to the one used for *Caenorhabditis elegans* dauer recovery (Hawdon and Datu, 2003). Among the major proteins secreted by host-activated hookworm L3 is a zinc containing metalloprotease of the astacin class (Zhan *et al.*, 2003), and two cysteine-rich secretory proteins known as ancylostoma secreted proteins (ASPs), which belong to the pathogenesis related protein (PRP) superfamily (Hotez and Hawdon, 1996; Hotez *et al.*, 2003). The function of these molecules has not been definitively established although it is believed that they have a role in tissue invasion and host-immunomodulation (see section 2.3.3). The host-activated L3 then migrate through the vasculature and are swept via the afferent circulation to the right side of the heart and then to the pulmonary vasculature. The L3 break out of lung capillaries and into the lung parenchyma where they ascend the alveoli, bronchioles, bronchi and trachea when they are coughed and swallowed. L3 enter the gastrointestinal tract where they molt twice and develop to the adult stage. Approximately 5-8 weeks pass from the time L3 first infect humans until they reach sexual maturity and mate. Each female hookworm produces thousands of eggs daily. Intestinal blood loss begins just prior to egg production and deposition, and continues for the life of the hookworm. The molecules associated with parasite-induced blood feeding have been extensively characterized (see section 2.3.1). The eggs are transparent, thin-shelled and ovoid, with blunt, rounded ends, measuring approximately 60 um × 40 um. Hookworms live in the human intestine on average from 1-3 years for *A. duodenale* and 3-10 years for *N. americanus* (Hoagland and Schad, 1978), with

a maximum life-span of 18 years (Beaver, 1988). Hookworm eggs exit the body in faeces. When deposited in soil with adequate warmth, shade and moisture, the eggs hatch within 24-48 h and give rise to first-stage larvae (L1) that feed on organic debris and bacteria in the soil. These L1s molt twice to the L3 stages. The L3 live can live for several weeks in the soil until they exhaust their lipid metabolic reserves.

There are significant biological differences between the two major human hookworms (Hoagland and Schad, 1978; Hotez, 1995), which are summarized in Table 1. As noted above, *A. duodenale* is transmitted both by skin penetration of L3 as well as oral ingestion, whereas *N. americanus* usually infects only via the former route. *N. americanus* is smaller than *A. duodenale* and produces fewer eggs, and causes less blood loss (Albonico *et al.*, 1998). Therefore, some investigators believe that *N. americanus* more adept at immune invasion, produces less blood loss, and therefore, better adapted to human parasitism (Hoagland and Schad, 1978; Pritchard and Brown, 2001). Generally speaking, *A. duodenale* is associated with greater intestinal blood loss than any other hookworm. This accounts for the observation, best documented in Tanzania, that the species of hookworm being transmitted in a community strongly influences the burden of iron deficiency anaemia in the community (Albonico *et al.*, 1998). However, *N. americanus* is more widespread worldwide and, therefore, more significant as a cause of disease burden.

Unlike *N. americanus, A. duodenale* also has the unique ability to undergo arrested development in humans (Schad *et al.*, 1973) and may, under certain conditions, enter human mammary glands during pregnancy prior to lactogenic transmission (Hotez, 1989; Yu *et al.*, 1995). The occurrence of neonatal ancylostomiasis has been documented the best in Asia and Africa (Yu *et al.*, 1995).

2.3. Molecular biology and pathogenesis of hookworm

In recent years, a variety of new molecules have been discovered and cloned, and sometimes expressed and isolated from *Ancylostoma* and *Necator* hookworms. Most of these molecules can be grouped into three major categories: adult hookworm secreted molecules; molecules that line the brush border membrane of the adult hookworm alimentary canal; and proteins secreted by L3 (Table 2). Each of them has importance either in the molecular pathogenesis of hookworm infection or in the host-parasite relationship; some are also promising vaccine targets.

2.3.1. Adult hookworm molecules—Work over the past two decades has determined that at their site of attachment in the gut mucosa and submucosa, adult hookworms secrete a variety of molecules, which thwart several host processes. The parasite's cephalic glands and esophageal glands are the origin of most of these molecules. Studies conducted by Zhan *et al.* (2002a) have revealed that the most abundant protein secreted by adult ancylostoma hookworms resembles mammalian tissue inhibitor of metalloprotease (TIMP). Hookworm TIMP is a 16 kDa protein that so far has not been shown to exhibit protease inhibitor activity. However, work conducted over the last decade has determined that mammalian TIMPs function in a number of biological processes that go beyond mere protease inhibition. Of particular interest is the finding by Guedez *et al.* (2001) that mammalian TIMP regulates interleukin 10 production by B cells. Measured IL-10 levels in humans infected with hookworm are extremely high (Bethony J., unpublished observation) suggesting that hookworm TIMP could have a role in host immunomodulation. In this case, hookworm TIMP would join a list of hookworm-derived molecules that have a role in either modulating or down-regulating the host inflammatory response, including neutrophil inhibitory factor (NIF), which serves as an integrin antagonist of host CD11b/CD18 (Moyle *et al.*, 1994; Muchowski *et al.*, 1994); hookworm proteases, which cleave eotaxin (Culley *et*

al., 2000); a calreticulin that interacts with host C1q (Kasper *et al.*, 2001); a retinol binding protein (Basavaraju *et al.*, 2003); a collagen-binding protein (Viaene *et al.*, 2001); a C-type lectin (Loukas *et al.*, 2002); an acetylcholinesterase (Brown and Pritchard, 1993); a glutathione S-transferase (Brophy *et al.*, 1995); a Cu/Zn superoxide dismutase (Taiwo *et al.*, 1999); and molecules that induce host T cell apoptosis (Chow *et al.*, 2000). In addition adult, hookworms produce and release at least four different ASPs, which are similar in structure to the two L3 ASPs (Zhan *et al.*, 2003). Preliminary experiments in vitro indicate that the ASPs also have immunomodulatory properties.

Adult hookworms secrete pharmacologically active peptides that facilitate blood feeding. The most potent are novel serine protease inhibitors that anticoagulate blood by inhibition of factor Xa (Harrison *et al.*, 2002) and factor VIIa/tissue factor (Lee and Vlasuk, 2003). The VIIa/tissue factor inhibitor is known as nematode anticoagulant protein c2 (NAPc2). It is a highly potent (Ki = 10 pm) inhibitor of the factor VIIa/tissue factor complex that requires the prerequisite binding of zymogen or activated factor X (Vlasuk *et al.*, 2003). This high affinity interaction with circulating factor X is critical to its bioactivity. The factor Xa inhibitor, known as NAP 5 is also potent (Stannsens *et al.*, 1996). Less potent is the corresponding anticoagulant from *A. ceylanicum* (Harrison *et al.*, 2002) a species generally believed to be much less dependent on blood feeding than either *A. duodenale* or *A. caninum* (Carroll and Grove, 1986). In addition to anticoagulants, ancylostoma hookworms also release a platelet inhibitor that binds to the integrin receptors glycoprotein Iib/IIIa and GPIa/Iia (del Valle *et al.*, 2003).

To facilitate intestinal mucosal and submucosal tissue invasion adult hookworms release several connective itissue hydrolases including a metalloprotease belonging to the astacin class similar to L3 MTP-1 (see 2.3.3.), known as MTP-2 (Zhan, B., unpublished observation), a cysteinyl protease known as CP-1, an aspartic protease known as APR-1 (Williamson *et al.*, 2003a), and a hyaluronidase (Hotez *et al.*, 1994). Hookworms also release protease inhibitors of the Kunitz-type of unknown function (Milstone *et al.*, 2000; Hawdon *et al.*, 2003).

2.3.2. Molecules lining the brush border membrane—Lining the hookworm intestinal tract of adult hookworms are proteolytic enzymes of several different types including MEP-1, a zinc metalloprotease of the neprilysin class (Jones and Hotez, 2002), APR-2, an aspartic proteases (Williamson *et al.*, 2003a, b), and a cysteine protease (Williamson *et al.*, 2003b). Work conducted by Angela Williamson and Alex Loukas has shown that these enzymes function to degrade haemoglobin in a carefully orchestrated cascade, which resembles the haemoglobin-degrading pathways from schistosomes and malaria parasites (Williamson *et al.*, 2003a, b). Inhibition of this pathway with antibody has proven to be a successful vaccine strategy against hookworm infection in laboratory animals (Hotez *et al.*, 2002; Loukas *et al.*, 2004), just as it has for vaccines against the sheep nematode *Haemonchus contortus* (Hotez *et al.*, 2002; Knox *et al.*, 2003).

2.3.3. Third-stage infective larval (L3) hookworm proteins—Work over the last decade has determined that hookworm L3 release several proteins upon stimulation with host serum, glutathione, and incubation at 37 °C (Hawdon and Hotez, 1996). It is believed that these molecules are produced upon host entry and in response to host-specific factors. Therefore, they function in the transition from the external environment into a parasitc milleau, and are linked to the developmental biology of the parasite (Hawdon and Hotez, 1996). The most abundant L3 secreted proteins are two unique ASPs (Hawdon *et al.*, 1996; Hawdon and Hotez, 1996; Hawdon *et al.*, 1999). Host-stimulated L3 also produce a metalloprotease of the astacin class known as MTP-1 (Zhan *et al.*, 2002b). Studies conducted in a variety of laboratory animals have determined that the ASPs and MTP-1 are

promising vaccine candidates against hookworm infection (Hotez *et al.*, 2003), and that antibodies to these molecules inhibit larval invasion in vitro (A. Williamson, unpublished observation). Of particular promise for purposes of vaccine development is the molecule ASP-2, which is protective in laboratory hamsters and dogs (Goud *et al.*, 2003), and is reactogenic with human sera (Bethony J. unpublished observations). The details of current vaccination strategies are discussed later in this review.

2.3.4. The genome and genetics of hookworm—Whereas the study of other parasites has benefited from the revolution in genomics and bioinformatics, limited hookworm genome projects are underway. However, through the parasitic nematode expressed sequence tag (EST) project, a collaboration between the University of Edinburgh and the Wellcome Trust Sanger Institute in the UK and the Genome Sequencing Center, St. Louis, MO, USA, over 400,000 nematode ESTs are now available (Blaxter, 2000; Parkinson *et al.*, 2003). To date, there are 7,328 ESTs available from *A. caninum*, 1,109 from *A. ceylanicum*, 800 from *A. duodenale*, and 961 from *N. amerianus* (www.nematode.net). Plans to sequence additional ESTs are underway. Two phylogenetic analyses, one using 53 small subunit ribosomal DNA sequences from a wide range of nematodes (Blaxter *et al.*, 1998) and another using mitochondrial genomes (Hu *et al.*, 2002), have resulted in a molecular evolutionary framework that demonstrates a close association between strongylids such as hookworms and the free-living nematode *Caenorhabditis elegans*. This information suggests that several modern approaches used to explore the molecular and cellular biology of *C. elegans* could be applied to hookworms (Blaxter, 2000, 2003).

Data on mitochondrial DNA (mtDNA) diversity and structure are increasingly available for several nematode species (Anderson *et al.*, 1998), including hookworm. Complete sequencing of mitochondrial genomes has now been conducted for both *A. duodenale* and *N. americanus* (Hu *et al.*, 2002), and are being used to investigate phylogeny and genetic diversity (Blaxter, 2000). For example, characterization of internal transcribed space (ITS) ribosomal DNA sequences of different hookworm species has recently been used to infer the evolutionary relationships of members of Ancylostomatidae family (Chilton and Gasser, 1999). Recent studies of *N. americanus* indicate substantial genetic variation in parasite populations (Hawdon *et al.*, 2001; Hu *et al.*, 2003), and a lack of correlation between geographical and genetic structure (Hawdon *et al.*, 2001). Such genetic heterogeneity indicates that it may be difficult to predict the development of anthelmintic resistance (Section 7.2.) or identify where populations are likely to differ at genetic loci targeted by specific recombinant vaccines (Section 7.3).

2.3.4. Molecular diagnosis—Several standard quantitative techniques, including the Kato-Katz thick smear method, are available to estimate hookworm egg output, which are valuable for epidemiological studies because they provide indirect measures of worm burden. The observation that IgG4 antibodies against hookworm antigens may serve as a serologic marker for the presence of active hookworm infection (Palmer *et al.*, 1996), has not yet been translated into a clinically reliable diagnostic test.

The eggs of *A. duodenale* and *N. americanus* are morphologically almost identical. Where it is desirable to differentiate the two species, however, a polymerase chain reaction (PCR) using primers derived from different genetic markers a hookworm cAMP-dependent protein kinase gene, followed by restriction fragment length polymorphism (RFLP) has recently been successfully employed (Hawdon, 1996). Another PCR-based method is based on the mitochrondrial cytochrome oxidase I gene (Zhan *et al.*, 2001). PCR and RFLP analysis of ITS rDNA has successfully been used to distinguish between *A. caninum, A. braziliense* and *A. ceylanicum* in animals (Gasser *et al.*, 1996; Traub *et al.*, 2003). This molecular method has additionally been used to distinguish human hookworm infection from infection with

Oesophagostomum biurcum, whose eggs are morphologically indistinguishable from hookworm, and whose prevalence exceeds 50% in northern Togo and Ghana, where it is co-endemic with *N. americanus* (Verweij *et al.*, 2001).

3. IMMUNE RESPONSES TO HOOKWORM

Immunological responses to hookworm infection in both human and experimental animal hosts have extensively reviewed by Behnke (1991) and more recently by Loukas and Prociv (2001). There is a notable absence of suitable animal models for hookworm infection, and extrapolating from immunological models of an abnormal host is unreliable. For example, *N. americanus* will mature in hamsters, but there is a wide variability on the number of L3 that develop to adult hamsters (Rose and Behnke, 1990; Xue *et al.*, 2003). Hasmsters frequently acquire resistance and do not develop patent infections (Rajasekariah *et ali*, 1985; Xue *et al.*, 2003). Similarly, *A. duodenale* will develop in dogs only with the administration of exogenous steroids (Leiby *et al.*, 1987). This may be because the two species that account for almost all human infections, *A duodenale* and *N americanus*, are highly host specific (Beaver *et al.*, 1984). As such, the focus here will be on recent contributions to hookworm immunology that derive from immunoepidemiological findings in human populations, referring to findings from animal models where they may provide useful insights into the immune response against these parasites.

The most studied aspect of the human immune response to hookworm infection is antibody levels to crude larval (L3E) and adult (AE) soluble extracts or adult excretory/secretory (ES) products (Loukas and Prociv, 2001). The methods used to identify antibody responses range from early observations by Sarles, Otto and colleagues of immuno-precipitates around oral opening of larval and adult worms (Sarles, 1938; Otto *et al.*, 1942; Sheldon and Groover, 1942) to more recent and detailed analysis of Ig subclasses by enzyme-linked immunosorbent assays (ELISA) and Western Blot analysis. These analyses have shown that extensive and vigorous antibody responses of all five of the human immunoglobulin (Ig) isotypes are mounted against crude antigen preparations in naturally infected individuals (Carr and Pritchard, 1987; Behnke, 1991; Loukas and Prociv, 2001).

A consistent observation from infected individuals is the marked heterogeneity in the quantity and composition of the antibody response among individuals of the same age, gender, and level of infection (Bethony, J., unpublished results). This heterogeneity has been shown in the (1) antigens recognized (Carr and Pritchard, 1987), (2) composition of the isotype profile (Pritchard *et ali*, 1992), and (3) levels of total and specific isotypes (Pritchard *et al.*, 1992). As antibody isotypes also differ in their biological properties, including their ability to mediate or block the killing of helminths (Shackelford *et al.*, 1988; Khalife *et al.*, 1989; Dunne *et al.*, 1993), heterogeneity in antibody isotype response has been thought to offer some clue as to the source of the marked variation in intensity of hookworm infection observed in individuals from the same endemic area (see Section 4.2).

As with most helminths, the antibody response to hookworm consists predominantly of the Th2 antibody isotypes, IgG1, IgG4, and IgE, with most attention on IgE. During hookworm infection, serum levels of IgE increase 100-fold (Jarrett and Bazin, 1974). IgE participates in an orchestrated IgE network (Sutton and Gould, 1993; Garraud *et al.*, 2003), with activation of this system often leading to cellular (i.e. mast cell, basophil, eosinophil) degranulation with subsequent toxic activity against helminths (Garraud *et ali*, 2003). Interestingly, much of the IgE elicited during hookworm infection is not directed against the parasite; in fact, total levels of this isotype have been shown to correlate with a reduction in parasite weight and fecundity (Pritchard *et al.*, 1995). This observation has led to the speculation that helminth parasites secrete pro-allergic mediators that induce polyvalent, non-parasite

specific IgE, thus saturating IgE receptors on effector cells (Pritchard, 1993). Another interpretation for the high levels of non-specific IgE found in the serum if infected individuals is a reduction in the risk of anaphylaxis (Hagan, 1993), even though the response may still be sufficient to eliminate the parasite. The small proportion of the serum IgE that is raised against *Necator* is highly specific (Pritchard and Walsh, 1995). Pritchard and colleagues (Pritchard and Walsh, 1995) found that IgE antibodies were more specifically directed against *N. americanus* epitopes than other Ig isotypes. Similarly, in human intestinal infection with canine hookworm *A. caninum*, IgE responses proved to be more specific than the IgG responses to adult ES antigens, with selected patients generating detectable levels of IgE but not IgG to a diagnostic *A. caninum* ES antigen (Loukas *et al.*, 1994).

Levels of IgG1 and IgG4 level are also elevated in hookworm infection, with IgG4 against L3E suggested to be a marker of active infection with *N. americanus* (Palmer *et al.*, 1996), and *A. duodenale* (Xue *et al.*, 2000). The role of IgG4 is poorly understood, although like IgE it is upregulated in atopic conditions and helminth infections. It is thought to downregulate the immune response by competitively inhibiting IgE mediated mechanisms; e.g. blocking mast cell activation (Rihet *et al.*, 1991). Adult hookworm also induce the production of secretory IgE, IgG and IgM but not IgA,and the levels of these Igs return to normal after anthelminthic treatment. The absence of secretory IgA is intriguing and may reflect the secretion of hookworm proteases that specifically cleave IgA (Loukas and Prociv, 2001).

Despite the extensive antibody response to infection, there is limited conclusive evidence that these antibodies offer any protection (Pritchard *et al.*, 1995), by either significantly reducing larval or adult hookworm numbers, similar to that found for schistosome infections (Hagan *et al.*, 1991; Dunne *et al.*, 1992). Mark Woolhouse has shown the theoretical difficulty of detecting protective immunity from levels of antibodies from human cross-sectional studies: in particular, the positive correlations often observed between usually protective antibody responses (such IgG1 and IgE) and parasite burden because of the numerous uncontrolled variables such as exposure, behavioral modifications, and coinfection with other helminths (Woolhouse, 1992; Woolhouse, 1993). The clinical manifestations of the small numbers of volunteers infected with *Necator* (Maxwell *et al.*, 1987) have varied from none to severe intestinal disturbance, further highlighting the heterogeneity in the response to hookworm infection in humans.

Another hallmark feature of the immune response to helminth infection is peripheral blood eosinophilia (Loukas and Prociv, 2001). Eosinophils predominate in the inflammatory responses to hookworm L3 in tissues and, with sufficient larval dose, can be reflected in peripheral blood eosinophilia (Behnke, 1991). Circulating eosinophils from human volunteers infected with *N. americanus* were functional and secreted superoxide (White *et al.*, 1986). Peripheral eosinophil responses in experimental human infections with either *N. americanus* (Maxwell *et al.*, 1987) or *A. doudenale* (Nawalinski and Schad, 1974) were boosted greatly by the arrival and development of worms in the gut, probably reflecting accelerating antigenic output by feeding L4 and adult stages. In vitro, human eosinophils binding to *Necator* L3 increase in the presence of complement and antibodies, although it is not known if this adherence is larvicidal (Desakorn *et al.*, 1987). The contribution of eosinophils to the in vivo destruction of helminths is unclear, but evidence suggests that these cells can kill infected larval stages but not the adults of most helminths species investigated (Meeusen and Balic, 2000).

Mast cell degranulation in response to IgE allergen interaction plays a critical role in local mobilization and activation of eosinophils. While mast cell proteases degrade cuticular collagens of adult *N. americanus* (McKean and Pritchard, 1989) and are considered crucial

to the host response to hookworms, they have attracted sparse research attention in humans. Hamsters infected with host-adapted *A. ceylanicum* quickly developed to resistance to infection with this species, characterized by increased antibody production and mucosal mastocytosis. Hookworms appear to be more resistant to intestinal inflammation than are most other intestinal nematodes, perhaps reflecting their attachment and feeding strategies. Hamsters infected with *Trichinella spiralis, A ceylanicum*, or *N. americanus* produced more intense mucosal response that cleared *T. spiralis*, while sparing the hookworms (Behnke *et al.*, 1994). Indeed, depressed anti-*T. spiralis* antibody levels indicate that hookworms might protect other parasites by generally suppressing immune responses. This observation had been shown in field studies of humans of mono-infected and co-infected with *S. mansoni* and *N. americanus* in Brazil (Bottazzi, M.E., personal communication). Patients mono-infected with *S. mansoni* showed a much higher proliferative capacity than patients coinfected with *N. americanus* and *S. mansoni*. Furthermore, the level of IL-10 in co-infected patients was 10 times higher than *S. mansoni* mono-infected patients, an indication of the possible reasons for the general suppression of the immune system during *Necator* infection.

Eosinophilia, mastocytosis, and IgE stimulation are the three main immune alterations observed during a hookworm infection in humans. But the overall immune responses of human hosts to hookworm infection are remarkably similar to infections with other helminths: dominated by the production of T-helper-2 (Th2) cytokines interleukin (IL)-4, IL-5, IL-9, IL-10, and IL-13, which is consistent with the development of strong IgE, eosinophil, and mast cell responses mentioned above. Indeed, the inherent ability of helminths to induced Th2 responses has led to interest in them from both the perspective of the elucidation of the underlying mechanisms that lead to Th2 responses and in terms of understanding Th2 response function (MacDonald *et al.*, 2002). Few studies have been done on the human cellular immune response to hookworm infection. In general, PBMCs from chronically infected individuals reacted strongly to mitogen, but weekly to crude antigen preparations. Recent studies show that the proliferative response of human PBMCs to L3E, AE and adult ES products increases dramatically after treatment and before reinfection. A concomitant observation was the high level of IL-10 compared to other cytokines (IL-4, IL-5, and IL-13) that accompany chronic hookworm infection and the decline that accompanies treatment. It is thought by many that IL-10 plays an important role in the regulation of the proliferative response of human PBMCs to crude antigen extracts, such that treatment of infection results in a decline in relative IL-10 levels and increase in proliferative response, as seen in other helminth infections, notably infection with the platyhelminth *Schistosoma mansoni* (MacDonald *et al.*, 2002). These high levels of IL-10 observed in the sera and in vitro cultures with crude antigen extracts are thought to play a principal role in minimizing what is effectively an immediate (type 1) hypersensitive (allergic) responses (Pritchard and Brown, 2001).

Experimental systems have demonstrated that the host protection to nematode infection may be a CD4 + T cell-dependent process, with the IL-4 secreted by these cells has an essential or very important role in the process (Finkelman *et al.*, 1997; Lawrence *et al.*, 1998). However, the steps from IL-4 secretion to worm elimination are still not clear. Multiple and not mutually exclusive mechanisms might be involved in immune response against nematode infections. Activated eosinophils plus immune serum has been able to kill helminth larvae *in vitro* and also in some experimental models, especially in parasites that have a systemic migration in the life cycle (reviewed in Cara *et al.* (2000)). However, IL-5 knockout mice showed that the eosinophil mediated mechanism is not essential to the protection (Cara *et al.*, 2000). Similarly, mastocytosis reported at the intestinal site of nematode infected animals would shown to be an important element to the protective immunity against *S. ratti* but not against *N. brasiliensis* (Nawa *et al.*, 1994).

Much of the basic cellular immunology of hookworm in humans remains to be done, especially the elucidation of the roles of cytokines, chemokines, and cell surface markers during infection. While hookworm immunology has benefited greatly by the immunology of other helminth infections in humans, notably schistosomiasis and filariasis, it cannot be assumed that cellular immune response to this nematode infection will be the same. Preliminary data (Botazzi, M.E., unpublished observation) already have established that the cytokine profile for hookworm infection in individuals living in *Necator* endemic areas of Brazil differs greatly in the composition and quantity of Th2 cytokine profile produced during *S. mansoni* infections, e.g. higher levels of IL-10 expressed. Furthermore, proteins secreted during third stage infective larvae upon encountering host serum factors (the Ancylostoma Secreted Proteins or ASPs) have recently shown to be potent Th2 adjuvants in mice (Gause *et al.*, 2003), suggesting that nematode ASPs may be one of the classes of molecules that contribute to the strongly polarized Th2 response observed during chronic helminth infections in humans. In addition, much work on the humoral immune response has shown differences in the immune response to somatic and secreted antigens and a marked difference in the immune response to different developmental stage of the parasite within the host, as seen in filariasis (and cells Mahanty *et al.*, 1996). This search will be aided by the production of stage-specific recombinant antigens. Finally, available data suggests that parasitic nematode ASPs bind to host proteins thereby interfering with various ligand-receptor interactions (Loukas, A., unpublished observation).

3.1. Co-infection with other pathogens and immune modulation

The overlapping distribution of infection with hookworm and other pathogens results in a high rate of co-infection (Petney & Andrews, 1998). In laboratory studies there is evidence suggestive of both synergism and antagonism in nematode and other pathogens (Cox, 2001), and this may have implications for the epidemiology of multiple species in humans. It has been speculated recently that helminth infections, including hookworm, may adversely influence host immune responses to other infections, especially to intracellular pathogens such as *Mycobacterium tuberculosis*, and to viral infection, such as the Human Immunodeficiency Virus (HIV) (Bentwhich *et al.* 1995; Borkow and Bentwich, 2000). This has lead to the suggestion that helminths may increase the suspectibility to, and quicken the disease progression of, bacterial and viral diseases. This hypothesis is based on the observation that helminth infections result in eosinophilia, elevated IgE and mast cell activation, suggestive of the ascendance of a Th2 cytokine response and a concomitant suppression of a Th1 type response, which is often involved in protection against intracellular pathogens. Furthermore, the activation of eosinophils may also result in upregulation of CD4+ molecule on the cell surface, adding yet another facilitator for enhancement of HIV infection. This hypothesis remains at present to be fully supported by available evidence and there dissent from this view (reviewed in Fincham *et al.* 2003). An interesting recent study among HIV-1 infected individuals in Uganda provided evidence that changes in viral load and CD4+ T cell counts did not differ between individuals infected with *Schistosoma mansoni* and individuals uninfected (Elliott *et al.*, 2003). However, individuals treated for helminths, whether or not the helminth was still present, showed sustained CD4+ T cell counts. Determing the influence of hookworm and other helminth species and the effect of anthelmintic treatment on the development and disease progression of HIV/AIDS is clearly an important area for future investigation.

It has also been suggested that helminths may adversely affect immune responses to vaccine antigens. Ascariasis and onchocerciasis have been reported to reduce post-vaccination immune responses tetanus and cholera vaccine, respectively (Cooper *et al.*, 1999, 2001), and it has been suggested that the relatively low effiacacy of bacille Calmette-Guerin (BCG) vaccination in the tropics may result from infection with helminth species (Borkow and

Bentwich, 2000). Recent studies that show that immune responses to mycobacterial antigen purified protein derivative (PPD) are reduced in persons with concurrent helminth infection (Malhotra *et al.*, 1999; Elias *et al.*, 2001; Quinnell *et al.*, in press) lend support to this hypothesis. The study conducted in Papua New Guinea (Quinnell *et al.*, in press) provides evidence for a suppressive effect of both hookworm and *Plasmodium* infection on responses to PPD. Recent data from Brazil and Uganda suggest that BCG immunization may have a long term influence on susceptibility to helminth as demonstrated by a negative association of infection with hookworm and scar of BCG vaccination (Elliott *et al.*, 1999; Barreto *et al.*, 2000); although another study in Malawi found no evidence for an association (Randall *et al.*, 2002). It has also been suggested that maternal helminth infection may have an equally converse effect, suppressing Th-1 immune responses in infancy (Malhotra *et al.*, 1999).

Finally, helminths have recently been implicated in individual differences in suspectibility to malaria. Results obtained recently in Thailand by Mathieu Nacher and colleagues provide evidence that helminths are associated with protection against malaria-associated renal failure and jaundice, and that helminth infected patients had reduced sequestration and higher nitric oxides concentrations than individuals uninfected with helminths (reviewed in Nacher, 2002). It is suggested that helminth-infected patients were protected from severe malaria because differences of IgE concentrations. In another study on the western Thai border, where malaria transmission is low, it was demonstrated that the incidence of *P. falciparum* was increased in helminth-infected individuals, possibly mediated through a bias towards Th2 cytokines. The potential importance of these findings for the epidemiology of multiple parasite species in humans and for future malaria vaccines highlighted for the need for further investigation in well-characterised populations in other transmission settings. Working in southwestern Uganda, where malaria transmission is low and epidemic-prone, Sharpiro *et al.* (in press) investigated whether helminths increased susceptibility to clinical malaria. They found no association between the risk of malaria and helminth species after controlling for socio-economic status and household clustering of infections; this was true for individual helminth species or helminths as a whole. Unfortunately, this study may have been confounded by unobserved differences in nutritional status and further studies are clearly warranted. Interestingly, Quinnell *et al.* (in press) have shown that immune responses to *N. americanus* were downregulated by concomitant infection with *Plasmodium* spp. or *Wuchereria bancrofti*. In this study involving 81 individuals aged 5 years and over in village in Papua New Guinea, IL-4 and IL-5 responses to hookworm antigen were significantly lower in people coinfected with *Plasmodium* or filarial infeciton, and that total IgE levels were lower in people infected with *Plasmodium* spp..

4. EPIDEMIOLOGY AND TRANSMISSION DYNAMICS

Observed epidemiological patterns of hookworm infection are generated by the relative magnitudes of the many transmission, reproductive and mortality rates involved in the parasites's life cycle (see Section 2.2), as well as the dynamic interactions between populations of parasites and hosts in human communities (reviewed in Anderson & May, 1991)). These various rates and interactions can be summarized into a single composite measure of transmission, known as the basic reproductive number (R_0). For helminths, which do not replicate within their hosts, this is defined as the average number of female offspring produced by one adult female parasite that attain reproductive maturity, in the absence of density dependent constraints. It implies that transmission cannot be maintained unless R_0 is greater than one. It also means that the basic unit of transmission is the individual worm, and that understanding patterns of infection and disease is dependent on understanding why worm burdens vary in communities (Bundy and Medley, 1992).

Our knowledge of such transmission dynamics has been greatly aided by the development of mathematical models, which have been described and expanded in the work over the last 25 years of Roy Anderson and Robert May (Anderson and May, 1991). Coupled with decades of careful epidemiological investigation, this body of work has revealed that certain population characteristics can now be recognised as common to the epidemiology of hookworm and other helminth species: (1) age-intensity profiles are typically convex or reach a plateau in adulthood; (2) the distribution of worm burdens per host is extremely over-dispersed; (3); some individuals are predisposed to heavy (or light) infections; and (4) after chemotherapy reinfection rapidly occurs (see Section 7.2). For a more detailed description of the transmission dynamics of hookworm the reader is referred to reviews by Anderson (1982), Anderson and May (1991), and Bundy (1990). Here, the current focus is on summarizing epidemiological phenomenon and relating this to factors underlying observed patterns, as well as highlighting recent evidence.

4.1. Epidemiological patterns by age and sex

Studies have shown that children can be infected with hookworm as young as 6 months (e.g. Brooker *et al.*, 1999). Subsequently, infection prevalence typically rises monotonically with increasing age to a plateau in adulthood (Figure 1.a). Interestingly, recent evidence from studies of populations in China and Southeast Asia suggest that peak prevalence is observed among the middle aged, or even individuals over the age of 60 years (Figure 1.b). Such age-profiles of hookworm contrast to the rapid increase in prevalence seen for *A. lumbricoides* and *T. trichiura* where maximum prevalence attained in 5-10 year age classes, and prevalence remains relatively stable during adulthood (Figure 1.a).

However, the well-described non-linear relationship between prevalence and intensity of infection (Guyatt *et al.*, 1990) means that observed age-prevalence profiles provide little indication of the underlying profiles of age-intensity (Bundy, 1990). For *A. lumbricoides* and *T. trichiura* age-intensity profiles are strongly convex, with intensity rising to a peak around 5-10 years old and declining in adults (Bundy, 1990). In contrast, there appears to be considerable variation in age-intensity profiles seen for hookworm (Bundy and Keymer, 1991). In West Africa, for example, convex age-intensity profiles are observed (Udonsi, 1984; Behnke *et al.*, 2000), while in China intensity increases continues to rise throughout life and is highest among the elderly (Gandhi *et al.*, 2001; Bethony *et al.*, 2002a). However, these studies rely on counts of hookworm eggs in faecal samples and thus provide only an approximation of true hookworm burden (Anderson and Schad, 1985). To our knowledge, there have only been five estimates of hookworm burden using anthelminthic expulsion in age-stratified human populations (Figure 2). Generally, these limited data suggest that *N. americanus* worm burdens tend to increase in hosts up to age 15-25 years and remain constant thereafter. An exception to this trend is the study in China, which shows that worm burdens tend to continuously increase with age (Ye *et al.*, 1994). The observation that the elderly are at-risk for heavy hookworm burdens has potentially important implications for the rapidly changing demographics in the developing world.

Given that observed age profiles of worm burdens are known to reflect the balance between (1) the rate of exposure to infective stages, (2) the rate of successful establishment in the face of host immune responses (see Section 3), and (3) the rate of adult parasite mortality, and how these processes vary with host age (Anderson and May, 1991; Bundy and Medley, 1992), apparent geographical differences seen for hookworm age-intensity profiles presumably reflect variation in some of these processes. One possible explaination could be higher levels of exposure among the elderly in Asia. Though estimates of hookworm exposure are notoriously difficult to obtain (Hominick *et al.*, 1987), better assessment of infection exposure may provide further.

In accordance with other infectious diseases (Goble and Konopka, 1973), it has been suggested that males are more commonly infected with hookworm than females (Bundy, 1988a). However this suggestion has yet to be comprehensively investigated. Recent studies among non-human mammals, using meta-analysis techniques to synthesize published parasitological data, have reported a consistent male-bias in helminth parasitism, which is suggested to reflect differential susceptibility to infection arising from immunosuppression associated with male hormones (Poulin, 1996; Moore and Wilson, 2002). To investigate sex-differentials in helminth infections of humans a literature search was made of studies, which reported infection prevalence according to sex. To reduce age bias only studies of school-aged children were included. In the analysis of these published data prevalence of infection with hookworm infection was significantly higher overall in males than females with more negative (male-biased) than positive (female-biased) differences in prevalence (Figure 3). By contrast, the prevalence of *A. lumbricoides* and *T. trichiura* showed no significant difference between males and females, with remarkably little variation between the studies. This meta-analysis provides empirical support for an earlier assertion that males are more commonly infected with hookworm infection than females (Bundy, 1988a).

It is suggested that the sex-difference in hookworm infection is largely a consequence of sex-related differences in exposure rather than differences in susceptibility to infection. In particular, because hookworm infection is more prevalent among adults occupational exposure is likely to be important. In Mali, for example, males are more commonly infected than females (Behnke *et al.*, 2000) - an observation explained by the fact that males are involved in constructing houses which frequently incorporate human faeces into materials to strengthen household structure. In contrast, in South China (Gandhi *et al.*, 2001; Bethony *et al.*, 2002a) and Vietnam (Needham *et al.*, 1998) females exhibit higher hookworm intensities among specific age groups. In the case of Vietnam, elderly women were observed to be responsible for most of the night-soil use and the higher infection levels could be explained, in this instance on occupational exposure (Humphries *et al.*, 1997). Sex-based differences in infection patterns may not always reflect occupational exposure, however. For example, in Hainan, China women exhibit a higher prevalence and intensity of hookworm compared to males despite the absence of night-soil use (Needham *et al.*, 1998; Gandhi *et al.*, 2001; Bethony *et al.*, 2002). Disentangling the relative contributions of exposure and susceptibility to infection remains enigmatic, although careful consideration of sex differences in exposure patterns may provide useful insights.

4.2. Heterogeneity in worm burdens

As well as differences in infection patterns by age and sex, a key feature of hookworm and other parasites is heterogeneity in worm burdens per person (Anderson and May, 1991). Specifically, frequency distributions of hookworms within human populations are highly aggregated such that a minority of people will harbour many and most people will harbour few. This well-described phenomenon has implications for both parasite population dynamics and the planning of control programmes (reviewed in Anderson and May, 1991; also see Section 7.2).

An increasing number of reinfection studies also provide evidence that individuals are predisposed to heavy (or light) hookworm infection (Schad and Anderson, 1985; Haswell-Elkins *et al.*, 1987, 1988; Bradley and Chandiwana, 1990; Behnke *et al.*, 2000; Quinnell *et al.*, 1993, 2001). Evidence of significant predisposition to hookworm can be detected as long as 6-8 years after a single round of treatment, as demonstrated by results obtained recently in Papua New Guinea by Quinnell *et al.* (2001). In a reinfection study of *N. americanus* worm burdens were collected at 6 years and 8 years after treatment. Interestingly, however, no predisposition could be detected in individuals who had received repeated chemotherapy, suggesting that differences in hosts, in either exposure or susceptibility, which contribute

towards predisposition, are only consistent over the short-term. Other evidence shows that certain families are predisposed to heavy infection with STH than would be expected by chance (Forrester *et al.*, 1988; Behnke *et al.*, 2000). Interesting recent data from Brazil and Uganda show that the statistical degree of household clustering of heavy infection is less for hookworm than for either *A. lumbricoides* or *T. trichiura* (Sharpiro *et al.*, in press; Brooker, S and Bethony, J, unpublished observation). A final epidemiological feature of hookworm is geographical variation between populations and regions in the level of infection. For example, national school surveys in Cameroon and Uganda showed that marked variation in the prevalence of infection between schools, ranging from 0-100% (Figure 4).

The underlying causation of these patterns of aggregation, predisposition, clustering and geographical variation remains poorly understood. Heterogeneity in worm burdens may arise from either differences in exposure to infection (due to household, social-economic, climatic, environmental and occupational factors) or differences in susceptibility to infection and the ability to mount effective immune responses (due to genetic and nutritional factors). It is most likely that observed heterogeneities arise as a result of the combined effect of differences in environmental exposure and in suspectibility to infection between individuals (Bundy, 1988b; Anderson & May, 1991; Bundy and Medley, 1992). The next three sections will review these factors, highlighting recent evidence; although it is evident that more research is needed.

4.3. Household, socio-economic and occupational risk factors

Since the transmission of hookworm involves contamination of the environment by hookworm eggs, it is expected that risk factors for infection may include poor personal hygiene and household sanitation, which in turn are influenced by differences in socio-economic status. Some studies have demonstrated that hookworm infection is associated with the absence of a latrine (Olsen *et al.*, 2001; Chongsuvivatwong *et al.*, 1996), and low socio-economic status (Holland *et al.*, 1988). However the relationship between helminth infection and socio-economic status is by no means clear: work in Madagascar shows that *A. lumbricoides* is more influenced by age and sex than socio-economic status (Kightlinger *et al.*, 1998); a study in Kenya showed that variation in household income and education level of the head of household were not associated with any helminth infection (Olsen *et al.*, 2001).

Because of the high rates of hookworm infection among adults, occupation also has an important influence on hookworm epidemiology. In particular, engagement in agricultural pursuits remains a common denominator for human hookworm infection. Heavy infections in Sichuan Province, China and in Vietnam, for instance, are attributed to widespread use of faeces as night-soil fertilizer (Humphries *et al.*, 1997). Hookworm has also been noted to be more common in families who are involved with agricultural pursuits. The Chinese nationwide survey of 1988-1992, for instance, found the highest prevalence among vegetable growers and farmers (Hotez *et al.*, 1997). Even when faeces are not used deliberately, heavy faecal pollution of plantations occurs where few toilets are available resulting in high levels of hookworm infection (Schad *et al.*, 1983). For instance, in India, Bangladesh, and Sri Lanka, high rates of infection are observed among workers and their families in the tea gardens (Gilgen *et al.*, 2001).

4.4. Climatic and environmental factors

Since hookworm larval stages have limited motility, their rate of development and survival are dependent on the surrounding environmental humidity, temperature and ultra-violet light, such that geographical differences in transmission will be influenced by these factors and related factors such as rainfall, soil type and altitude. The influence of climate on

hookworm transmission was reviewed by Chandler (1929) and much can profitably still be gained by reading of this work. Chandler concluded that 20-30 °C was optimal for transmission with larvae reaching maturity in five days, with the lower limit lying between 8-10 °C and the upper limit 40-45 °C. Available experimental data indicate that above temperatures of 35-40 °C development of eggs arrests and death occurs (Nwosu, 1978; Udonsi and Atata, 1987; Smith and Schad, 1990). For example, Udonsi and Atata (1987) showed that at temperatures of 35 °C larvae of *Necator americanus* were all dead, with the highest cumulative hatching rates obtained at 30 °C. The apparent difference between these experimental findings and Chandler's observation can be explained by the ability of hookworm larvae to migrate downwards in the soil and to areas of shade. Thus, although the ambient temperature may exceed 35 °C hookworm larvae can still find refuge in cooler microhabitats.

Recent use of geographical information systems and satellite sensor data (remote sensing) has lead to a better understanding of the thermal geography of helminths. In Cameroon, Chad, Mali and Uganda, for example, *A. lumbricoides* and *T. trichiura* do not occur in areas where estimates of land surface temperature, derived from satellite sensors, exceed 37-40 °C (Ratard *et al.*, 1991, 1992; Brooker *et al.*, 2002a,b; Brooker *et al.*, in press). Hookworm however occurs throughout Cameroon and Uganda, most of Mali and the southern parts of Chad, suggesting hookworm has greater thermal geographical limits than *A. lumbricoides* and *T. trichiura*, lying around 45 °C. The lower thermal limit of hookworm in the tropics is often determined by altitude: altitudinal and coastal transects across KwaZulu-Natal Province, South Africa show that hookworm transmission is confined to the coastal plain below 150 m above sea level (Appleton and Gouws, 1996; Appleton *et al.*, 1999; Mabaso *et al.*, 2003). Above these altitudes low temperatures (<20 °C) limit the transmission of the parasite. The negative association between hookworm prevalence and altitude is corroborated by studies throughout Africa (see Brooker and Michael (2000)).

It has been noted, that *A. duodenale* will occur in some areas where *N. americanus* L3 cannot survive during the winter months. This includes Anhui Province of China where temperatures will on occasion drop below freezing (Yu, 1995). It has been postulated that the unique ability of *A. duodenale* L3 to undergo arrested development in the human host, may allow this species to survive during the cold winter months (Schad *et al.*, 1973).

Since hookworms are unable to survive desiccation, the amount of rainfall in an area is also an important factor influencing hookworm transmission, both spatially and temporally. The spatial relationship between rainfall (both total and distribution) and hookworm prevalence is well-established (Brooker and Michael, 2000). In some endemic areas, hookworm infection exhibits marked seasonality. For *A. duodenale*, which undergoes arrested development following the transmission during the rainy season, new infections appear 8-10 months after (Schad *et al.*, 1973). This pattern is believed to be an adaptation to a short transmission season in dry or cool places. The seasonal transmission pattern of *N. americanus* is generally considered to be longer in length and differs from that of *A. duodenale*. Studies in West Africa show that populations of L3 larvae are highest during the rainy season (Udonsi *et al.*, 1980) and faecal egg counts are highest 2-7 months after the rainy season (Knight and Merrett, 1981; Nwosu, 1981). However, although seasonal fluctutations in transmission occur, such fluctuations may be of little significance to the overall persistence of hookworm populations (Anderson, 1982). This is because the adult hookworm life-span is typically much longer (1-10 years) than the periods in the year during which the basic reproductive rate (R_o) is less than unity, and R_o will on average will greater than one, maintaining overall endemicity.

A final environmental factor that may influence the transmission of hookworm is soil type. It has long been believed that hookworm thrive in areas with sandy soils because of the small particle size and well-aerated texture of sandy soil. So although infective larvae quickly die on the surface of sandy soil in direct sunlight, they are able to rapidly migrate into the soil and during the rains are able to migrate vertically as moisture permits. In contrast, clay soils inhibit larval migration. Early evidence of an association between soil type and hookworm was provided by studies in the southern American States (reviewed in Brooker and Michael (2000)). Recent confirmation of this association has been provided by two studies in South Africa: Mabaso *et al.* (2003) found that high hookworm prevalence was significantly associated with well-drained sandy soil types whereas low prevalence was associated with clay soils; and Saathoff *et al.* (2002) found that hookworm infection is positively associated with sandy soils, while controlling for other environmental factors and socio-economic and behavioural factors.

4.5. Genetics of susceptibility

A growing number of studies are pointing to genetic factors in influencing susceptibility to helminth infections, including hookworm (Quinnell, 2003). Bethony and colleagues (Bethony J., unpublished results) using multi-household extended pedigrees from *N. americanus* endemic area in northeastern Brazil found that additive genetic factors play an important role in regulating infection and the humoral immune response to *Necator* infection. For example, additive genetic factors account for a significant proportion of the variation in fecal egg count: 49% heritability when modeled without household effects and 26% heritability when modeled with household effects. The significantly better fit of a model that included shared residence (household) and shared genes (genetics) indicate that both play important roles in determining the variance in fecal egg counts in the study population.

These data are consistent with quantitative genetic analyses of hookworm (Williams-Blangero *et al.*, 1997) and other helminth infections in humans, including *Ascaris lumbricoides* (Williams-Blangero *et al.*, 1999) and *Schistosoma mansoni* (Bethony *et al.*, 2001; Bethony *et al.*, 2002b). For example, Williams-Blangero *et al.* (1997) quantified the influence of genetic factors on patterns of hookworm infection intensity among 279 individuals of 62 families in a rural population in Zimbabwe. Using a variance decomposition approach, they estimated the heritability of hookworm intensity to be 37% (+/-9%), after correcting for possible confounding covariates. The relative importance of additive genetic effects in the Brazil population can be seen when they are compared to the role of the often studied covariate effects of age or sex, which accounted for only 4% of the variation in fecal egg counts.

Further, quantitative genetic analysis on this same Brazilian multi-household extended pedigree demonstrated that nearly half (~45%) of the variation in the IgG1, IgG4, and IgE responses to third stage larval or adult somatic antigen preparations were explained by the additive effect of genes. Bivariate genetic analyses showed strong genetic correlations between these *Necator*-related traits, suggesting that a common set of genes coordinately regulates the variation in the IgG1, IgG4, and IgE responses to larval and adult somatic antigen preparations. Conversely, no additive genetic effects were observed for the antibody responses to adult excretory secretory (ES) products. The study in Brazil by Bethony *et al.* (unpublished observations) also demonstrated strong genetic correlations between the IgG1, IgG4, and IgE responses to somatic antigen preparations and faecal egg counts. The magnitudes of the heritabilities for these traits as well as the strong genetic correlations indicate that antibody response to somatic antigen preparations and the intensity of the infection are driven by shared genes. While these studies do not identify the specific loci responsible for these observations, they quantify the large extent to which variation in the

infection intensity and the committing antibody response is regulated by additive genetic effects that may have an underlying set of common genes.

5. PUBLIC HEALTH CONSEQUENCES

The morbidity associated with hookworm infection is varied and ranges from mild, transient clinical signs and symptoms to severe clinical disease, as well chronic and insidious effects on the growth, learning and productivity of individuals. The precise form of morbidity is closely related to the chronology of cutaneous infection, migration and development (for a review of the older literature, see Miller, 1979), and, as mentioned above (Section 4), the severity of morbidity depends on the intensity of hookworm burden. In overall public health terms, the overall impact of hookworm infection will ultimately depend on the underlying health status of certain populations, making them particularly vulnerable.

5.1. Acute infection

In areas of high transmission, repeated waves of *N. americanus* and *A. duodenale* L3 entry through the skin can result in a cutaneous syndrome known as ground itch. This comprises a pruritic erythematous papulovesicular rash. Ground itch appears most commonly on the hands and feet. In contrast to infection by the anthropophilic hookworm, zoonotic infection with *A. braziliense* L3 results in cutaneous larva migrans (CLM), which is characterized by serpiginous burrows appearing most frequently on the feet, buttocks, and abdomen (Blackwell and Vega-Lopez, 2001). It is not known whether other animal hookworms, such as *A. caninum* and *Uncinaria stenocephala* are also significant causes of CLM; however, in an experimental infection, the placement of 200 *A. caninum* L3 on the skin did not result in the appearance of serpiginous cutaneous lesions (Landmann and Prociv, 2003). CLM has been reported with increased frequency among travellers returning from the Caribbean resorts and among residents along the Atlantic and Gulf coasts of the United States (Yosipovitch *et al.*, 2002). A second form of CLM associated with folliculitis has also been reported (Caumes *et al.*, 2002).

Following their entry, human hookworm L3 undergo larval pulmonary migration, which can be accompanied by cough, sore throat and fever (Miller, 1979). Pulmonary hookworm infection resembles Loeffler's pneumonitis because of its association with lung eosinophils. Hookworm pneumonitis is usually not severe although it may last for more than a month until the L3 leave the lungs and enter into the gastrointestinal tract. Entry of hookworm L3 entry into the gastrointestinal tract and their development into adult hookworms frequently results in epigastric pain (Anyaeze, 2003). Monitoring of the clinical responses of five normal human volunteers exposed percutaneously 50 *N. americanus* L3 revealed that abdominal pain, as well as other gastrointestinal symptoms (e.g., flatulence, and nausea) peak between days 30 to 45 post-infection; abdominal pain precedes the appearance of eggs in the faeces, which appear between days 48-58 post-infection (Maxwell *et al.*, 1987). Blood eosinophil counts peak beginning with the onset of gastrointestinal symptoms and continue until egg patency (Maxwell *et al.*, 1987).

When *A. duodenale* infection occurs via the oral route, the early L3 migrations sometimes produce a syndrome known as Wakana disease, which is characterized by nausea, vomiting, pharyngeal irritation, cough, dyspnea and hoarseness (Harada, 1962). It is not clear whether the pulmonary manifestations of Wakana disease occur because of pulmonary migrations following oral ingestion, or because of allergenic responses to orally ingested L3. In contrast, oral ingestion of 100 *A. caninum* L3 was shown to produce only mild gastrointestinal discomfort and no respiratory symptoms (Landmann and Prociv, 2003).

5.2. Hookworm-induced protein loss

In their review of the nutritional consequences of hookworm infection, Albonico and Savioli (1997) concluded that although hookworm may cause a loss of protein by plasma being directly ingested by the adult worm, hookworm rarely contributes to malnutrition with the important exception of iron deficiency anaemia (see section 5.3). Consquently, observed improvements in appetite and food intake and subsequent weight gain following anthelminthic treatment from studies in schoolchildren (Stephenson *et al.* 1989; Hadju *et al.*, 1996; Stoltzfus *et al.*, 1997a) may reflect treatment-associated changes in iron-deficiency. However, in some areas of high transmission with heavy worm burdens hookworm-induced protein loss is substantial and may result in hypoproteinemia leading to edema, or even anasarca. This phenomenon was well described in the older accounts of hookworm disease in China and elsewhere (Hotez, 2002). In addition, hookworm-associated protein loss results in weight loss among vulnerable populations. For example, data from a study among hookworm-infected pregnant women in Sierra Leone show weight gain following treatment (Torlesse, 1999).

5.3. Hookworm anaemia

Intestinal blood loss is the major clinical manifestation of human hookworm infection (for a review of studies, see Roche and Layrisee (1966), Miller (1979), and Crompton and Stephenson (1990)). Heavy hookworm infections or moderate infections in patients with underlying iron and protein nutritional deficiencies results in hookworm disease, the clinical entity that specifically refers to the resulting iron deficiency and microcytic, hypochromic anaemia (Beaver *et al.*, 1984). The attachment of hookworms' cutting organs to the intestinal mucosa and submucosa and the subsequent rupture of intestinal capillaries and arterioles causes blood loss. The secretion of factor Xa and VIIa/TF inhibitors, and anti-platelet agents by the parasite helps to maintain continuous oozing of blood at the hookworm attachment site and the free flow of blood through the parasite alimentary canal (Stanssens *et al.*, 1996; del Valle *et al.*, 2003). The hookworm ingests some of the extravasated blood. The extent of red blood cell lysis during passage through the parasite gut is controversial. Although many cells pass out of the worm still intact, many others are also lysed. The free haemoglobin is digested through the concerted action of aspartic-, cysteine- and metallo-hemoglobinases (Jones and Hotez, 2002; Williamson *et al.*, 2003a; 2003b).

Because of their underlying iron and nutritional status, women of child-bearing age, pregnant women and children are frequently the ones most susceptible to developing hookworm anaemia. Among school-aged children and adults in resource-poor countries, where host iron stores are often depleted, there is a well-established intensity-related relationship between hookworm infection and blood loss (Roche and Layrisee, 1966; Crompton and Whithead, 1993; Stoltzfus *et al.*, 1996) and haemoglobin (Crompton and Stephenson, 1990; Stoltzfus *et al.*, 1997b). Hookworm burdens of 40 to 160 worms are usually sufficient to cause anemia, although this depends on host iron stores (Lwambo *et al.*, 1992), and the species of hookworm (Albonico *et al.*, 1998).

With the increased physiological demands for iron during pregnancy, pregnant women are particularly at risk from anaemia. In many developing countries, severe anaemia in pregnancy is an important contributor to maternal mortality, especially around the time of delivery. It also adversely affects women's health, making women tired, breathless and less able to work and care for their children (Shulman 1999). Severe anaemia is also associated with reduced birthweight, which in turn is an important risk factor for infant mortality. Studies conducted amongst pregnant women suggest that hookworm in pregnant women can be prevalent and can contribute significantly to anaemia. Of the five studies located in the literature, there was a strong association between intensity of infection and anaemia, with an

increasing severity of anaemia at higher intensities of hookworm infection (Jackson and Jackson, 1987; Sill *et al.* 1987; Shulman *et al.* 1996; Stoltzfus *et al.* 1997c; Egwunyenga *et al.*, 2001). The study in Kenya also highlighted the importance of parity group, where multigravidae were shown to have higher intensities of hookworm infection than primigravidae (Shulman *et al.* 1996). Subsequent analysis of the data from the Kenyan coast demonstrated that whereas malaria was only a significant predictor of haemoglobin levels in primigravidae, hookworm was only a significant predictor in multigravidae (Geissler *et al.* 1998).

Hookworm has traditionally been considered relatively unimportant in contributing towards the anaemia among pre-schoolchildren. More typically *Plasmodium falciparum* malaria is the important aetiological factor. Two recent analyses of anaemia in pre-school populations have challenged this perception however and shed new light on the aetiology of anaemia in these populations. In coastal Kenya, Brooker *et al.* (1999) found that 28% of pre-schoolchildren were infected with hookworm, that 76% were anaemic and that egg counts of >200 epg were associated with anaemia and severe anaemia (Figure 5). In Zanzibar, Tanzania, Stoltfuz *et al.* (2000) found that low haemoglobin was associated with malaria parasitaemia in children <30 months, and with hookworm intensity in children aged 30-71 months (Figure 5). The aetiological differences between the two age groups were suggested to reflect the relatively light hookworm infection among younger children and the development of partial immunity to malaria among older children. Importantly, this study also assessed indicators of iron deficiency, serum ferritin and erythrocyte protoporphyrin, and found a similar negative association between these indicators and hookworm intensity.

5.4. Perinatal hookworm

Hookworm anemia during pregnancy has also been linked to adverse maternal-fetal consequences including prematurity, low birth weight, and impaired lactation (Miller, 1979). Results of a prospective study in Sri Lanka showed that treatment mebendazole was associated with a lower proportion of stillbirths, perinatal deaths and low-birthweight babies (de Silva *et al.*, 1999). A study of Nigerian pregnant women, found that mothers with malaria infection but without helminths had neonates of higher mean birth-weights than those infected with malaria and helminths and that this effect was more pronounced in primigravids (Egwunyenga *et al.*, 2001). Although the authors they did not differentiate between separate helminth species, the effect is likely to be due to hookworm because of its specific effect on anaemia. In China, hookworm infection during pregnancy has been reported to result in lactogenic transmission to neonates (Yu *et al.*, 1995). This occurs because *A. duodenale* L3 can arrest their development in human tissues (Schad *et al.*, 1973); with parturition the L3 enter the mammary glands and milk. Neonatal infection resulting from vertical transmission of hookworm results in severe disease associated with profound anaemia (Yu *et al.*, 1995).

5.5. School performance and productivity in adulthood

In addition to clinical effects, severe and chronic infection with hookworm and other STH during children's development also has consequences for their cognitive performance and ultimately their educational achievement. As early as the first half of the twentieth century it has been suggested that hookworm infection might impair educational performance (Waite and Neilson, 1919; Smillie and Spencer, 1925), and recent studies conducted throughout the developing world have provided evidence that schoolchildren infected with helminths perform poorly in tests of cognitive function (Watkins and Pollitt, 1997; Drake *et al.*, 2000). For example, Sakti *et al.* (1999) studying 432 children from 42 primary schools in Indonesia found that children infected with hookworm performed significantly worse in tests

associated with working memory than children without hookworm infection, even after controlling for nutritional status and socio-economic status.

The effect on cognitive function may occur as a result of one or a combination of symptoms associated with infection, namely iron deficiency anaemia (IDA) and growth retardation. In turn, there is much evidence that these conditions are associated with impaired cognitive performance and educational achievement (Lozoff, 1990; Mendez and Adair, 1999; Grantham-McGregor and Ani, 2001). The most persuasive evidence for a causal relationship between helminth infection and cognitive function is provided by randomised controlled trials. Trials conducted in school-age populations suggest that anthelmintic treatment improves cognitive function for children with only the heaviest worm burdens or those afflicted with nutritional deficiencies in addition to worm infections (Drake *et al.*, 2000). Notwithstanding the general trend of these results, we still lack unequivocal evidence that anthelmintic treatment improves cognitive function (Dickson *et al.*, 2000). It is likely that for most children, treatment alone cannot eradicate the cumulative effects of lifelong infection nor compensate for years of missed learning opportunities. Deworming does not lead inevitably to improved cognitive development but it does provide children with the potential to learn. A recent study conducted by the Partnership for Child Development in Tanzania has shown that children who were given deworming treatment did not improve their performance in various cognitive tests but did benefit more from a teaching session in which they were shown how to perform the tests (Partnership for Child Development, unpublished observation). This suggests that children are more ready to learn after treatment for worm infections and that they may be able to catch up with uninfected peers if this learning potential is exploited effectively in the classroom.

Because hookworm typically occurs in agricultural settings in which cash crops are cultivated in plantation style (e.g., coffee, cocoa, sugar, tea, rubber), the older literature contains numerous references to its effects on workers (Chandler, 1929). In her comprehensive review, Guyatt (2000) highlights the ways in which infection can affect productivity and wage-earning capacity in adults, either directly or indirectly through an effect of early ill-health in childhood (Figure 6), but notes that there is little or no direct evidence that helminth infections lower the productivity of adults, in part due to the lack of well-designed studies. However, some of the health consequences of hookworm, especially anaemia and undernutrition, are known to affect productivity: several field studies show a strong impact of IDA on decreased aerobic work capacity and to a lesser extent on economic productivity (reviewed in Haas and Brownlie (2001). A recent study among Bangladeshi tea pluckers found a negative association between intensity of major nematode species (*A. lumbricoides, T. trichiura* and hookworm) and both haemoglobin and measures of labour productivity, and that haemoglobin and productivity were positively associated (Gilgen *et al.*, 2001). However, in a four-way trial design both anthelminthic treatment and iron supplementation increased haemoglobin but did not have a measurable effect on labour productivity, although this was suggested to reflect seasonal nutritional stress which occurred in the latter part of the trial (Gilgen and Mascie-Taylor, 2001). Even where IDA does not result in reduced productivity, the higher energetic cost of undertaking work may reduce non-economic activities such as childcare and leisure.

An area which remains inadequately investigated is the link between early childhood infection and ill-health and subsequent productivity. An illustration of the potential relationship is provided by the experience of hookworm control in southern United States. At the dawn of the 20th century, the prevalence of hookworm in the rural southern United States was between estimated to be 44-59% among school-age children (McGuire and Elman, 2003). By examining data from surveys of the Rockefeller Commission, Bleakley (2002) has undertaken a non-experimental evaluation of the economic impact of hookworm

control in the southern States. His study estimated that children cured of hookworm were 25% more likely to attend school than similar children who weren't, and by 1940 a hookworm-free childhood translated into 45% higher earnings. Little is known at present about the economic impact of hookworm and its control in modern-day resource poor countries, and longitudinal studies investigating the long-term effects of hookworm and the benefits of anthelmintic treatment are needed to provide a clear understanding of the non-health impact of hookworm.

6. GLOBAL DISTRIBUTION AND DISEASE BURDEN

6.1. Paleoparasitological evidence

Human intestinal parasites have found their ecological niche in humans for possibly thousands of years. In studying parasites in archaeological material, paleoparasitiology provides evidence on the historical occurrence of parasites. Summarizing the available literature Gonçalves *et al.* (2003), report finds of *N. americanus* and *A. duodenale* from sites in both the New and Old World, including North and South America and Europe. No finds have been made in Asia or Africa but this is a probable reflection of an absence of investigation than an absence of occurrence.

An ongoing controversy in paleoparasitology is the occurrence of hookworm in the New World prior to 1492. Gonçalves *et al.* (2003) present several lines of evidence of hookworm finds dating back before pre-Columbian times, with the oldest find in faecal material from Brazil, dating to about 7230 ± 80 years ago. Such finds had previously been disputed by Fuller (1997) who argues that these finds represent mis-identification and that hookworm was one of the many parasites brought to the New World after contact in 1492. The debate crucially centres on how hookworm arrived in the New World. Given the precise climatic requirements for the completion of the hookworm life cycle, and in contrast to the robustness of the eggs of *T. trichiura* and *A. lumbricoides* (Bundy and Cooper, 1989), it is unlikely that *N. americanus* was transported along a cold trans-Berengian route. However, it has been postulated that through the process of arrested development in human tissues, *A. duodenale* infections could survive this passage (Hawdon & Johnston, 1996). Other workers suggest a trans-Pacific route (Gonçalves *et al.*, 2003) - a claim yet to be substantiated.

6.2. Contemporary global distribution

The current global picture of hookworm prevalence is shown in Figure 7. This indicates that hookworm is particularly prevalent throughout much of sub-Saharan Africa as well as in South China, the Pacific and Southeast Asia. However, this picture may underestimate the true global distribution. For example, very little information has been published in the international literature on current levels of infection in the former Soviet Union and Eastern Europe. However, a recent national survey of schoolchildren in Afghanistan found that although *A. lumbricoides* ranged from 12-69% and *T. trichiura* ranged from 2-27%, hookworm only occurred in selected schools in the eastern part of the country, at 6% prevalence (WHO, unpublished observations). It is also worth emphasizing that country-level estimates of prevalence are not meant to reflect accurately small-scale within country variation in distributions, or necessarily be representative of a country as a whole.

Regarding species distribution, *Necator americanus* is the predominant hookworm worldwide, except in some defined locations where *A. duodenale* is focally endemic. The major regions endemic for *N. americanus* include South and Southwest China, South India, Southeast Asia, sub-Saharan Africa, and Central and South America. Coastal areas of these regions are especially associated with high necator transmission (Lwambo *et al.*, 1992). The *A. duodenale* predominant regions include more northerly latitudes of South and West China (e.g., Anhui, Sichuan Provinces) and India (e.g., Kanpur) where *N. americanus* may not

survive the relatively harsh conditions. *A. duodenale* may survive in these harsher climates because of its ability to undergo arrested development in host tissues during periods of dryness or cold. *A. duodenale* infections also occur in Egypt, Northern Australia and in a few localities in Latin America including Northern Argentina, Paraguay (Labiano-Abello *et al.*, 1999), Peru, and in a region bordering El Salvador and Honduras (Kaminsky, R., unpublished results). Throughout the world, mixed infections with both major hookworm species are common.

6.3. Disease burden

Updated 2003 numerical estimates suggest that hookworm infections remain exceedingly common with over 740 million infections globally (de Silva *et al.*, 2003; Table 3). China and sub-Saharan Africa have the largest number of cases of hookworm with about 200 million infections each. Noteworthy, these figures differ from previous estimates, which, using older data, estimated 1297 million infections (Chan *et al.*, 1994a). The decrease is most apparent in Asia and Latin America, presumably due to improvements in socio-economic status and water and sanitation, as well as control efforts. Somewhat disappointing from a public health perspective, there appears to have been little change in prevalence rates in sub-Saharan Africa.

In the absence of direct measurements, current estimations of potential morbidity due to hookworm have followed the approach developed for *A. lumbricoides* by Guyatt *et al.* (1990). This work determined the relationship between prevalence of *A. lumbricoides* infection and mean worm burden and showed that the relationship is non-linear and is determined by the overdispersed frequency distribution of worms within human communities. The approach assumes that people with higher intensity infections are at greater risk of morbidity. A threshold worm burden is defined above which morbidity is likely to occur (Guyatt and Bundy, 1991). This approach was subsequently employed by Lwambo and colleagues (1992) to estimate the morbidity to hookworm in East Africa. Investigation of the relationships between prevalence, worm burden and haemoglobin allowed the authors to estimate hookworm burdens associated with hookworm anaemia. These methods were expanded by Chan and colleagues (Chan *et al.*, 1994a) who developed a mathematical framework for estimating the global potential morbidity due to hookworm and other intestinal nematodes based on the observed prevalence of infection. This approach included age-dependent worm burden thresholds above which morbidity occurs, as well as incorporating spatial heterogeneity in levels of infection. The estimates for hookworm showed that between 1.5-2.4% of the exposed population suffered from morbidity due to hookworm, and as expected, morbidity is much higher in adults.

This quantification has allowed comparisons with other diseases to be made. Recently, the Global Burden of Disease (GBD) study has used a common method of assessment of disease burden (Murray and Lopez, 1994). This provides a standardized evidence base for comparing the impact on morbidity and mortality of the various packages of health care that may be provided for specific diseases. The indicator used to quantify the global burden of disease is termed the ‘disability-adjusted life year’ (DALY), and is a summary measure of population health, which combines in a single indicator years of life lost from premature death (YLL) and years of life lived with disability (Murray and Lopez, 1994). DALYs translate disabilities into years of healthy life lost by giving each disease state a disability weight ranging from 0 (healthy) to 1 (death). Estimates are provided for each sex and five different age groups, thus take into account changes in the distribution and severity of disease over the life span by including age-specific incidences, discounting and age disability weights, and are also provided for different regions of the world.

According to the 2002 GBD study, hookworm caused the loss of 1.8 million DALYs worldwide in 2001, of which 23% (0.41 million DALYs) were lost in sub-Saharan Africa and 47% (0.86 million DALYs) were lost in Southeast Asia (WHO, 2002). The range of sequelae specified in these estimates included anaemia and cognitive impairment, which are both assigned a disability weight of 0.024. Table 4 lists the estimated DALYs and deaths from the major communicable diseases included in the GBD study and indicates that nearly 24.5% of the total world disease burden (due to all causes) was contributed by communicable diseases, including helminth infections. These data reveal that hookworm is the most significant parasitic infections of humans with the exceptions of malaria, leishmaniasis and lymphatic filariasis.

However, even these DALY measurements probably underestimate the true disease burden due to hookworm. Iron-deficiency anaemia is one of the leading causes of morbidity in the developing world, associated with more than 12 million DALYs annually. Accurate incorporation of the contribution of hookworm to anaemia together with anticipated progress in the elimination of lymphatic filariasis, suggest that hookworm may soon emerge as the second most important parasitic infection of humans, after malaria.

The most severe consequence of hookworm infection is severe iron-deficiency anaemia (IDA), and this is suggested is responsible for 20% of maternal deaths worldwide (Crompton, 2000). It is difficult however to identify the specific aetiology of anaemia-related deaths. In terms of numerical estimates, widely differing rates have been reported for the frequency with which hookworm-related anaemia causes death, ranging from 0.1 to 0.5 per 1000 infections per year (Miller, 1979; Pawlowski *et al.*, 1991). In 1992, WHO estimated that 65,000 deaths per year are due to hookworm (WHO, 1992), and more recently, the Global Burden of Disease study has attributed 3,000 deaths due to hookworm (WHO, 2002). However the data sources and methods by which these different estimates are derived are not described, and it remains unclear why such wildly different estimates of mortality exist.

6.4. Impact of economic development and poverty

In common with most parasitic diseases, hookworm is intimately linked to poverty and economic underdevelopment. While is it widely assumed that the Rockefeller Hookworm Eradication Campaigns were responsible for eradicating hookworm among the southern states of the United States in the first half of the twentieth century, almost certainly it was the overall economic development of the new South that finally halted hookworm transmission. Similar economic reforms that transformed South Korea and Japan during the 1960s and 1970s combined with specific control programmes, helped eradicate STH from these countries (Hara, 2001).

Because the 1990s has been one of the most productive periods in China's modern economic history, it is of interest to determine how China's development has affected hookworm prevalence (Hotez, 2002). China's eastern province of Jiangsu illustrates the possible impact of economic development in the reduction of hookworm prevalence (Sun *et al.*, 1998). As part of China's nationwide survey of human parasites conducted in 1990, the overall prevalence of hookworm infection in Jiangsu was 32%. However, in the village of Yaojiakon (Wujian County) the prevalence was determined to be 55%. In 1990, this southern Jiangsu village was heavily dependent on agriculture, with the major crops being rice, wheat, rapeseed, and mulberry leaf cultivation (for the silkworm industry). When the residents of Yaojiakon were examined seven years later, there was a dramatic reduction of their hookworm prevalence to 12%. The dramatic reduction is believed to have resulted from its economic prosperity (Sun *et al.*, 1998). Because of its southern Jiangsu location with close proximity to greater metropolitan Shanghai, Yaojiakon and surrounding areas

underwent dramatic economic development between 1990 and 1997. The impact was particularly great for young adults between the ages of 20 and 40 years who left agricultural pursuits in favor of commercial enterprises; the heaviest hookworm infections were in residents over the age of 50 (Sun *et al.*, 1998). The economic reforms in southern Jiangsu starkly contrast with the situation in many rural parts of Sichuan, Yunnan, and Hainan Provinces where both the economy as well as the prevalence and intensity of hookworm infection have remained stagnant between the early 1990s when first surveyed and later in the decade when these regions were re-examined (Hotez, 2002).

Globally, a negative correlation between hookworm infections and income level is clearly demonstrated in cross-country comparisons (de Silva *et al.*, 2003). However, causality cannot be inferred from this established relationship, since lower incomes and higher poverty promote higher infection levels, and there remain few well-documented studies of the impact of economic well-being and poverty on hookworm, or vice versa.

7. STRATEGIES FOR CONTROL

In the past, the objective of hookworm control was to eradicate the infection. However, hookworm and other helminths are extremely difficult to eradicate in communities where poverty and inadequate water and sanitation prevail, due to the high transmission potential of these parasites (Anderson and May, 1991). As a consequence, the current control strategies for hookworm and other STH have shifted from eradication to controlling morbidity, using chemotherapy, supported by health education and improved water and sanitation. The design and implementation of chemotherapy programmes has greatly benefited from an improved understanding of the transmission dynamics of helminths, and mathematical models have been used to explore the consequences of control (Anderson and May, 1991). Furthermore, recent positive experiences of large-scale control programmes have shown that morbidity caused by hookworm can be significantly reduced through regular chemotherapy, and has now resulted in major global initiatives being launched. There are also now research efforts to develop anti-hookworm vaccines.

7.1. Sanitation and footwear

Undoubtedly improved sanitation and hygiene are essential for the long-term control of parasitic diseases. However, although the availability of latrine facilities is associated with lower hookworm intensities (Chongsuvivatwong *et al.*, 1996), the impact of introducing sanitation on infection levels may only be evident after decades however (Esrey *et al.*, 1991) and may not be completely effective. For example, in one study hookworm prevalence declined only 4% after the introduction of latrines (Huttly, 1990). Despite associations between hookworm infection and use of footwear to protect from exposure to infective larvae, there is debate as to whether promotion of footwear is an effective control strategy (Albonico *et al.*, 1999). Furthermore, the impact of footwear on interrupting hookworm transmission has probably been overestimated, given that *N. americanus* infective larvae penetrate all aspects of the skin and *A. duodenale* larvae are orally infective. The long time required for improved sanitation and behaviour change to occur necessitates a need for a quick-acting, medium-term measure to control helminth infections, namely chemotherapy (Albonico *et al.*, 1999).

7.2. Chemotherapy

Their broad spectrum of activity, low cost, high efficacy and ease of administration mean benzimidazole anthleminthics are the current cornerstone of helminth control (Savioli *et al.*, 2002). This is particularly true for resource-poor nations that cannot afford expensive sanitation measures. The range of recommended drugs and their mode of action,

pharmacokinetics, efficacy and side-effects is well summarized by Albonico *et al.* (1999) and Utzinger and Keiser (2004). Four anthleminthics are available for the treatment of hookworm: albendazole, levamisole, mebendazole and pyrantel pamoate. Previously, the use of benzimidazole drugs was contraindicated in pregnancy because of uncertainty over possible teratogenicity. However, a recent WHO consulatation have reviewed the evidence and concluded that pregnant and lactating women should be considered a high-risk group and included in treatment campaigns (Allen *et al.*, 2002).

Because albendazole and mebendazole are provided as a single-dose tablet and patients do not need to be weighed, most control programmes are based on the use of these drugs. Two recent comprehensive reviews have assessed the efficacy of albendazole and mebendazole against hookworm and other STH (Bennett and Guyatt, 2000; Horton, 2000). In their review Bennett and Guyatt (2000) assessed relative efficacy of 400mg single-dose albendazole, 500mg single-dose mebendazole and multiple-dose mebendazole. Whereas all drug regimens were highly effective against *A. lumbricoides*, the patterns for *T. trichiura* and hookworm were more variable. New studies conducted in Zanzibar, Tanzania indicate that the efficacy of mebendazole decreases with frequent and repeated use (Albonico *et al.*, 2003). Reviewing albendazole alone, Horton (2000) found a wide variation in cure rates (33.3-100%) but remarked that substantial reduction in egg count was observed. He noted that very heavy infection with *N. americanus* may require more than one dose to achieve complete cure.

Varying cure and egg reducation rates have also been reported for levamisole and pyrantel pamoate (Utzinger and Keiser, 2004). Interestingly, results from recent trials conducted among Zanziabri schoolchildren show that pyrantel-oxantel (Albonico *et al.*, 2002) and levamisole (Albonico *et al.*, 2003) have poor efficacy rates in curing hookworm (9-12%) and only moderate efficacy in reducing hookworm egg count rates (66-67%). Studies show that ivermectin, best known for treating filariasis, is also effective against *A. lumbricoides* but it less efficacious against *T. trichiura* and hookworm (Utzinger and Keiser, 2004). Diethylcarbamazine (DEC), another anti-filariasis treatment, has negligible effect on hookworm egg counts (Meyrowitsch and Simonsen, 2001; Mani *et al.*, 2001). An interesting study from Cote d'Ivoire has suggested that praziquantel, the drug of choice for the treatment of schistosomiasis, may also reduce hookworm intensity (Utzinger *et al.*, 2002). Finally, a new anthelmintic, tribendimidine resulted in cure and egg reduction rates of 66-99% (Sun *et al.* 1999); findings which clearly warrant further investigated. There is also a need for research and development of novel anthelmintics, as well as further studies on the comparative efficacies of different drug combindations versus single drug treatments (Utzinger and Keiser, 2004).

The current and planned widespread use of benzimidazoles for treating hookworm and other STH in human populations has raised concerns of the potential of drug resistance (Albonico, 2003). Although there have been recent reports of mebendazole drug failures in *N. americanus* in Mali (De Clerq *et al.*, 1997), and pyrantel pamoate failure against *A. duodenale* in Western Australia (Reynoldson *et al.*, 1997), as well as diminished efficacy with repeated targeted treatments in Zanziibar (Albonico *et al.*, 2003), conclusive evidence of drug resistance in humans has yet to be provided (Geerts and Gryseels, 2001). By contrast, benzimidazole drug resistance as a consequence of mutations in nematode tubulin alleles is now a well-described phenomenon among parasitic nematodes of sheep and cattle (Conder and Campbell, 1995). Although findings of drug resistance in veterinary helminths cannot to extrapolated directly to human studies, they can provide important lessons on measures to offset the development of drug resistance, including treatment frequency, population targeting of treatment, underdosing and switching the drug used (Geerts and Gryseels, 2001).

As mentioned above, how best to use available anthelminthics to control disease within human communities has benefited from an improved understanding of helminth transmission dynamics (Anderson and May, 1982; Anderson and Medley, 1985; Anderson and May, 1991; Chan *et al.*, 1994b). Theoretical studies have shown that the precise impact of drug treatment will depend on the proportion of the population treated and the efficacy of the anthleminthic. Because distributions of helminths are highly aggregated within human communities, targeted treatment of the heavily infected groups will have the greatest impact. Experience over the last two decades across a range of epidemiological settings has shown that the simplest and most practical form of targeting involving school-based treatment (Partnership for Child Development, 1997; Savioli *et al.*, 2002). Based on this principle, at the World Health Assembly in 2001 a resolution was put forward (Resolution 54.19) urging member states to provide regular treatment to high-risk groups. The global target is to regularly treat at least 75 percent of all school-aged children and other high-risk groups with either albendazole or mebendazole alone or together with praziquantel by 2010. A practical rationale for focusing on school-age children is that this population has the highest intensity of overall helminth infections of any single age group, and that schools provide a cost-effective way to deliver anthelminthics (Warren *et al.*, 1993). Recent analysis has demonstrated that the cost of school-based treatment, including delivery costs, where both STH and schistosomiasis are treated is less than $1 per child treated per year, and that STH treatment alone can cost as little as $0.10 per child treated per child per year (Partnership for Child Development, 1999). For further details of the costs and cost-effectiveness of control, the reader is referred to the reviews by Guyatt (2001) and Bundy *et al.* (2002). Notwithstanding, the low costs of treatment, fulfilling the mandate of Resoution 54.19 would still be an expensive venture, and would become the largest and most ambitious human health program ever undertaken (Horton, 2003).

In contrast to other STH, hookworm is more common in adults such that school-based treatment programmes may be less effective in the control of morbidity due to hookworm. Modelling work by Chan *et al.* (1997a) of hookworm transmission dynamics with varying mixing patterns and rates of contamination and infection between children and adults has explored the consequences of different age-targeting treatment strategies. Analysis of field data from Zimbabwe found that that some degree of mixing between age groups does occur, which will reduce the impact of either schoolchild or adult targeted treatment since an untreated reservoir of infection remains. Targeting only adults had a bigger impact on schoolchildren than the other way round and a school-based strategy may be unlikely to limit morbidity due to hookworm among adults. The practical implication of this work is that in areas of high hookworm prevalence school-based treatments may be insufficient and community treatment, although logistically more difficult, is preferred.

In public health terms, several treatment trials have clearly shown that regular chemotherapy can reduce the morbidity associated with hookworm infection. This impact is most clearly demonstrated for anaemia. In Kenya, anthelminthic treatment reduced intensities of hookworm and other helminth infections and improved haemoglobin among schoolchildren (Stephenson *et al.*, 1989). In Zanzibar thrice-yearly treatment yielded a 55% reduction in severe anaemia among schoolchildren relative to controls and significant improvements in serum ferritin (Stoltzfus *et al.*, 1998). This improvement compared to a 23% reduction among children who received twice-yearly treatment. Treatment additionally led to small improvements in growth (Stoltzfus *et al.*, 1997a). Not only children can benefit from treatment. In a recent study among pregnant women in Sierra Leone anthelmintic treatment increased haemoglobin concentration by 6.6 g/L, relative to controls (Torlesse and Hodges, 2000). In Sri Lanka treatment improved both the health of mothers and their birth outcomes (de Silva *et al.*, 1999).

As well as evidence from trials, several operational programmes demonstrate the impact on anaemia of regular treatment, delivered through school health programmes or community-based programmes: in Tanzania, a school-based treatment programme caused a 26% reduction in anaemia and 47% reduction in severe anaemia 15 months post-treatment (Guyatt *et al.*, 2001); in Western Australia, a six and half year community-based treatment programme resulted in a 38% reduction in anaemia over six and half years (Garrow *et* al., 2001).

A more immediate insight into the potential benefits of treatment in other settings can be ascertained from attributable-fraction methods (Booth, 1998). This approach requires cross-sectional data on infection and disease status and provides some measure of the disease cases (e.g. anaemia) due to a given infection (e.g. hookworm). Table 5 presents estimates of attributable-fraction for selected school-aged populations in East Africa. Although the estimates will clearly vary with other factors such as dietary intake and other parasitic infections, they provide an approximation of the potential maximum reduction in anaemia cases if infection was removed. Estimates among pregnant women in Kenya suggest that hookworm may be causing 10% of anaemia cases (<110 g/L), 30% of moderate anaemia cases (<90 g/L) and 17% of severe anaemia cases (<70 g/L) in women of all parities (Guyatt *et al.*, 2000). Importantly these effects varied with parity group, with hookworm being a more important contributor to moderate and severe anaemia in multigravidae than in primigravidae. A further study of pregnant women in Nepal found that 41% of moderate-to-severe anaemia (haemoglobin < 90 g/L) was due to this infection (Stoltzfus *et al.* 1997c).

The major limitation of chemotherapy is that it provides only a short-term measure since rapid reinfection after chemotherapy is a common feature of helminth transmission, including hookworm (Figure 8). For instance, a study in a highly endemic area of Tanzanian found that reinfection with hookworm among schoolchildren to pre-treatment levels occurred within 4-12 months (Albonico *et al.*, 1995), whereas another study, conducted in Papua New Guinea, showed that reinfection following treatment occurred more slowly (Quinnell *et al.*, 1993). The varying re-infeciton rates can be explained by population dynamic models of transmission which show that the rate of re-infection depends on treatment efficacy and coverage, and the intrinsic transmission potential of the parasite in the population (Anderson and Medley, 1985). The rate of re-infection is inversely correlated with the magnitudes of each of these factors. Thus, the high rates of re-infection observed in Tanzania are due, in part, to the high transmission potential and low population coverage of treatment.

7.3. Vaccination

The benefits of an anti-hookworm vaccine used either in place of, or alongside anthelminthic drugs has been reviewed elsewhere (Hotez *et al.*, 2003). Proof-of-principle for the feasibility of developing anti-hookworm vaccines is based on the success of employing live or irradiation-attenuated Ancylostoma L3 as vaccines in laboratory animals (Miller, 1971, 1978; Hotez *et al.*, 1999). Critical to the protection of these vaccines were the antigens secreted by living L3 after their inoculation into animals (Hotez *et al.*, 1999). Because living L3 cannot be produced under Good Manufacturing Practice (GMP) for use in humans, an effort was initiated to reproduce its effect by substituting recombinant antigens corresponding to the major L3 secreted products (Hotez *et al.*, 2003), as well as an L3 surface antigen (Zhan *et al.*, in press). As indicated above, the most abundant L3 secreted antigens are the ASPs. Evidence that ASP-2, a 21-22 kDa antigen produced by all members of the genus *Ancylostoma* and *Necator* is an attractive vaccine candidate was based on multiple lines of converging evidence including 1) human correlates of hookworm immunity in China and Brazil; 2) animal protection data; and 3) supporting data from a parallel

veterinary trichostrongyle nematode (*Haemonchus contortus*) vaccine development program based in Europe (Knox *et al.*, 2003).

7.3.1. Human correlates of hookworm immunity—In immuno-epidemiology studies in two geographically diverse hookworm endemic regions, Minas Gerais, Brazil and Hainan Province China, an important association between the Th2 humoral immune response to ASP-2 and lower faecal egg excretion rates was identified. Over the course of two years individuals with high IgE levels against ASP-2 do not become reinfected. This contrasts with 60% reinfection rate in the remainder of the study sample (Bethony, J., unpublished observations).

The presence of a Th2 humoral immune response against ASP-2 in individuals with lower egg counts means three things: 1) individuals in endemic areas, where natural infection occurs, "see" the quantities large enough quantities of the ASP-2 molecule to develop a strong antibody response among a variety of isotypes; i.e., ASP-2 is secreted in large enough amounts to be a target of attack; 2) a small group of individuals in endemic areas develop a Th2 humoral immune response to ASP-2 that results in lower intensity of infection; and 3) after treatment, individuals with a high level of Th2 humoral immune response become "putatively immune" to further infection.

7.3.2. Animal protection data—Recombinant ASP-2 from different hookworm species was cloned and expressed either in the methanol utilizing yeast *Pichia pastoris* (*A. ceylanicum*) or Sf9 insect cells (*A. caninum*). Pichia-derived *A. ceylanicum* ASP-2 formulated with Quil A adjuvant was successfully used to vaccinate hamsters, and resulted in antibody titers that exceeded 1:100,000 dilution. Following oral challenge with *A. ceylanicum* L3, the vaccinated hamsters demonstrated statistically significant reductions in adult hookworm burden, hookworm size, and hookworm-induced blood loss relative to hamsters vaccinated with adjuvant alone (Goud *et al.*, 2004). Similarly insect-cell derived *A. caninum* ASP-2 formulated with the Glaxo SmithKline adjuvant system 3 (ASO3), resulted in high antibody titers when employed as a canine vaccine. Following skin challenge with *A. caninum* L3, the vaccinated dogs exhibited statistically significant reductions in hookworm burdens.

7.3.3. Parallel veterinary vaccine development studies—Work by Schallig *et al.* (1997) and Kooyman *et al.* (2001) in the Netherlands has shown that vaccination of sheep with an ASP-2 homologue from *Haemonchus contortus* (Hc24) elicits high levels (>80%) worm burden reduction relative to adjuvant injected control sheep.

7.3.4. Future plans for combination vaccine development—Process development on ASP-2 from *Necator americanus* using the yeast expression vector *Pichia pastoris* is underway, with GMP manufacture of the molecule scheduled for early 2004 and the first (Phase 1) human trials by 2005.

It is ultimately anticipated that ASP-2 will comprise a "cocktail vaccine" comprised of this L3 antigen in addition to one of the proteases used by adult hookworms in blood feeding. Therefore, major cysteine-, aspartic-, and metalloproteases inovled required for host hemoglobin degradation (hemoglobinases) are under evaluation (Hotez *et al.*, 2003). As noted above, vaccination of dogs with CP-2 and other adult hookworm proteases results in the production of antibody that localizes to the hookworm gut (Loukas *et al.*, 2004). By so doing, the anti-protease antibody diminishes parasite size, fecundity, and blood-feeding ability (Williamson *et al.*, 2003a). These experiments provide proof-of-principle of Asa Chandler's early prediction that anti-enzyme antibodies could be employed as a means to embark on anti-hookworm immunotherapies (Hotez *et al.*, 2003; Loukas *et al.*, 2004).

A critical component of testing a first-generation anthelminthic vaccine will be to determine baseline infection rates in endemic areas. It is anticipated that anti-hookworm vaccines will result in reduced worm burdens and hookworm-associated blood loss. They would also diminish the reliance on anthelminthic drugs for STH infection control and possibly delay the emergence of anthelminthic drug resistance. Therefore a vaccine would complement existing approaches to helminth control that use anthelminthics.

Notwithstanding the progress towards developing a hookworm vaccine, there remain uncertainties as to the design of optimal vaccination programmes (Woolhouse, 1995; Chan *et al.* 1997b) and cost-effectiveness of vaccination relative to chemotherapy (Tanner and Evans, 1994; Bundy *et al.*, 1995). The development of a vaccine for an infection such as hookworm for which naturally acquired immunity does not frequently occur and where reinfection is a common occurrence (Maxwell *et al.*, 1987; Olatunde and Onyemelukwe, 1994), also raises new challenges, not least of which is the rigorous evaluation of vaccine efficacy. This will not be easy and will require innovative evaluative approaches. In particular, planned Phase 1 and 2 trials will need to consider those heterogeneities that influence age and sex specific rates of infection and reinfection, and the distribution of worm burden within human communities. There is also a need to identify appropriate outcome measures for vaccine efficacy; one generally accepted measure of efficacy for helminth vaccines is the recovery rate of infection intensity after treatment, comparing reinfection between vaccinated and non-vaccinated populations (Gryseels, 2000).

8. FUTURE DIRECTIONS

Although tremendous progress has been made in the study of hookworm in recent decades, several important issues remain to be investigated, and require detailed and integrated study.

Host immune responses

In contrast to other helminth species, little is still known about the host immune response to hookworm. Better understanding is needed on the cellular immune response in general and the cellular and humoral immune response to stage specific hookworm antigens. There is also a need to investigate the effect of host genetics, parasite genetics, aging, and co-infections on the immune response during hookworm infection. Finally, the evidence that hookworm influence susceptibility to other pathogens and immune response to vaccines remains speculative, and detailed studies that separate the effect of hookworm versus other helminths are required.

Hookworm and host genetics

Although there is evidence for diminishing efficacy of benzimidazole anthelminthic chemotherapy with repeated and frequent use (Albonico *et al.*, 2003), there is still no direct evidence for benzimidazole drug resistance among the human hookworms. Better monitoring and evaluation during mass chemotherapy programs, which includes analyses of novel genetic markers, such as hookworm tubulin alleles (Albonico *et al.*, 2004), are needed. Further, a better understanding of the genetic basis of resistance and susceptibility to hookworm is required in order to explain the basis for hookworm predisposition in humans.

Estimation of disease burden

There is a need to revise estimation of DALYs that incorporate the impact of infection on anemia and iron deficiency during pregnancy and pre-schools years is required. If hookworm is shown to influence the susceptibility to other pathogens, this may also require including. There is also a need for well-designed studies on the economic impact of hookworm.

Hookworm control

The long-term nutritional and educational benefits of treatment have yet to be fully established and this will require careful longitudinal study over several years. The goal of 54th World Health Assembly to provide anthelminthic drugs to 75% of schoolchildren in endemic areas is laudable, yet highly ambitious. Because this would be the largest health program ever attempted (Horton, 2003), serious attention must be given to monitoring, especially for diminished anthelminthic efficacy because of drug resistance (Albonico *et al.*, 2003), as well as adult and pre-school populations that will not benefit from school-based programs. Rather than relying on existing anthelminthic alone, there is a need to develop a new generation of broad-spectrum agents and further assess the efficacy of combination therapy. Alternatively, and complementary to treatment, the development of an anti-hookworm vaccine could be a major breakthrough, and diminish our current reliance on frequent and periodic deworming with drugs.

After decades of neglect, hookworm is beginning to once again be the subject of scientific study. A new era of scientific activity on hookworm, similar to that of the early 20th century, would likely to reveal a number of new insights on the complicated nature of host-parasite relationships, and establish new paradigms for helminth control.

Acknowledgments

Work was supported by the Human Hookworm Vaccine Initiative (HHVI) of the Sabin Vaccine Institute and the Bill and Melinda Gates Foundation, NIH grant AI-32726, March of Dimes Clincial Research Grant (6FY-00-791), and a grant from the China Medical Board of New York, Inc. SB is supported by a Wellcome Trust Advanced Training Fellowship (073656). JB is supported by an International Research Scientist Development Award (IRSDA) (1K01 TW00009) from the John E. Fogarty International Center, NIH. We also are grateful to Rupert Quinnell for helpful comments on a draft of this review.

REFERENCES

Albonico M. Methods to sustain drug efficacy in helminth control programmes. Acta Tropica. 2003; 86:233–242. [PubMed: 12745140]

Albonico M, Bickle Q, Haji HL, Ramsan M, Khatib KJ, Montresor A, Savioli L, Taylor M. Evaluation of the efficacy of pyrantel-oxantel for the treatment of soil-transmitted nematodes infections. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2002; 96:685–690. [PubMed: 12625151]

Albonico M, Bickle Q, Ramsan M, Montresor A, Savioli L, Taylor M. Efficacy of mebendazole and levamisole alone or in combination against intestinal nematode infections after repeated targeted mebendazole treatment in Zanzibar. Bulletin of the World Health Organization. 2003; 81:343–352. [PubMed: 12856052]

Albonico M, Crompton DWT, Savioli L. Control strategies for human intestinal nematode infections. Advances in Parasitology. 1999; 42:278–341. [PubMed: 10050275]

Albonico M, Savioli L. Hookworm infection and disease: advances for control. Ann Ist Super Sanita. 1997; 33:567–579. [PubMed: 9616967]

Albonico M, Stoltzfus RJ, Savioli L, Tielsch JM, Chaway HM, Ercole E, Cancrini G. Epidemiological evidence for a differential effect of hookworm species, *Ancylostoma duodenale* and *Necator americanus*, on iron status of schoolchildren. International Journal of Epidemiology. 1998; 27:530–537. [PubMed: 9698148]

Albonico M, Smith PG, Ercole E, Hall A, Chwaya HM, Alawi KS, Savioli S. Rate of reinfection with intestinal nematodes after treatment of children with mebendazole or albendazole in a highly endemic area. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1995; 89:538–541. [PubMed: 8560535]

Albonico M, Wright V, Bickle Q. Molecular analysis of the beta-tubulin gene of human hookworms as a basis for possible benzimidazole resistance on Pemba Island. Molecular and Biochemical Parasitolgy. 2004; 134:281–284. [PubMed: 15003848]

Allen HE, Crompton DWT, de Silva N, LoVerde PT, Olds GR. New policies for using anthelmintics in high risk groups. Trends in Parasitology. 2002; 18:381–382. [PubMed: 12377247]

Anderson, RM. The population dynamics and control of hookworm and roundworm infection. In: Anderson, RM., editor. Population Dynamics of Infectious Diseases: Theory and Applications. London: Chapman and Hall; 1982. p. 67-109.

Anderson RM, May RM. Population dynamics of human helminth infections: control by chemotherapy. Nature. 1982; 297:557–563. [PubMed: 7088139]

Anderson, RM.; May, RM. Infectious Diseases of Humans: dynamics and control. Oxford: Oxford University Press; 1991.

Anderson RM, Medley GF. Community control of helminth infections of man by mass and selective chemotherapy. Parasitology. 1985; 90:629–660. [PubMed: 3892436]

Anderson RM, Schad GA. Hookworm burdens and faecal egg counts: an analysis of the biological basis of variation. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1985; 79:812–825. [PubMed: 3832493]

Anderson TJC, Blouin MS, Beech RN. Population biology of parasitic nematodes: application of genetic markers. Advances in Parasitology. 1998; 41:219–283. [PubMed: 9734295]

Anyaeze CM. Reducing burden of hookworm disease in the management of upper abdominal pain in the tropics. Tropical Doctor. 2003; 33:174–175. [PubMed: 12870611]

Appleton CC, Gouws E. The distribution of common intestinal nematodes along an altitudinal transect in Kwa-Zulu Natal, South Africa. Annals of Tropical Medicine and Parasitology. 1996; 90:181–188. [PubMed: 8762408]

Appleton CC, Maurihungirire M, Gouws E. The distribution of helminth infections along the coastal plain of Kwazulu-Natal province, South Africa. Annals of Tropical Medicine and Parasitology. 1999; 93:859–868. [PubMed: 10715680]

Ashford, BK. A Soldier in Science. William Morrow and Company Inc; San Juan: Universidad de Puerto Rico; 1934. p. 83Reprinted in 1998

Basavaraju S, Zhan B, Kennedy MW, Liu Y, Hawdon J, Hotez PJ. Ac-FAR-1, a 20 kDa fatty acid- and retinol-binding protein secreted by adult Ancylostoma caninum hookworms: gene transcription pattern, ligand binding properties and structural characterization. Molecular and Biochemical Parasitology. 2003; 126:63–71. [PubMed: 12554085]

Barreto ML, Rodrigues LC, Silva RCR, Assis AMO, Reis MG, Santos CAST, Blanton RE. Lower hookworm incidence, prevalence and intensity of infection in children with a Bacillus Calmette-Guérin vaccination scar. Journal of Infectious Diseases. 2000; 182:1800–1803. [PubMed: 11069259]

Beaver, PC.; Jung, RC.; Cupp, EW. Clinical Parasitology. 9th Edition. Philadelphia: Lea and Febiger; 1984. p. 284-285.p. 733-750.

Beaver PC. Light, long-lasting *Necator* infection in a volunteer. American Journal of Tropical Medicine and Hygiene. 1988; 39:369–372. [PubMed: 3189697]

Behnke, J. Human parasitic diseases: hookworm infections. In: Gilles, HM.; Ball, P., editors. Immunology. Amsterdam: Elsevier; 1991. p. 93-155.

Behnke JM, De Clercq D, Sacko M, Gilbert FS, Ouattara DB, Vercruysse J. The epidemiology of human hookworm infections in the southern region of Mali. Tropical Medicine and International Health. 2000; 5:343–354. [PubMed: 10886798]

Behnke JM, Rose R, Little J. Resistance of the hookworms *Ancylostoma ceylanicum* and *Necator americanus* to intestinal inflammatory responses induced by heterologous infection. International Journal of Parasitology. 1994; 24:91–101. [PubMed: 8021111]

Bennett A, Guyatt H. Reducing intestinal nematode infection: efficacy of albendazole and mebendazole. Parasitology Today. 2000; 16:71–74. [PubMed: 10652492]

Bentwich Z, Kalinkovich A, Weisman Z. Immune activation is a dominant factor in the pathogenesis of African AIDS. Immunology Today. 1995; 16:187–191. [PubMed: 7734046]

Bethony J, Chen J, Lin S, Xiao S, Zhan B, Li S, Xue H, Xing F, Humphries D, Yan W, Chen G, Foster V, Hawdon JM, Hotez P. Emerging patterns of hookworm infection: influence of aging on the intensity of *Necator* infection in Hainan Province, People's Republic of China. Clinical Infectious Disease. 2002a; 35:1336–1344. [PubMed: 12439796]

Bethony J, Gazzinelli A, Lopes A, Pereira W, Alves-Oliveira L, Willams-Blangero S, Blangero J, Loverde P, Correa-Oliveira R. Genetic epidemiology of fecal egg excretion during Schistosoma mansoni infection in an endemic area in Minas Gerais, Brazil. Memórias do Instituto Oswaldo Cruz. 2001; 96:49–55.

Bethony J, Williams JT, Blangero J, Kloos H, Gazzinelli A, Soares-Filho B, Coelho L, Alves-Fraga L, Williams-Blangero S, Loverde PT, Correa-Oliveira R. Additive host genetic factors influence fecal egg excretion rates during Schistosoma mansoni infection in a rural area in Brazil. American Journal of Tropical Medicine and Hygiene. 2002b; 67:336–343. [PubMed: 12452486]

Blackwell V, Vega-Lopez F. Cutaneous larva migrans: clinical features and management of 44 cases in the returning traveler. British Journal of Dermatology. 2001; 145:434–437. [PubMed: 11531833]

Blaxter M. Genes and genomes of Necator americanus and related hookworms. International Journal of Parasitology. 2000; 10:347–355. [PubMed: 10731559]

Blaxter M. Nematoda: genes, genomes and the evolution of parasitism. Advances in Parasitology. 2003; 54:101–195. [PubMed: 14711085]

Blaxter ML, De Ley P, Garey JR, Liu LX, Scheldeman P, Vierstraete A, Vanfleteren JR, Mackey LY, Dorris M, Frisse LM, Vida JT, Thomas WK. A molecular evolutionary framework for the phylum Nematoda. Nature. 1998; 392:71–75. [PubMed: 9510248]

Bleakely H. Disease and development: evidence from hookworm eradication in the American South. 2003http://web.mit.edu/hoyt

Booth M. The application of attributable risk analysis in helminth epidemiology. Parasitology Today. 1998; 14:497–450. [PubMed: 17040864]

Borkow G, Bentwich Z. Eradication of helminth infections may be essential for successful vaccination against HIV and tuberculosis. Bulletin of the World Health Organization. 2000; 78:1368–1369. [PubMed: 11143198]

Bradley M, Chandiwana SK. Age-dependency in predisposition to hookworm infection in the Burma valley area of Zimbabwe. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1990; 84:826–828. [PubMed: 2136254]

Bradley M, Chandiwana SK, Bundy DAP, Medley GF. The epidemiology and population biology of Necator americanus infection in a rural community in Zimbabwe. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1992; 86:73–76. [PubMed: 1566314]

Brooker S, Beasley NMR, Ndinaromtan M, Madjiouroum EM, Baboguel M, Djenguinabe E, Hay SI, Bundy DAP. Use of remote sensing and a geographical information system in a national helminth control programme in Chad. Bulletin of the World Health Organization. 2002b; 80:783–789. [PubMed: 12471398]

Brooker S, Hay SI, Tchuem Tchuenté LA, Ratard R. Using NOAA-AVHRR data to model helminth distributions for planning disease control in Cameroon, West Africa. Photogrammetric Engineering and Remote Sensing. 2002a; 68:175–179.

Brooker S, Kabatereine NB, Tukahebwa EM, Kazibwe F. Spatial analysis of the distribution of intestinal nematode infections in Uganda. Epidemiology and Infection. in press.

Brooker S, Michael E. The potential of geographical information systems and remote sensing in the epidemiology and control of human helminth infections. Advances in Parasitology. 2000; 47:245–288. [PubMed: 10997209]

Brooker S, Peshu N, Warn PA, Mosobo M, Guyatt H, Marsh K, Snow RW. Epidemiology of hookworm infection and its contribution to anaemia among pre-school children on the Kenyan coast. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1999; 93:240–246. [PubMed: 10492749]

Brophy PM, Patterson LH, Brown A, Pritchard DI. Glutathione S-transferase (GST) expression in the human hookworm *Necator americanus*: potential roles for excretory-secretory forms of GST. Acta Tropica. 1995; 59:259–263. [PubMed: 7572433]

Brown A, Pritchard DI. The immunogenicity of hookworm (*Necator americanus*) acetylcholinesterase (AchE) in man. Parasite Immunology. 1993; 15:195–203. [PubMed: 8506114]

Bundy DAP. Sexual effects on parasite infection. Parasitology Today. 1988a; 4:186–189. [PubMed: 15463089]

Bundy DAP. Population ecology of intestinal helminth infections in human communities. Philosphical Transactions of the Royal Society Series B. 1988b; 321:405–420. [PubMed: 2907151]

Bundy, DAP. Is the hookworm just another geohelminth?. In: Schad, GA.; Warren, KS., editors. Hookworm disease current status and new directions. London: Taylor and Francis; 1990. p. 147-164.

Bundy DAP, Chan M-S, Guyatt HL. The practicality and sustainability of vaccination as an approach to parasite control. Parasitology. 1995; 110:S51–S58. [PubMed: 7784130]

Bundy DAP, Cooper ES. *Trichuris* and trichuriasis in humans. Advances in Parasitology. 1989; 28:107–173. [PubMed: 2683614]

Bundy, DAP.; Keymer, AE. The epidemiology of hookworm infection. In: Gilles, HM.; Ball, PAJ., editors. Human Parasitic Diseases Volume 4. Hookworm Infections. Amsterdam: Elsevier; 1991. p. 157-178.

Bundy DAP, Medley GF. Immuno-epidemiology of human geohelminthiasis: ecological and immunological determinants of worm burden. Parasitology. 1992; 104:S105–S119. [PubMed: 1589298]

Bundy, DAP.; Michael, E.; Guyatt, HL. Epidemiology and control of nematode infection and disease in humans. In: Lee, DL., editor. The Biology of Nematodes. London: Taylor and Francis; 2002. p. 599-617.

Cara DC, Negrao-Correa D, Teixeira MM. Mechanisms underlying eosinophil trafficking and their relevance in vivo. Histology and Histopathology. 2000; 15:899–920. [PubMed: 10963133]

Carr A, Pritchard DI. Antigen expression during development of the human hookworm, *Necator americanus* (Nematoda). Parasite Immunology. 1987; 9:219–234. [PubMed: 2437516]

Carroll SM, Grove D. Experimental infection of humans with *Ancylostoma ceylanicum*: clinical, parasitological, hematological and immunological findings. Tropical and Geographic Medicine. 1986; 38:38–45. [PubMed: 3961908]

Caumes E, Ly F, Bricaire F. Cutaneous larva migrans with folliculitis: report of seven cases and review of the literature. British Journal of Dermatology. 2002; 146:314–316. [PubMed: 11903247]

Chan M-S, Bradley M, Bundy DAP. Transmission patterns and the epidemiology of hookworm. International Journal of Epidemiology. 1997a; 26:1392–1400. [PubMed: 9447422]

Chan M-S, Guyatt HL, Bundy DAP, Medley GF. The development annd validation of an age-stratified mode for the evaluation of disease control strategies for intestinal helminths. Parasitology. 1994b; 109:389–396. [PubMed: 7970893]

Chan MS, Medley GF, Jamison D, Bundy DAP. The evaluation of potential global morbidity attributable to intestinal nematode infections. Parasitology. 1994a; 109:373–387. [PubMed: 7970892]

Chan M-S, Woolhouse MEJ, Bundy DAP. Human schistosomiasis: potential long term consequences of vaccination programmes. Vaccine. 1997b; 15:1545–1550. [PubMed: 9330466]

Chandler, AC. Its distribution, biology, epidemiology, pathology, diagnosis, treatment and control. New York: Macmillan; 1929. Hookworm disease.

Chilton NB, Gasser RB. Sequence differences in the internal transcribed spacers of DNA among four species of hookworm (Ancylostomatoidea: *Ancylostoma*). International Journal of Parasitology. 1999; 29:1971–1977. [PubMed: 10961853]

Conder GA, Campbell WC. Chemotherapy of nematode infections of veterinary importance, with special reference to drug resistance. Advances in Parasitology. 1995; 35:1–84. [PubMed: 7709851]

Chongsuvivatwong V, Pas-Ong S, McNail D, Greater A, Duerawee M. Predictors for the risk of hookworm infection: experience from endemic villages in southern Thailand. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1996; 90:630–633. [PubMed: 9015498]

Chow SC, Brown A, Pritchard D. The human hookworm pathogen *Necator americanus* induces apoptosis in T lymphocytes. Parasite Immunology. 2000; 22:21–29. [PubMed: 10607288]

Cooper PJ, Chico M, Sandoval C, Espinel I, Guevara A, Levine MM, Griffin GE, Nutman TB. Human infection with Ascaris lumbricoides is associated with suppression of the interleukin-2 response to recombinant cholera toxin B subunit following vaccination with the live oral cholera vaccine CVD 103-HgR. Infection and immunity. 2001; 69:1574–1580. [PubMed: 11179329]

Cooper PJ, Espinel I, Wieseman M, Paredes W, Espinel M, Guderian RH, Nutman TB. Human onchocerciasis and tetanus vaccination: impact on the postvaccination antitetanus antibody response. Infection and immunity. 1999; 67:5951–5957. [PubMed: 10531253]

Cox FEG. Concomitant infections, parasites and immune responses. Parasitology. 2001; 122:S23–S38. [PubMed: 11442193]

Crompton DWT. The public health significance of hookworm disease. Parasitology. 2001; 121:S39–S50. [PubMed: 11386690]

Crompton, DWT.; Stephenson, LS. Hookworm infection, nutritional status and productivity. In: Schad, GA.; S, K., editors. Hookworm disease current status and new directions. London: Taylor and Francis; 1990. p. 231-264.

Crompton DWT, Whitehead RR. Hookworm infections and human iron metabolism. Parasitology. 1993; 107:S137–S145. [PubMed: 8115178]

Culley FJ, Brown A, Conroy DM, Sabroe I, Pritchard DI, Williams TJ. Eotaxin is specifically cleaved by hookworm metalloproteases preventing its action in vitro and in vivo. Journal of Immunology. 2000; 165:6447–6453. [PubMed: 11086084]

de Clerq D, Sacko M, Behnke J, Gilbert F, Dorny P, Vercruysse J. Failure of mebendazole in treatment of human hookworm infection inn the southern region of Mali. American Journal of Tropical Medicine and Hygiene. 1997; 57:25–30. [PubMed: 9242313]

del Valle A, Jones BF, Harrison LM, Chadderdon RC, Cappello M. Isolation and molecular cloning of a secreted hookworm platelet inhibitor from adult Ancylostoma caninum. Molecular and Biochemical Parasitology. 2003; 129:167–177. [PubMed: 12850261]

Desakorn V, Suntharasamai P, Pukrittayakamee S, Migasena S, Bunnag D. Adherence of human eosinophils to infective filariform larvae of *Necator americanus* in vitro. Southeast Asian Journal of Tropical Medicine and Public Health. 1987; 18:66–72. [PubMed: 3660070]

de Silva NR, Brooker S, Hotez PJ, Montresor A, Engels D, Savioli L. Soil-transmitted helminth infections: updating the global picture. Trends in Parasitology. 2003; 19:547–551. [PubMed: 14642761]

de Silva NR, Sirisena JL, Gunasekera DP, Ismail MM, de Silva HJ. Effect of mebendazole therapy during pregnancy on birth outcome. Lancet. 1999; 353:1145–1149. [PubMed: 10209979]

Dickson R, Awasthi S, Williamson P, Demellweek C, Garner P. Effects of treatment for intestinal helminth infection on growth and cognitive performance in children: systematic review of randomised trials. British Medical Journal. 2000; 320:1697–1701. [PubMed: 10864543]

Dock, G.; Bass, CC. Hookworm Disease. St Louis: C.V. Mosby Company; 1910. p. 115-154.

Drake LJ, Jukes MCH, Sternberg RJ, Bundy DAP. Geohelminth infections (Ascariasis, Trichuriasis and Hookworm): cognitive and developmental impacts. Seminars in Pediatric Infectious Diseases. 2000; 11:245–251.

Dunne DW, Butterworth AE, Fulford AJ, Kariuki HC, Langley JG, Ouma JH, Capron A, Pierce RJ, Sturrock RF. Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. European Journal of Immunology. 1992; 22:1483–1494. [PubMed: 1601036]

Dunne DW, Richardson BA, Jones FM, Clark M, Thorne KJ, Butterworth AE. The use of mouse/human chimaeric antibodies to investigate the roles of different antibody isotypes, including IgA2, in the killing of *Schistosoma mansoni* schistosomula by eosinophils. Parasite Immunology. 1993; 15:181–185. [PubMed: 8316412]

Egwunyenga AO, Ajayi JA, Nmorsi OPG, Duhlinska-Popova DD. *Plasmodium*/intestinal helminth co-infections among pregnant Nigerian women. Memórias do Instituto Oswaldo Cruz. 2001; 96:1055–1059.

Elias D, Wolday D, Akuffo H, Petros B, Bronner U, Britton S. Effect of deworming on human T cell responses to mycobacterial antigens in helminth-exposed individuals before and after bacille Calmette-Guerin (BCG) vaccination. Clinical & Experimental Immunology. 2001; 123:219225. [PubMed: 11207651]

Elliott AM, Mawa PA, Joseph S, Namujju PB, Kizza M, Nakiyingi JS, Watera C, Dunne DW, Whitworth JAG. Associations between helminth infection and CD4+ T cell count, viral load and

cytokine responses in HIV-1 infected Ugandan adults. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2003; 97:103–108. [PubMed: 12886815]

Elliott AM, Nakiyingi J, Quigley MA, French N, Gilks CF, Whitworth JAG. Inverse association between BCG immunisation and intestinal nematodes infection among HIV-1 positive individuals in Uganda. Lancet. 1999; 354:1000–1001. [PubMed: 10501367]

Esrey SA, Potash JB, Roberts L, Shiff C. Effects of improved water supplies and sanitation on ascariasis, diarrhoea, dracunculiasis, hookworm infection, schistosomiasis and trachoma. Bulletin of the World Health Organization. 1991; 69:609–621. [PubMed: 1835675]

Fee, E.; Acheson, A. A History of the Johns Hopkins School of Hygiene and Public Health, 1916-1939. Baltimore: Johns Hopkins Univeristy Press; 1987. Disease and Discovery; p. 104-109.

Fincham JE, Markus MB, Adams VJ. Could control of soil-transmitted helminthic infection influence the HIV/AIDS pandemic. Acta Tropica. 2003; 86:315–333. [PubMed: 12745148]

Finkelman FD, Shea-Donohue T, Goldhill J, Sullivan CA, Morris SC, Madden KB, Gause WC, Urban JF Jr. Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. Annual Review of Immunology. 1997; 15:505–533.

Forrester JE, Scott ME, Bundy DAP, Golden MHN. Clustering of *Ascaris lumbricoides* and *Trichuris trichiura* infections within households. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1988; 82:282–288. [PubMed: 3188157]

Fuller K. Hookworm: not a pre-Columbian pathogen. Medical Anthropology. 1997; 17:297–308. [PubMed: 9241990]

Gandhi NS, Chen JZ, Koshnood K, Xing FY, Li SW, Liu YR, Bin Z, Haechou X, Chong-Jin T, Wang Y, Wensen W, Dungxing H, Chong C, Shuhua X, Hawdon JM, Hotez PJ. Epidemiology of *Necator americanus* hookworm infections in Xiulongkan Village, Hainan Province, China: High prevalence and intensity among middle-aged and elderly residents. Journal of Parasitology. 2001; 87:739–743. [PubMed: 11534635]

Garraud O, Perraut R, Riveau G, Nutman TB. Class and subclass selection in parasite-specific antibody responses. Trends in Parasitology. 2003; 19:300–304. [PubMed: 12855380]

Garrow SC, McCarthy JC, Thompson RCA, Reynoldson JA, Hopkins R, Musk B. Hookworm control programmes and anaemia. Medical Journal of Australia. 2001; 175:442–443. [PubMed: 11700841]

Gasser RB, Stewart LE, Speare R. Genetic markers in ribosomal DNA for hookworm identification. Acta Tropica. 1996; 62:15–21. [PubMed: 8971275]

Gause WC, Xiao SY, Liu ZG, Zhan B, Goud G, Loukas A, Hawdon J, Urban JF, Hotez P. A human hookworm recombinant protein can function as a Th2 adjuvant in mice. FASEB J. 2003; 17(Suppl):C194–C194.

Geissler PW, Shulman CE, Prince RJ, Mutemi W, Mnazi C, Friis H, Lowe B. Geophagy, iron status and anaemia among pregnant women on the coast of Kenya. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1998; 92:549–553. [PubMed: 9861377]

Geerts S, Gryseels B. Drug resistance in human helminths: current situation and lessons from livestock. Clinical Microbiology Reviews. 2001; 13:207–222. [PubMed: 10755998]

Gilgen D, Mascie-Taylor CGN. The effect of anthelmintic treatment on helminth infection and anaemia. Parasitology. 2001; 122:105–110. [PubMed: 11197758]

Gilgen D, Mascie-Taylor CGN, Rosetta L. Intestinal helminth infections, anaemia and labour productivity of female tea pluckers in Bangladesh. Tropical Medicine and International Health. 2001; 6:449–457. [PubMed: 11422959]

Goble FC, Konopka EA. Sex as a factor in infectious disease. Transactions of New York Academy of Science. 1973; 35:325–46.

Gonçalves MLC, Araújo A, Ferreira LF. Human intestinal parasites in the past: new findings and a review. Memórias do Instituto Oswaldo Cruz. 2003; 98:S103–S118. [PubMed: 12687769]

Goud GN, Zhan B, Ghosh K, Loukas A, Hawdon J, Dobardzic A, Deumic V, Liu S, Dobardzic R, Zook BC, Qun J, Liu YY, Hoffman L, Chung-Debose Sl. Patel R, Mendez S, Hotez PJ. Cloning, yeast expression, isolation and vaccine testing of recombinant Ancylostoma secreted protein 1 (ASP-1) and ASP-2 from Ancylostoma ceylanicum. Journal of Infectious Diseases. in press. [PubMed: 14976610]

Grantham-McGregor S, Ani C. A review of studies on the effect of iron deficiency on cognitive development in children. Journal of Nutrition. 2001; 131:S649–S666. [PubMed: 11160596]

Guedez L, Mansoor A, Birkedal-Hansen B, Lim MS, Fukushima P, Venzon D, Stetler-Stevenson WG, Stetler-Stevenson M. Tissue inhibitor of metalloproteinases 1 regulation of interleukin 10 in B-cell differentiation and lymphomagenesis. Blood. 2001; 97:1796–802. [PubMed: 11238122]

Guyatt H. Do intestinal nematodes affect productivity in adulthood? Parasitology Today. 2000; 16:153–158. [PubMed: 10725902]

Guyatt, HL. The economics of worm control. In: Holland, CV.; Kennedy, MW., editors. The Geohelminths. Ascaris, Trichuris and Hookworm. Boston: Kluwer Academic Publishers; 2001. p. 75-87.

Guyatt HL, Brooker S, Hall A, Kihamia CK, Bundy DAP. Evaluation of efficacy of school-based anthelmintic treatments against anaemia in children in the United Republic of Tanzania. Bulletin of the World Health Organization. 2001; 79:695–703. [PubMed: 11545325]

Guyatt HL, Brooker S, Peshu N, Shulman CE. Hookworm and anaemia prevalence. The Lancet. 2000; 356:2101. [PubMed: 11145522]

Guyatt HL, Bundy DAP. Estimating prevalence of community morbidity due to intestinal helminths: prevalence of infection as an indicator of the prevalence of disease. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1991; 85:778–782. [PubMed: 1801353]

Guyatt HL, Bundy DAP, Medley GF, Grenfell BT. The relationship between the frequency distribution of Ascaris lumbricoides and the prevalence and intensity of infection in human communities. Parasitology. 1990; 101:139–143. [PubMed: 2235069]

Gryseels B. Schistosomiasis vaccines: a devil's advocate view. Parasitology Today. 2000; 16:46–48. [PubMed: 10652485]

Haas JD, Brownlie T. Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. Journal of Nutrition. 2001; 131:S676–S690. [PubMed: 11160598]

Hadju V, Stephenson LS, Abadi K, Mohammed HO, Bowman DD, Parker RS. Improvements in appetite and growth in helminth-infected schoolboys three and seven weeks after a single dose of pyrantel pamoate. Parasitology. 1996; 113:497–504. [PubMed: 8893536]

Hagan P. IgE and protective immunity to helminth infections. Parasite Immunology. 1993; 15:1–4. [PubMed: 8433850]

Hagan P, Blumenthal UJ, Dunn D, Simpson AJ, Wilkins HA. Human IgE, IgG4 and resistance to reinfection with Schistosoma haematobium. Nature. 1991; 349:243–245. [PubMed: 1898985]

Hara, T. Large-scale control against intestinal helminthic infections in Japan, with special reference to the activities of Japan Association of Parasite Control. In: Hayashi, S., editor. Collected Papers on the Control of Soil-transmitted Helminthiases. Vol. VII. Tokyo: ACOP; 2001. p. 267-271.

Harada Y. Wakana disease and hookworm allergy. Yonago Acta Medica. 1962; 6:109–118.

Harrison LM, Nerlinger A, Bungiro RD, Cordova JL, Kuzmic P, Cappello M. Molecular characterization of Ancylostoma inhibitors of coagulation factor Xa. Hookworm anticoagulant activity in vitro predicts parasite bloodfeeding in vivo. Journal of Biological Chemistry. 2002; 277:6223–6229. [PubMed: 11741914]

Harrop SA, Prociv P, Brindley PJ. Amplification and characterization of cysteine proteinase genes from nematodes. Tropical Medicine and Parasitlogy. 1995; 46:119–122. [PubMed: 8525283]

Haswell-Elkins M, Elkins D, Anderson R. Evidence for predisposition in humans to infection with *Ascaris*, hookworm, *Enterobius* and *Trichuris* in a South Indian fishing community. Parasitology. 1987; 95:323–337. [PubMed: 3696767]

Haswell-Elkins MR, Elkins DB, Manjula K, Michael E, Anderson RM. An investigation of hookworm infection and reinfection following mass anthelminthic treatment in the south Indian fishing village. Parasitology. 1988; 96:565–578. [PubMed: 3405641]

Hawdon JM. Differentiation between the human hookworms *Ancylostoma duodenale* and *Necator americanus* using PCR-RFLP. Journal of Parasitology. 1996; 82:642–647. [PubMed: 8691377]

Hawdon JM, Hotez PJ. Hookworm: developmental biology of the infectious process. Current Opinion in Genetics and Development. 1996; 6:618–623. [PubMed: 8939719]

Hawdon JM, Datu B. The second messenger cyclic GMP mediates activation in *Ancylostoma caninum* infective larvae. International Journal for Parasitology. 2003; 33:787–793. [PubMed: 12865078]

Hawdon JM, Datu B, Crowell M. Molecular cloning of a novel multidomain Kunitz-type proteinase inhibitor from the hookworm *Ancylostoma caninum*. Journal of Parasitology. 2003; 89:402–407. [PubMed: 12760667]

Hawdon JM, Jones BF, Hoffman DR, Hotez PJ. Cloning and characterization of Ancylostoma-secreted protein. A novel protein associated with the transition to parasitism by infective hookworm larvae. Journal of Biological Chemistry. 1996; 22:6672–6678. [PubMed: 8636085]

Hawdon JM, Johnston SA. Hookworm in the Americans: an alternative to trans-Pacific contact. Parasitology Today. 1996; 12:72–74. [PubMed: 15275258]

Hawdon JM, Li T, Zhan B, Blouin MS. Genetic structure of populations of the human hookworm, *Necator americanus*, in China. Molecular Ecology. 2001; 10:1433–1437. [PubMed: 11412366]

Hawdon JM, Narasmihan S, Hotez PJ. Ancylostoma secreted protein 2: cloning and characterization of a second member of a family of nematode secreted proteins from *Ancylostoma caninum*. Molecular and Biochemical Parasitology. 1999; 30:149–165. [PubMed: 10340481]

Hill RB. The estimation of the number of hookworm harboured by the use of the dilution egg count method. American Journal of Hygiene. 1926; 6:19–41.

Hoagland KE, Schad GA. *Necator americanus* and *Ancylostoma duodenale*: Life history parameters and epidemiological implications of two sympatric hookworms of humans. Experimental Parasitology. 1978; 44:36–49. [PubMed: 627275]

Holland CV, Taren DL, Crompton DWT, Nesheim MC, Sanjur D, Barbeau I, Tucker K, Tiffany J, Rivera G. Intestinal helminthiases in relation to the socioeconomic environment of Panamanian children. Social Science and Medicine. 1988; 26:209–213. [PubMed: 3347848]

Hominick WM, Dean CG, Schad GA. Population biology of hookworms in West Bengal: analysis of numbers of infective larvae recovered from damp pads applied to the soil surface of defaecation sites. Transaction of the Royal Society of Tropical Medicine and Hygiene. 1987; 81:978–986. [PubMed: 3503420]

Horton J. Albendazole: a review of anthelmintic efficacy and safety in humans. Parasitology. 2000; 121:S113–S132. [PubMed: 11386684]

Horton J. Global anthelmintic chemotherapy programs: learning from history. Trends in Parasitology. 2003; 19:405–409. [PubMed: 12957517]

Hotez PJ. Hookworm infection in children. Pediatric Infectious Diseases Journal. 1989; 8:516–20. [PubMed: 2671907]

Hotez, PJ. Human hookworm infection. In: Farthing, MJG.; Keusch, GT.; Wakelin, D., editors. Enteric Infection: Mechanisms, Manifestations, and Management. Vol. II. London: Chapman and Hall; 1995. p. 129-150.Intestinal Helminths

Hotez PJ. China's hookworms. China Quarterly. 2002; 172:1029–1041.

Hotez PJ, Cappello M, Hawdon J, Beckers C, Sakanari J. Hyaluronidases from thegastrointestinal invasive nematodes *Ancylostoma caninum* and *Anisakis simplex*: Their function in the pathogenesis of human zoonoses. Journal of Infectious Diseases. 1994; 170:918–926. [PubMed: 7930737]

Hotez PJ, Feng Z, Xu LQ, Chen MG, Xiao SH, Liu SX, Blair D, McManus DP, Davis GM. Emerging and reemerging helminthiases and the public health of China. Emerging Infectious Diseases. 1997; 3:303–310. [PubMed: 9284374]

Hotez PJ, Ghosh K, Hawdon JM, Narasimhan S, Jones B, Shuhua X, Sen L, Bin Z, Haechou X, Hainan R, Heng W, Koski RA. Experimental approaches to the development of a recombinant hookworm vaccine. Immunological Reviews. 1999; 171:163–171. [PubMed: 10582170]

Hotez PJ, Ghosh K, Hawdon JM, Narasimhan S, Jones B, Shuhua X, Sen L, Bin Z, Haechou X, Hainan R, Heng W, Koski RA. Experimental approaches to the development of a recombinant hookworm vaccine. Immunological Reviews. 1999; 171:163–171. [PubMed: 10582170]

Hotez PJ, Hawdon J, Schad GA. Hookworm larval amphiparatenesis: the Caenorhabditis elegans Daf-c paradigm. Parasitology Today. 1993; 9:23–6. [PubMed: 15463660]

Hotez PJ, Narasimhan S, Haggerty J, Milstone L, Bhopale V, Richards FF. A hyaluronidase from Ancylostoma hookworm larvae and its function as a virulence factor in cutaneous larva migrans. Infection and Immunity. 1992; 60:1018–1023. [PubMed: 1541516]

Hotez PJ, Zhan B, Bethony JM, Loukas A, Williamson A, Goud G, Hawdon JM, Dobardzic A, Dobardzic R, Ghosh K, Bottazzi ME, Mendez S, Zook B, Wang Y, Liu S, Essiet-Gibson I, Chung-Debose S, Xiao SH, Knox D, Meagher M, Inan M, Correa-Oliveira R, Vilk P, Shepherd HR, Brandt W, Russell PK. Progress in the development of a recombinant vaccine for human hookworm disease: the human hookworm vaccine initiative. International Journal of Parasitology. 2003; 33:1245–1258. [PubMed: 13678639]

Hu M, Chilton NB, Abs El-Osta YG, Gasser RB. Comparative analysis of mitochondrial genome data for *Necator americanus* from two endemic regions reveals susbtantial genetic variation. International Journal of Parasitology. 2003; 33:955–963. [PubMed: 12906879]

Hu M, Chilton NB, Gasser RB. The mitochondrial genomes of the human hookworms, *Ancylostoma duodenale* and *Necator americanus* (Nematoda: Secernentea). International Journal of Parasitology. 2002; 32:145–158. [PubMed: 11812491]

Humphries DL, Stephenson LS, Pearce EJ, The PH, Dan HT, Khanh LT. The use of human faeces for fertilizer is associated with increased intensity of hookworm infection in Vietnamese women. Transaction of the Royal Society of Tropical Medicine and Hygiene. 1997; 91:518–520. [PubMed: 9463654]

Huttly SRA. The impact of inadequate sanitary conditions on health in developing countries. World Health Statistics Quarterly. 1990; 43:118–126. [PubMed: 2146815]

Jackson RT, Jackson LC. Biological and behvioural contributors to anemia during pregnancy in Liberia, West Africa. Human Biology. 1987; 59:585–597. [PubMed: 3623506]

Jarrett E, Bazin H. Elevation of total serum IgE in rats following helminth parasite infection. Nature. 1974; 251:613–614. [PubMed: 4547380]

Jones BF, Hotez PJ. Molecular cloning and characterization of Ac-mep-1, a developmentally regulated gut luminal metalloendopeptidase from adult *Ancylostoma caninum* hookworms. Molecular and Biochemical Parasitology. 2002; 119:107–116. [PubMed: 11755191]

Kasper G, Brown A, Eberl M, Vallar L, Kieffer N, Berry C, Girdwood R, Eggleton P, Quinnell R, Pritchard DI. A calreticulin-like molecule from the human hookworm *Necator americanus* interacts with C1q and the cytoplasmic signaling domains of some integrins. Parasite Immunology. 2001; 23:141–152. [PubMed: 11240905]

Khalife J, Dunne DW, Richardson BA, Mazza G, Thorne KJ, Capron A, Butterworth AE. Functional role of human IgG subclasses in eosinophil-mediated killing of schistosomula of Schistosoma mansoni. Journal of Immunology. 1989; 142:4422–4427. [PubMed: 2723436]

Kightlinger LK, Seed JR, Kightlinger MB. Ascaris lumbricoides intensity in relation to environmental, socioeconomic, and behavioral determinants of exposure to infection in children from southeast Madagascar. Journal of Parasitology. 1998; 84:480484. [PubMed: 9645843]

Kooyman FN, Schallig HD, Van Leeuwen MA, MacKellar A, Huntley JI, Cornelissen AW, Vervelde L. Protection in lambs vaccinated with *Haemonchus contortus* antigens is age related, and correlates with IgE rather than IgG antibody. Parasite Immunology. 2000; 22:13–20. [PubMed: 10607286]

Knight R, Merrett TG. Hookworm infection in rural Gambia. Seasonal changes, morbidity and total IgE levels. Annals of Tropical Medicine and Parasitology. 1981; 75:299–314. [PubMed: 7305500]

Knox DP, Redmond DL, Newlands GF, Skuce PJ, Pettit D, Smith WD. The nature and prospects for gut membrane proteins as vaccine candidates for *Haemonchus contortus* and other ruminant trichostrongylids. International Journal for Parasitology. 2003; 33:1129–1137. [PubMed: 13678629]

Labiano-Abello N, Canese J, Velazquez ME, Hawdon JM, Wilson ML, Hotez PJ. Epidemiology of hookworm infection in Itagua, Paraguay: a cross sectional study. Memórias do Instituto Oswaldo Cruz. 1999; 94:583–586.

Landmann JK, Prociv P. Experimental human infection with the dog hookworm, Ancylostoma caninum. Medical Journal of Australia. 2003; 178:69–71. [PubMed: 12526725]

Lawrence CE, Paterson JC, Higgins LM, MacDonald TT, Kennedy MW, Garside P. IL-4-regulated enteropathy in an intestinal nematode infection. European Journal of Immunology. 1998; 28:2672–2684. [PubMed: 9754555]

Lee AY, Vlasuk GP. Recombinant nematode anticoagulant protein c2 and other inhibitors targeting blood coagulation factor VIIa/tissue factor. Journal of Internal Medicine. 2003; 254:313–321. [PubMed: 12974870]

Leiby DA, el Naggar HM, Schad GA. Thirty generations of Ancylostoma duodenale in laboratory-reared beagles. Journal of Parasitology. 1987; 73:844–848. [PubMed: 3625436]

Lili Z, Bingxiang Z, Hong T, Shuhua X, Hotez P, Bing Z, Yangzhong L, Ying L, Haichou X, Hawdon J, Hui Y, Huizhen W, Zhen F. Epidemiology of human geohelminth infections (ascariasis, trichuriasis and necatoriasis) in Lushui and Puer Counties, Yunnan Province, China. Southeast Asian Journal of Tropical Medicine and Public Health. 2000; 31:448–453. [PubMed: 11288999]

Looss A. On the penetration of Ancylostoma larvae into the human skin. Centralblatt Backteriol. Parasitenkunde. 1901; 29:733–739.

Loukas A, Opdebeeck J, Croese J, Prociv P. Immunologic incrimination of *Ancylostoma caninum* as a human enteric pathogen. American Journal of Tropical Medicine and Hygiene. 1994; 50:69–77. [PubMed: 8304575]

Loukas A, Bethony JM, Williamson AL, Goud GN, Mendez S, Zhan B, Hawdon JM, Bottazzi ME, Brindley PJ, Hotez PJ. Vaccination of dogs with recombinant cysteine protease from the intestine of canine hookworms diminishes fecundity and growth of worms. Journal of Infectious Diseases. 2004; 189:1952–1961. [PubMed: 15122534]

Loukas A, Brown AP, Pritchard DI. Na-ctl-2 a cDNA encoding a C-type lectin expressed exclusively in adult Necator americanus hookworms. DNA Sequence. 2002; 13:61–65. [PubMed: 12180139]

Loukas A, Prociv P. Immune responses in hookworm infections. Clinical Microbiological Review. 2001; 14:689–703. [PubMed: 11585781]

Lozoff, B. Has iron deficiency been shown to cause altered behaviour in infants?. In: Dobbing, J., editor. Brain, Behaviour and Iron in the Infant. London: Springer-Verlag; 1990. p. 107-131.

Lwambo NJS, Brooker S, Siza JE, Bundy DAP, Guyatt H. Stunting and anaemia in schoolchildren: a cross-sectional study in Tanzania. European Journal of Clinical Nutrition. 2000; 54:36–40. [PubMed: 10694770]

Lwambo NJS, Bundy DAP, Medley GF. A new approach to morbidity risk assessment in hookworm endemic communities. Epidemiology and Infection. 1992; 108:469–481. [PubMed: 1601081]

Mabaso MLH, Appleton CC, Hughes JC, Gouws E. The effect of soil type and climate on hookworm (*Necator americanus)*. distribution in KwaZulu-Natal, South Africa. Tropical Medicine and International Health. 2003; 8:722–727. [PubMed: 12869093]

MacDonald AS, Araujo MI, Pearce EJ. Immunology of parasitic helminth infections. Infection and Immunity. 2002; 70:427–433. [PubMed: 11796567]

Mahanty S, Luke HE, Kumaraswami V, Narayanan PR, Vijayshekaran V, Nutman TB. Stage-specific induction of cytokines regulates the immune response in lymphatic filariasis. Experimental Parasitology. 1996; 84:282–290. [PubMed: 8932778]

Malhorta I, Mungai P, Wamacji A, Kioko J, Ouma JH, Kazura JW, King CL. Helminth- and Bacillus Calmette-Guérin-induced immunity in children sensitized *in utero* to filariasis and schistosomiasis. Journal of Immunology. 1999; 162:6843–6848. [PubMed: 10352306]

Mani TR, Rajendran R, Munirathinan A, Sunish IP, Abdullah SM, Augustin DJ, Satyanarayana. Efficacy of co-administration of albendazole and diethylcarbamazine against geohelminthiases: a study from South India. Tropical Medicine and International Health. 2001; 7:541–548. [PubMed: 12031078]

Maxwell C, Hussain R, Nutman TB, Poindexter RW, Little MD, Schad GA, Ottesen EA. The clinical and immunologic responses of normal human volunteers to low dose hookworm (*Necator americanus*) infection. American Journal of Tropical Medicine and Hygiene. 1987; 37:126–1234. [PubMed: 3605493]

McGuire, RA.; Elman, C. The Prevalence of Parasitic Intestinal Worms in the Early Twentieth-Century American South and their Demographic and Economic Correlates. University of Akron; 2003. Working Paper

McKean PG, Pritchard DI. The action of a mast cell protease on the cuticular collagens of Necator americanus. Parasite Immunology. 1989; 11:293–297. [PubMed: 2671869]

Meeusen EN, Balic A. Do eosinophils have a role in the killing of helminth parasites? Parasitology Today. 2000; 16:95–101. [PubMed: 10689327]

Mendez MA, Adair LS. Severity and timing of stunting in the first two years of life affect performance on cognitive tests in late childhood. Journal of Nutrition. 1999; 129:1555–1562. [PubMed: 10419990]

Meyrowitsch DW, Simonsen PE. Efficacy of DEC against *Ascaris* and hookworm infections in schoolchildren. Tropical Medicine and International Health. 2001; 6:739–742. [PubMed: 11555442]

Michaud C, Tantalean M, Ique C, Montoya E, Gozalo A. A survey for helminth parasites in feral New World non-human primate populations and its comparasiton with parasitiological data from man in the region. Journal of Medical Primatology. 2003; 32:341–345. [PubMed: 14641789]

Miller TA. Vaccination against the canine hookworm diseases. Advances in Parasitology. 1971; 9:153–183. [PubMed: 4932829]

Miller TA. Industrial development and field use of the canine hookworm vaccine. Advances in Parasitology. 1978; 16:333–342. [PubMed: 364958]

Miller TA. Hookworm infection in man. Advances in Parasitology. 1979; 17:315–384. [PubMed: 395835]

Milstone AM, Harrison LM, Bungiro RD, Kuzmic P, Cappello M. A broad spectrum Kunitz type serine protease inhibitor secreted by the hookworm *Ancylostoma ceylanicum*. Journal of Biological Chemistry. 2000; 275:29391–2939. [PubMed: 10893410]

Moore SL, Wilson K. Parasites as a Viability Cost of Sexual Selection in Natural Populations of Mammals. Science. 2002; 297:2015–2018. [PubMed: 12242433]

Moyle M, Foster DL, McGrath DE, Brown SM, Laroche Y, De Meutter, Stanssens P, Bogowitz CA, Fried VA, Ely JA. A hookworm glycoprotein that inhibits neutrophil function is a ligand of the integrin CD11b/CD18. Journal of Biological Chemistry. 1994; 269:1008–1015. [PubMed: 7908286]

Muchowski PJ, Zhang L, Chang ER, Soule HR, Plow EF, Moyle M. Functional interaction between the integrin antagonist neutrophil inhibitory factor and the I domain of CD11b/CD18. Journal of Biological Chemistry. 1994; 269:26419–26423. [PubMed: 7929363]

Murray, CJL.; Lopez, AD., editors. Global Comparative Assessments in the Health Sector: Disease Burden, Expenditures and Intervention Packages. Geneva: World Health Organization; 1994.

Nacher M. Worms and malaria: noisy nuisances and silent benefits. Parasite Immunolgy. 2002; 24:391–393. [PubMed: 12164826]

Nagahana M, Tanabe K, Yoshida Y, Kondo K, Ishikawa M, Okdada S, Sato K, Okamoto K, Ito S, Fukutome S. Experimental studies on the oral infection of *Necator americanus*. III. Experimental infection of three cases of human beings with *Necator americanus* larvae through the mucous membrane of the mouth. Japanese Journal of Parasitology. 1963; 12:162–167.

Nawa Y, Ishikawa N, Tsuchiya K, Horii Y, Abe T, Khan AI, Bing S, Itoh H, Ide H, Uchiyama F. Selective effector mechanisms for the expulsion of intestinal helminths. Parasite Immunology. 1994; 16:333–338. [PubMed: 7970872]

Nawalinski TA, Schad GA. Arrested development in *Ancylostoma duodenale*: course of a self-induced infection in man. American Journal of Tropical Medicine and Hygiene. 1974; 23:895–898. [PubMed: 4451228]

Nawalinski, TA.; Schad, GA. Historical Introduction. In: Gilles, HM.; Ball, PAJ., editors. Human Parasitic Diseases Volume 4. Hookworm Infections. Amsterdam: Elsevier; 1991. p. 1-4.

Needham C, Kim HT, Hoa NV, Cong LD, Michael E, Drake L, Hall A, Bundy DAP. Epidemiology of soil-transmitted nematode infections in Ha Nam Province, Vietnam. Tropical Medicine and International Health. 1998; 3:904–912. [PubMed: 9855404]

Nwosu ABC. Investigations into the free-living phase of the cat hookworm life-cycle. Zeitschrift fur Parasitenkunde. 1978; 56:243–249. [PubMed: 29389]

Nwosu ABC. The community ecology of soil-transmitted helminth infection of humans in a hyperendemic area of southern Nigeria. Annals of Tropical Medicine and Parasitology. 1981; 75:197–203.

Olatunde BO, Onyemelukwe GC. Immunosuppression in Nigerians with hookworm infection. African Journal of Medicine and Medical Science. 1994; 23:221–225. [PubMed: 7604745]

Olsen A, Samuelsen H, Onyango-Ouma W. A study of risk factors for intestinal helminth infections using epidemiological and anthropological approaches. Journal of Biosocial Science. 2001; 33:569–584. [PubMed: 11683225]

Orihel TC. *Necator americanus* infection in primates. Journal of Parasitology. 1971; 57:117–21. [PubMed: 4994803]

Otto G, Schugman N, Groover M. A precipitin reaction resulting from *Necator americanus* larvae in serum from hookworm infected individuals. Proceedings of the Helminthological Society of Washington DC. 1942; 9:25–26.

Palmer DR, Bradley M, Bundy DAP. IgG4 responses to antigens of adult Necator americanus: potential for use in large-scale epidemiological studies. Bulletin of the World Health Organization. 1996; 74:381–386. [PubMed: 8823959]

Parkinson J, Mitreva M, Hall N, Blaxter M, McCarter JP. 400000 nematode ESTs on the Net. Trends in Parasitology. 2003; 19:283–286. [PubMed: 12855373]

Partnership for Child Development. Better health, nutrition and education for the school-aged child. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1997; 91:1–2. [PubMed: 9093613]

Partnership for Child Development. The cost of large-scale school health programmes which deliver anthelmintics in Ghana and Tanzania. Acta Tropica. 1999; 73:183–204. [PubMed: 10465058]

Pawlowski, ZS.; Scahd, GA.; Stott, GJ. Approaches to prevention and control. Geneva: World Health Organization; 1991. Hookworm infection and anaemia.

Peduzzi R, Piffaretti JC. *Ancylostoma duodenale* and the Saint Gothard anemia. Lancet. 1983; 287:1942–1945. [PubMed: 6418279]

Petney TN, Andrews RH. Multiparasite communities in animals and humans: frequency, structure and pathogenic significance. International Journal of Parasitology. 1998; 28:377–393. [PubMed: 9559357]

Poulin R. Sexual inequalities in helminth infections: a cost of being male? American Naturalist. 1996; 147:287–295.

Pritchard DI. Immunity to helminths: is too much IgE parasite--rather than host-protective? Parasite Immunology. 1993; 15:5–9. [PubMed: 8433853]

Pritchard DI, Brown A. Is Necator americanus approaching a mutualistic symbiotic relationship with humans? Trends in Parasitology. 2001; 17:169–172. [PubMed: 11282505]

Pritchard DI, Quinnell RJ, Slater AF, McKean PG, Dale DD, Raiko A, Keymer AE. Epidemiology and immunology of Necator americanus infection in a community in Papua New Guinea: humoral responses to excretory-secretory and cuticular collagen antigens. Parasitology. 1990; 100:317–326. [PubMed: 2345664]

Pritchard DI, Quinnell RJ, Walsh EA. Immunity in humans to *Necator americanus*: IgE, parasite weight and fecundity. Parasite Immunology. 1995; 17:71–75. [PubMed: 7761110]

Pritchard DI, Walsh EA. The specificity of the human IgE response to Necator americanus. Parasite Immunology. 1995; 17:605–607. [PubMed: 8817608]

Pritchard DI, Walsh EA, Quinell RJ, Raiko A, Edmonds P, Keymer AE. Isotypic variation in antibody responses in a community in Papua New Guinea to larval and adult antigens during infection, and following reinfection, with the hookworm *Necator americanus*. Parasite Immunology. 1992; 14:617–631. [PubMed: 1470481]

Prociv P. Pathogenesis of human hookworm infection: insights from a ‘new’ zoonosis. Chemical Immunology. 1997; 66:62–98. [PubMed: 9103666]

Prociv P, Croese J. Human enteric infection with *Ancylostoma caninum*: hookworms reappraised in the light of a “new” zoonosis. Acta Tropica. 1996; 62:23–44. [PubMed: 8971276]

Quinnell RJ. Genetics of susceptibility to human helminth infections. International Journal of Parasitology. 2003; 30:1219–1231. [PubMed: 13678637]

Quinnell RJ, Griffin J, Nowell MA, Raiko A, Pritchard DI. Predisposition to hookworm infection in Papua New Guinea. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2001; 95:139–142. [PubMed: 11355543]

Quinnell RJ, Pritchard DI, Raiko A, Brown AP, Shaw M-A. Immune response to human necatoriasis: interleukin-5 responses are associated with resistance to reinfection. Journal of Infectious Diseases. in press.

Quinnell RJ, Slater AFG, Tighe P, Walsh EA, Keymer AE, Pritchard DI. Reinfection with hookworm after chemotherapy in Papua New Guinea. Parasitology. 1993; 106:379–385. [PubMed: 8316435]

Rajasekariah GR, Deb BN, Dhage KR, Bose S. Site of resistance to *Necator americanus* in hamsters. Acta Tropica. 1985; 42:333–340. [PubMed: 2868635]

Randall AE, Perez MA, Floyd S, Black GF, Crampin AC, Ngwira B, Pistoni WN, Mulawa D, Sichali L, Mwaungulu L, Bickle Q, Fine PEM. Patterns of helminth infection and relationship to BCG vaccination in Karonga District, northern Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2002; 96:29–33. [PubMed: 11925985]

Ratard RC, Kouemeni LE, Bessala MME, Ndamkou CN, Sama MT, Cline BL. Ascariasis and trichuriasis in Cameroon. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1991; 85:84–88. [PubMed: 2068772]

Ratard RC, Kouemeni LE, Ekani Bessala MK, Ndamkou CN. Distribution of hookworm infection in Cameroon. Annals of Tropical Medicine and Parasitology. 1992; 86:413–8. [PubMed: 1463363]

Reynoldson JA, Behnke JM, Pallant LJ, Macnish MG, Gilbert F, Giles S, Spargo RJ, Thompson RC. Failure of pyrantel in treatment of human hookworm infection (*Ancylostoma duodenale*). in the Kimberely region of North West Australia. Acta Tropica. 1997; 68:301–312. [PubMed: 9492915]

Rihet P, Demeure CE, Bourgois A, Prata A, Dessein AJ. Evidence for an association between human resistance to *Schistosoma mansoni* and high anti-larval IgE levels. European Journal of Immunology. 1991; 21:2679–86. [PubMed: 1936116]

Roche M, Layrisse M. The nature and causes of hookworm anaemia. American Journal of Tropical Medicine and Hygiene. 1966; 15:1031–110. [PubMed: 5334793]

Rose RA, Behnke JM. *Necator americanus* in the DSN hamster: density-dependent expulsion of adult worms during primary infection. Parasitology. 1990; 100:469–478. [PubMed: 2362772]

Saathoff, E. PhD Thesis. University of Copenhagen; 2002. Geohelminth and *Schistosoma haematobium* infection in schoolchildren from rural northern KwaZulu-Natal, South Africa.

Sakti H, Nokes C, Hertanto WS, Hendratno S, Hall A, Bundy DAP, Satoto. Evidence for an association between hookworm infection and cognitive function in Indonesian school children. Tropical Medicine and International Health. 1999; 4:322–334. [PubMed: 10402967]

Sarles MP. The in vitro action of immune rat serum on the nematode, *Nippostrongylus muris*. Journal of Infectious Diseases. 1938; 62:337–348.

Savioli L, Stansfield S, Bundy DAP, Mitchell A, Bhatia R, Engels D, Montresor A, Neira M, Shein AM. Schistosomiasis and soil-transmitted helminth infections: forging control efforts. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2002; 96:577–579. [PubMed: 12625126]

Schad GA, Anderson RM. Predisposition to hookworm infection in humans. Science. 1985; 228:1537–1539. [PubMed: 4012307]

Schad, GA.; Banwell, JG. Hookworm infection. In: Warren, KS.; Mahmoud, AAF., editors. Tropical and Geographical Medicine. New York: McGraw-Hill; 1984. p. 359-372.

Schad GA, Chowdhury AB, Dean CG, Kochar VK, Nawalinski TA, Thomas J, Tonascia A. Arrested development in human hookworm infections: an adaptation to a seasonally unfavorable external environment. Science. 1973; 180:52–54. [PubMed: 4700608]

Schad, GA.; Nawalinski, TA.; Kochar, V.; Cross, JH. Human ecology and the distribution and abundance of hookworm populations. In: Croll, N.; Cross, J., editors. Human ecology and infectious diseases. London: Academic Press; 1983. p. 187-223.

Schallig HD, van Leeuwen MA, Cornelissen AW. Protective immunity induced by vaccination with two Haemonchus contortus excretory secretory proteins in sheep. Parasite Immunology. 1997; 19:447–453. [PubMed: 9372512]

Shackelford PG, Nelson SJ, Palma AT, Nahm MH. Human antibodies to group A streptococcal carbohydrate. Ontogeny, subclass restriction, and clonal diversity. Journal of Immunology. 1988; 140:3200–3205. [PubMed: 3283244]

Shapiro S, Brooker S, Tukahebwa EM, Katsen J, Magnussen P, Olsen A, Clarke S, Kabatereine NB, Ndyomugyenyi R. Epidemiology of helminth infections and their relationship to clinical malaria in southwest Uganda. Transactions of the Royal Society of Tropical Medicine and Hygiene. in press. [PubMed: 15550257]

Sheldon AJ, Groover ME Jr. An experimental approach to the problem of acquired immunity in human hookworm (*Necator americanus*) infections. American Journal of Hygiene. 1942; 36:183–186.

Shield J, Anian G, Ostwald R, Arnhold R. Reinfection with intestinal helminthes after treatment with mebendazole and fluctuations in individual *Ascaris lumbricoides* infection with time. Papua New Guinea Medical Journal. 1984; 27:89–94. [PubMed: 6598553]

Shulman C. Malaria in pregnancy: its relevance to safe-motherhood programmes. Annals of Tropical Medicine and Parasitology. 1999; 93:S59–S66. [PubMed: 10715689]

Shulman CE, Graham WJ, Jilo H, Lowe BS, New L, Obiero J, Snow RW, Marsh K. Malaria is an important cause of anaemia in primigravidae: evidence from a district hospital in coastal Kenya. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1996; 90:535–539. [PubMed: 8944266]

Sill PR, Hill AVS, Igo JD. Multifactorial aetiology of anaemia of pregnancy in Port Moresby, Papua New Guinea. Papua New Guinea Medical Journal. 1987; 30:193–198. [PubMed: 3478921]

Smillie WG, Spencer CR. Mental retardation in school children infested with hookworms. Journal of Educational Psychology. 1925; 17:314–321.

Smith G, Schad GA. *Ancylostoma duodenale* and *Necator americanus*: effect of temperature on egg development and mortality. Parasitology. 1990; 99:127–132. [PubMed: 2797868]

Stanssens P, Bergum PW, Gansemans Y, Jespers L, Laroche Y, Huang S, Maki S, Messens J, Lauwereys M, Cappello M, Hotez PJ, Lasters I, Vlasuk GP. Anticoagulant repertoire of the hookworm *Ancylostoma caninum*. Proceedings of the National Academy of Science USA. 1996; 93:2149–2154. [PubMed: 8700900]

Steenhard NR, Storey PA, Yelifari L, Pit DS, Nansen P, Polderman AM. The role of pigs as transport hosts of the human helminths *Oesophagostomum bifurcum* and *Necator americanus*. Acta Tropica. 2000; 76:125–130. [PubMed: 10936571]

Stephenson LS, Latham MC, Kurz KM, Kinoti SN, Oduori ML, Crompton DWT. Relationship of *Schistosoma haematobium*, hookworm and malaria infections and metrifonate treatment to hemoglobin levels in Kenyan school children. American Journal of Tropical Medicine and Hygiene. 1985; 34:519–528. [PubMed: 4003667]

Stephenson LS, Latham MC, Kurz KM, Kinoti SN, Brigham H. Treatment with a single dose of albendazole improves growth of Kenyan schoolchildren with hookworm, *Trichuris trichiura*, and *Ascaris lumbricoides* infection. American Journal of Tropical Medicine and Hygiene. 1989; 41:78–87. [PubMed: 2764230]

Stiles CW. A new species of hookworm (Uncinaria Americana). parasite in man. American Medicine. 1902; 3:777–778.

Stoltzfus RJ, Albonico M, Chwaya HM, Savioli L, Tielsch JM, Schulze KJ, Yip R. Hemoquant determination of hookworm-related blood loss and its role in iron deficiency in African children. American Journal of Tropical Medicine and Hygiene. 1996; 55:399–404. [PubMed: 8916795]

Stoltzfus RJ, Albonico M, Chwaya HM, Tielsch JM, Schulze KJ, Savioli L. Effects of the Zanzibar school-based deworming program on iron status of children. American Journal of Clinical Nutrition. 1998; 68:179–186. [PubMed: 9665112]

Stoltzfus RJ, Albonico M, Tielsch JM, Chwaya HM, Savioli L. School-based deworming program yields small improvement in growth of Zanzibari school children after one year. Journal of Nutrition. 1997a; 127:2187–2193. [PubMed: 9349846]

Stoltzfus RJ, Chwaya HM, Montresor A, Albonico M, Savioli L, Tielsch JM. Malaria, hookworms and recent fever are related to anemia and iron status indicators in 0- to 5-y old Zanzibari children and these relationships change with age. Journal of Nutrition. 2000; 130:1724–1733. [PubMed: 10867043]

Stoltzfus RJ, Chwaya HM, Tielsch JM, Schulze KJ, Albonico M, Savioli L. Epidemiology of iron deficiency anemia in Zanzibari schoolchildren: the importance of hookworms. American Journal of Clinical Nutrition. 1997b; 65:153–159. [PubMed: 8988928]

Stoltzfus RJ, Dreyfuss M, Hababuu MPH, Chwaya HM, Albonico M. Hookworm control as a strategy to prevent iron deficiency. Nutrition Reviews. 1997c; 55:223–232. [PubMed: 9279058]

Sun FH, Wu ZX, Qian YX. Field trials on the efficacy of tribendimidine against hookworm infections. Chinese Journal of Parasitic Disease Control. 1999; 13:184–186.

Sun FH, Wu ZX, Qian YX, Cao HQ, Xue HC, Ren HN, Xiao SH, Zhan B, Hawdon JM, Feng Z, Hotez PJ. Epidemiology of human intestinal nematode infections in Wujiang and Pizhou Counties, Jiangsu Province, China. Southeast Asian Journal Tropical Medicine and Public Health. 1998; 29:605–610.

Sutton BJ, Gould HJ. The human IgE network. Nature. 1993; 366:421–428. [PubMed: 8247150]

Taiwo FA, Brophy PM, Pritcahrd DI, Brown A, Wardlaw A, Patterson LH. Cu/Zn superoxide dismutase in excretory-secretory products of the human hookworm *Necator americanus*. An electron paramagnetic spectrometry study. European Journal of Biochemistry. 1999; 264:434–438. [PubMed: 10491088]

Tanner M, Evans D. Vaccines or drugs: complementarity is crucial. Parasitology Today. 1994; 10:196–202. [PubMed: 15275473]

Tatala S, Svanberg U, Mduma B. Low dietary iron availability is a major cuase of anemia: a nutritional survey in Lindi District of Tanzania. American Journal of Clinical Nutrition. 1998; 68:171–178. [PubMed: 9665111]

Torlesse, H. PhD Dissertation. University of Glasgow; 1999. Parasitic infections and anaemia during pregnancy in Sierra Leone.

Torlesse H, Hodges M. Anthelminthic treatment and haemoglobin concentrations during pregnancy. The Lancet. 2000; 356:1083. [PubMed: 11009150]

Traub RJ, Robertson ID, Irwin P, Mencke N, Monis N, Thompson RCA. Humans, dogs and parasitc zoonoses - unravelling the relationship in a remote endemic community in northeast India using molecular tools. Parasitology Research. 2003; 90:S156–S157. [PubMed: 12928889]

Udonsi JK. *Necator americanus* infection: a cross-sectional study of a rural community in relation to some clinical symptoms. Annals of Tropical Medicine and Parasitology. 1984; 78:443–444. [PubMed: 6332584]

Udonsi JK, Atata G. *Necator americanus*: temperature, pH, light, and larval development, longevity, and desiccation tolerance. Experimental Parasitology. 1987; 63:136–142. [PubMed: 3569472]

Udonsi JK, Nwosu ABC, Anya AO. *Necator americanus*: Population Structure, Distribution, and Fluctuations in Population Densities of Infective Larvae in Contaminated Farmlands. Zeitschrift fur Parasitenkunde. 1980; 63:251–259. [PubMed: 7434873]

Utzinger J, Keiser J. Schistosomiasis and soil-transmitted helminthiasis: common drugs for treatment and control. Expert Opinion in Pharmacotherapy. 2004; 5:263–286. [PubMed: 14996624]

Utzinger J, Vounatsou P, N'Goran EK, Tanner M, Booth M. Reduction in the prevalence and intensity of hookworm infection after praziquantel treatment for schistosomiasis infection. International Journal of Parasitology. 2002; 32:759–765. [PubMed: 12062494]

Verweij JJ, Pit DSS, van Lieshout L, Baeta SM, Dery GD, Gasser RB, Polderman MA. Determing the prevalence of *Oesophagostomum bifurcum* and *Necator americanus* infections using specific PCR amplification of DNA from faecal samples. Tropical Medicine and International Health. 2001; 6:726–731. [PubMed: 11555440]

Viaene A, Crab A, Meiring M, Pritchard D, Deckmyn H. Identification of a collagen-binding protein from Necator americanus by using a cDNA-expression phage display library. Journal of Parasitology. 2001; 87:619–25. [PubMed: 11426727]

Vlasuk GP, Bradbury A, Lopez-Kinninger L, Colon S, Bergum PW, Maki S, Rote WE. Pharmacokinetics and anticoagulant properties of the factor VIIa-tissuefactor inhibitor recombinant Nematode anticoagulant protein c2 following subcutaneous administration in man. Dependence on the stoichiometric binding to circulating factor X. Thrombosis and Haemostasis. 2003; 90:803–812. [PubMed: 14597974]

Waite JH, Neilson IL. A study of the effect of hookworm infection upon the mental development of North Queensland schoolchildren. Medical Journal of Australia. 1919; 1:1–7.

Warren, KS.; Bundy, DAP.; Anderson, RM.; Davis, AR.; Henderson, DA.; Jamison, DT.; Prescott, N.; Senft, A. Helminth infections. In: Jamison, DT.; Mosley, WH.; Measham, AR.; Bobadilla, JL., editors. Disease Control Priorities in Developing Countries. Oxford: Oxford University Press; 1993. p. 131-160.

Watkins WE, Pollitt E. "Stupidity or Worms": do intestinal worms impair mental performance? Psychological Bulletin. 1997; 121:171–191. [PubMed: 9100486]

White CJ, Maxwell CJ, Gallin JI. Changes in the structural and functional properties of human eosinophils during experimental hookworm infection. Journal of Infectious Diseases. 1986; 154:778–783. [PubMed: 3021866]

Williams-Blangero S, Blangero J, Bradley M. Quantitative genetic analysis of susceptibility to hookworm infection in a population from rural Zimbabwe. Human Biology. 1997; 69:201–208. [PubMed: 9057344]

Williams-Blangero S, Subedi J, Upadhayay RP, Manral DB, Rai DR, Jha B, Robinson ES, Blangero J. Genetic analysis of susceptibility to infection with Ascaris lumbricoides. American Journal of Tropical Medicine and Hygiene. 1999; 60:921–926. [PubMed: 10403321]

Williamson AL, Brindley PJ, Knox DP, Hotez PJ, Loukas A. Digestive proteases of blood-feeding nematodes and other parasites. Trends in Parasitology. 2003a; 19:417–423. [PubMed: 12957519]

Williamson AL, Brindley PJ, Abbenante G, Prociv P, Berry C, Girdwood K, Pritcahrd DI, Fairlie DP, Hotez PJ, Zhan B, Loukas A. Hookworm aspartic protease, Na-APR-2 cleaves human haemoglobin and serum proteins in a host-specific fashion. Journal of Infectious Diseases. 2003b; 187:484–494. [PubMed: 12552433]

Williamson AL, Brindley PJ, Abbenante G, Prociv P, Berry C, Girdwood K, Pritcahrd DI, Fairlie DP, Hotez PJ, Dalton JP, Loukas A. Cleavage of hemoglobin by hookworm cathepsin D aspartic proteases and its potential to host specificity. FASEB Journal. 2003c; 16:1458–1460. [PubMed: 12205047]

Woolhouse ME. A theoretical framework for the immunoepidemiology of helminth infection. Parasite Immunology. 1992; 14:563–578. [PubMed: 1470476]

Woolhouse ME. A theoretical framework for immune responses and predisposition to helminth infection. Parasite Immunology. 1993; 15:583–594. [PubMed: 7877835]

Woolhouse MEJ. Human schistosomiasis: potential consequences of vaccination. Vaccine. 1995; 13:1045–1050. [PubMed: 7491810]

WHO. Global Health Situation and Projections, Estimates. Geneva: World Health Organization; 1992. WHO Technical Report Series no. 749

WHO. The World Health Report 2002. Geneva: World Health Organization; 2002. Reducing risks, promoting healthy life; p. 192Annex Table 3. Burden of disease in DALYs by cause, sex and mortality stratusm in WHO regions, estimates for 2001

Xue HC, Wang Y, Xiao SH, Liu S, Wang Y, Shen GJ, Wu WT, Zhan B, Drake L, Feng Z, Hotez PJ. Epidemiology of human ancylostomiasis among rural villagers in Nanlin County (Zhongzhou Village), Anhui Province, China: II. Seroepidemiological studies of the age relationships of serum antibody levels and infection status. Southeast Asian Journal of Tropical Medicine and Public Health. 2000; 31:736–741. [PubMed: 11414422]

Xue J, Liu S, Ren HN, Li TH, Xue HC, Hotez P, Xiao SH. Necator americanus: maintenance through one hundred generations in golden hamsters (Mesocricetus auratus). I. Hotst sex-associated differences in hookworm burden and fecundity. Experimental Parasitology. 2003; 104:62–66. [PubMed: 12932761]

Ye XP, Wu ZX, Sun FH. The population biology and control of *Nectator americanus* in a village community in south-eastern China. Annals of Tropical Medicine and Parasitology. 1994; 88:635–643. [PubMed: 7893178]

Yosipovitch G, Sugeng MW, Seow CS, Goh CL. Widespread and unusual presentations of cutaneous larva migrans acquired in tropical sandy beach resorts. Journal of the European Academy of Dermatology and Venerealogy. 2002; 16:284–285. [PubMed: 12195574]

Yu SH, Jian ZX, Xu LQ. Infantile hookworm disease in China. A review. Acta Tropica. 1995; 59:265–270. [PubMed: 8533661]

Zhan B, Badamchian M, Bo MH, Ashcom J, Feng JJ, Hawdon J, Xiao SH, Hotez PJ. Molecular cloning and purification of Ac-TMP a developmentally regulated putative tissue inhibitor of metalloprotease released in relative abundance by adult Ancylostoma hookworms. American Journal of Tropical Medicine and Hygiene. 2002a; 66:238–244. [PubMed: 12139214]

Zhan B, Hotez PJ, Wang Y, Hawdon JM. A developmentally regulated metalloprotease secreted by host-stimulated *Ancylostoma caninum* third-stage infective larvae is a member of the astacin family of proteases. Molecular and Biochemical Parasitology. 2002b; 120:291–296. [PubMed: 11897134]

Zhan B, Li T, Xiao S, Zheng F, Hawdon JM. Species-specific identification of human hookworms by PCR of the mitochondrial cytochrom oxidase I gene. Journal of Parasitology. 2001; 87:1227–1229. [PubMed: 11695411]

Zhan B, Liu Y, Badamchian M, Williamson A, Feng JJ, Loukas A, Hawdon JM, Hotez PJ. Molecular characterization of the Ancylostoma-secreted protein (ASP) family from the adult stage of *Ancylostoma caninum*. International Journal for Parasitology. 2003; 33:897–907. [PubMed: 12906874]

Zhan B, Wang Y, Liu YY, Williamson A, Loukas A, Xue HC, Xiao SH, Hotez PJ. Ac-SA-1, an immunodominant 16 kDa surface-associated antigen of infective larvae and adults of *Ancylostoma caninum*. International Journal for Parasitology. in press.

Europe PMC Funders Author Manuscripts

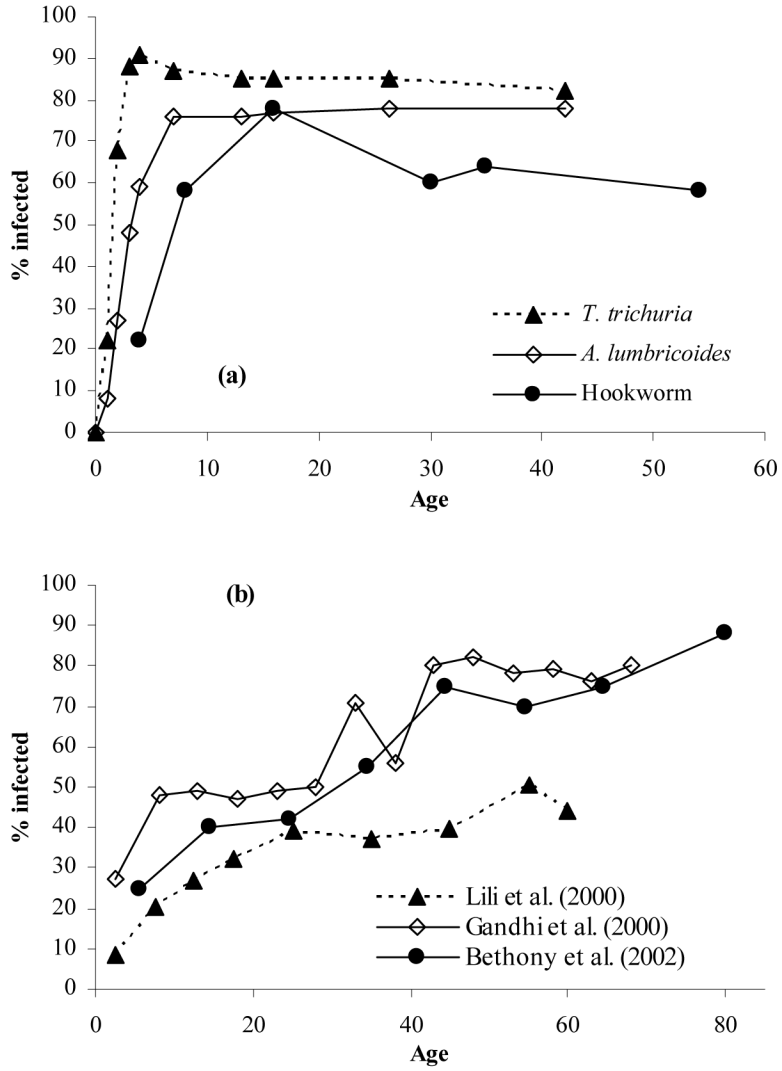

Figure 1.

(a) Typical age-prevalence profiles of hookworm and other STH. Data from Bundy (1988a) and Behnke *et al.* (2000) (females only). (b) Recent age-prevalence data from China (Lili *et al.*, 2000; Gandi *et al.*, 2001; Bethony *et al.*, 2002a)

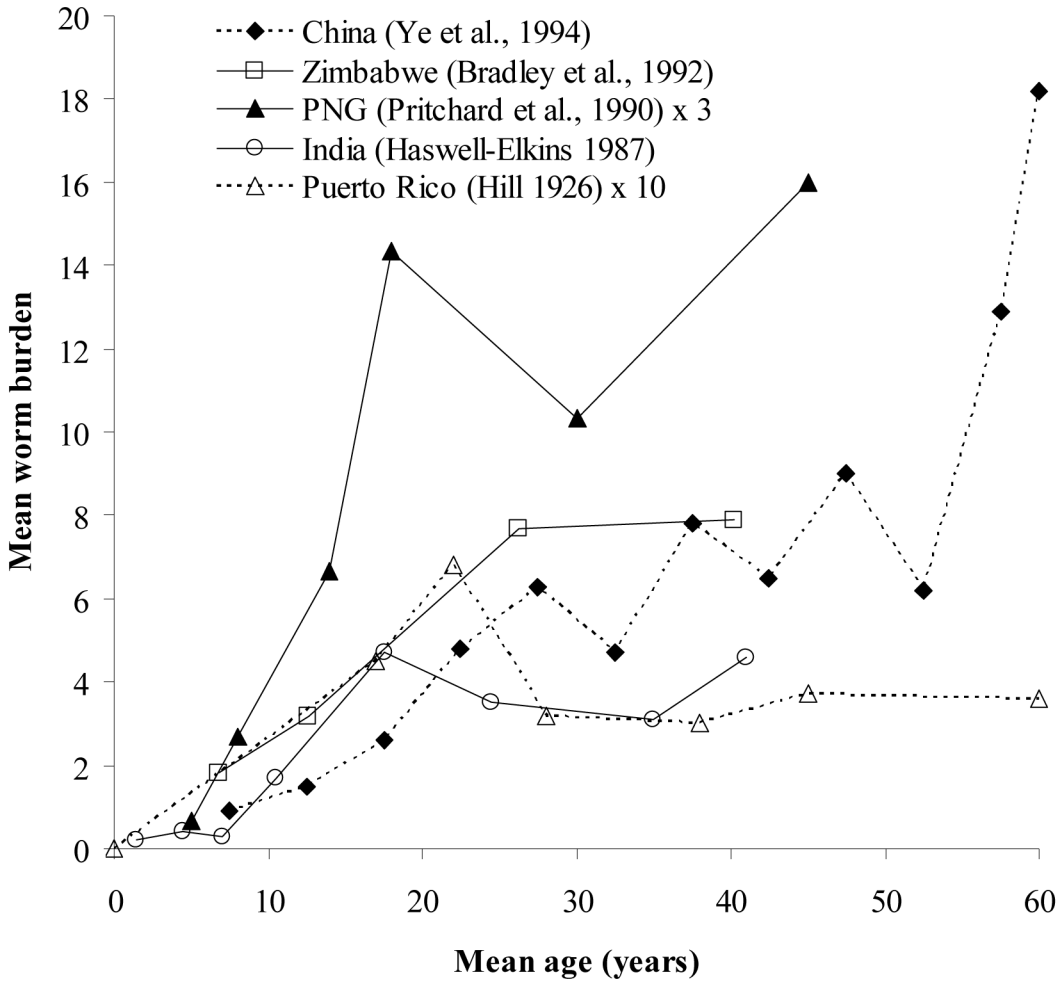

Figure 2.
The relationship between host age and mean *N. americanus* worm burden obtained by anthelmintic expulsion: India (Haswell-Elkins *et al.*, 1988), Papua New Guinea (Pritchard *et al.*, 1990), Zimbabwe (Bradley *et al.*, 1991), Puerto Rico (Hill, 1926) and China (Ye *et al.*, 1994). No comparable data are currently available for *A. duodenale* (Bundy & Keymer, 1991).

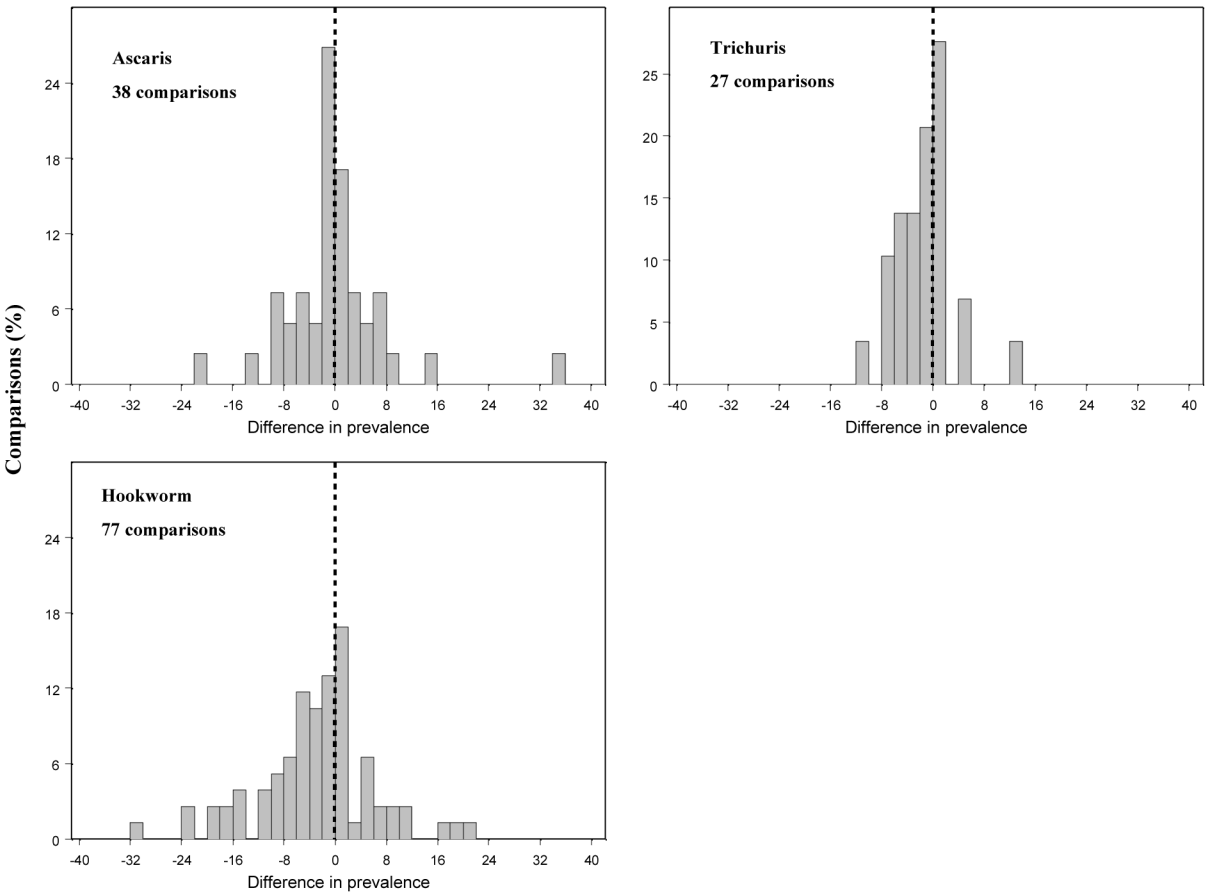

Figure 3.

Frequency distribution of differences in the prevalence of infection between male and female school-aged children for (a) *A. lumbricoides*, (b) *T. trichiura*, and (c) hookworm. Analysis follows that used by Poulin (1996) who employed a fixed effects meta-analysis. In brief, differences in prevalence were calculated using the following formula:

$$\left(p_f - p_m\right)(J), \text{where} \quad J=1-\frac{3}{4\left(N_f+N_m-2\right)-1}$$

which is the difference between prevalence in females (P_f) and that in males (P_m) weighted by a correction for small sample sizes (J). If there is no sex bias in levels of infection, differences in prevalence expected to be normally distributed around a mean of zero. Frequency distributions of differences were compared with χ^2 test. Values to the left of the line represent indicate higher prevalence in males, and values to the right indicate higher prevalence in females. Data sources available from authors.

Europe PMC Funders Author Manuscripts

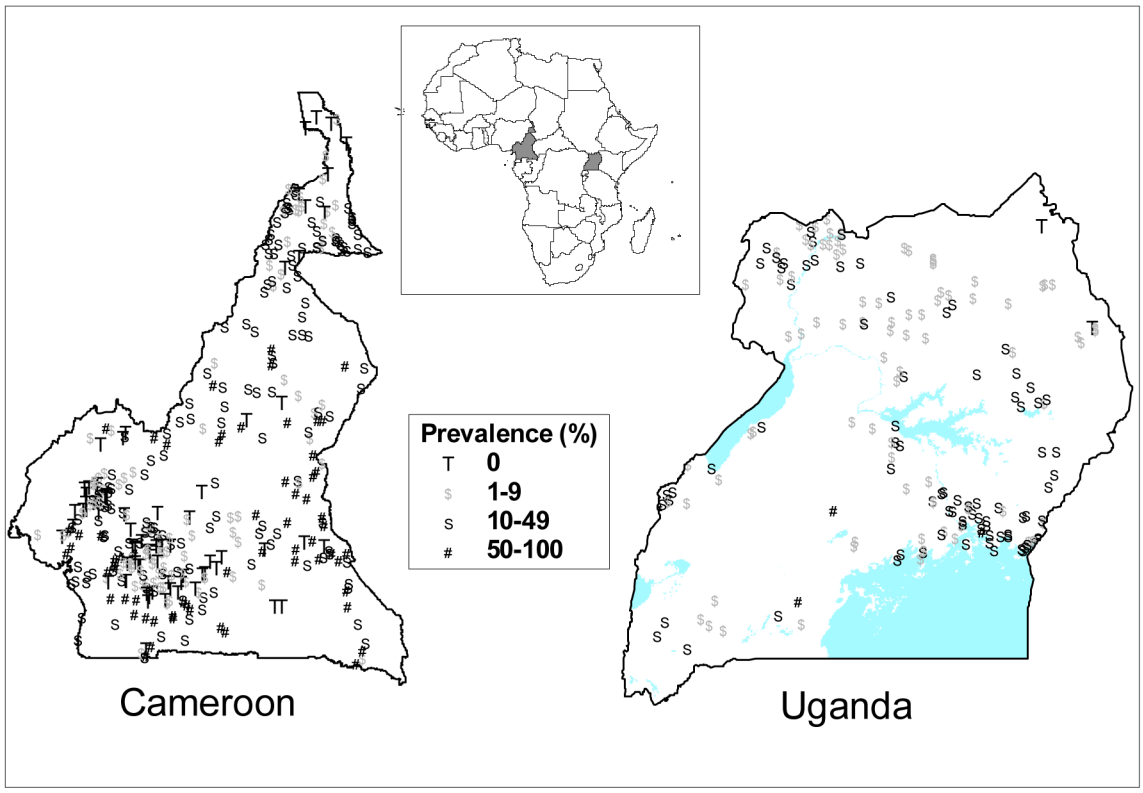

Figure 4.
The geographical distribution of hookworm in Cameroon (18,260 schoolchildren in 402 schools) and Uganda (13,378 children in 235 schools). Data source: Ratard *et al.*, (1992); Brooker *et al.*, in press).

Europe PMC Funders Author Manuscripts

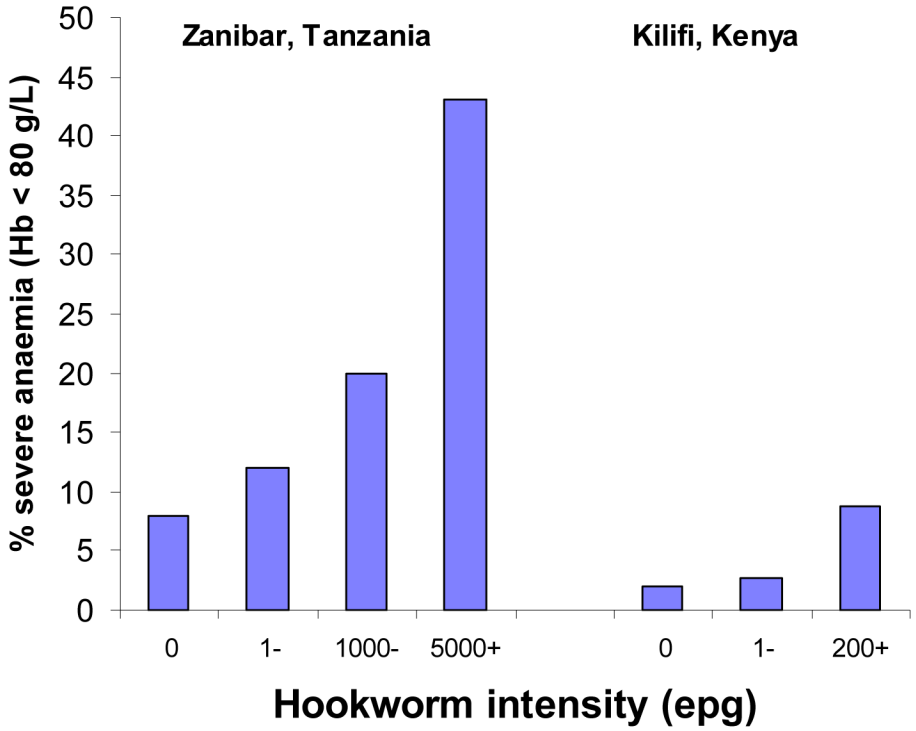

Figure 5.
Proportion of Zanzibari (aged 30 months or greater) and Kenyan (aged 6-76 months) pre-schoolchildren with severe anaemia (Hb<80 g/L) by hookworm egg counts. Taken from Brooker *et al.* (1999) and Stoltfuz *et al.* (2000) Chi-square test for trends of association were p=0.007 and p=0.0002, respectively.

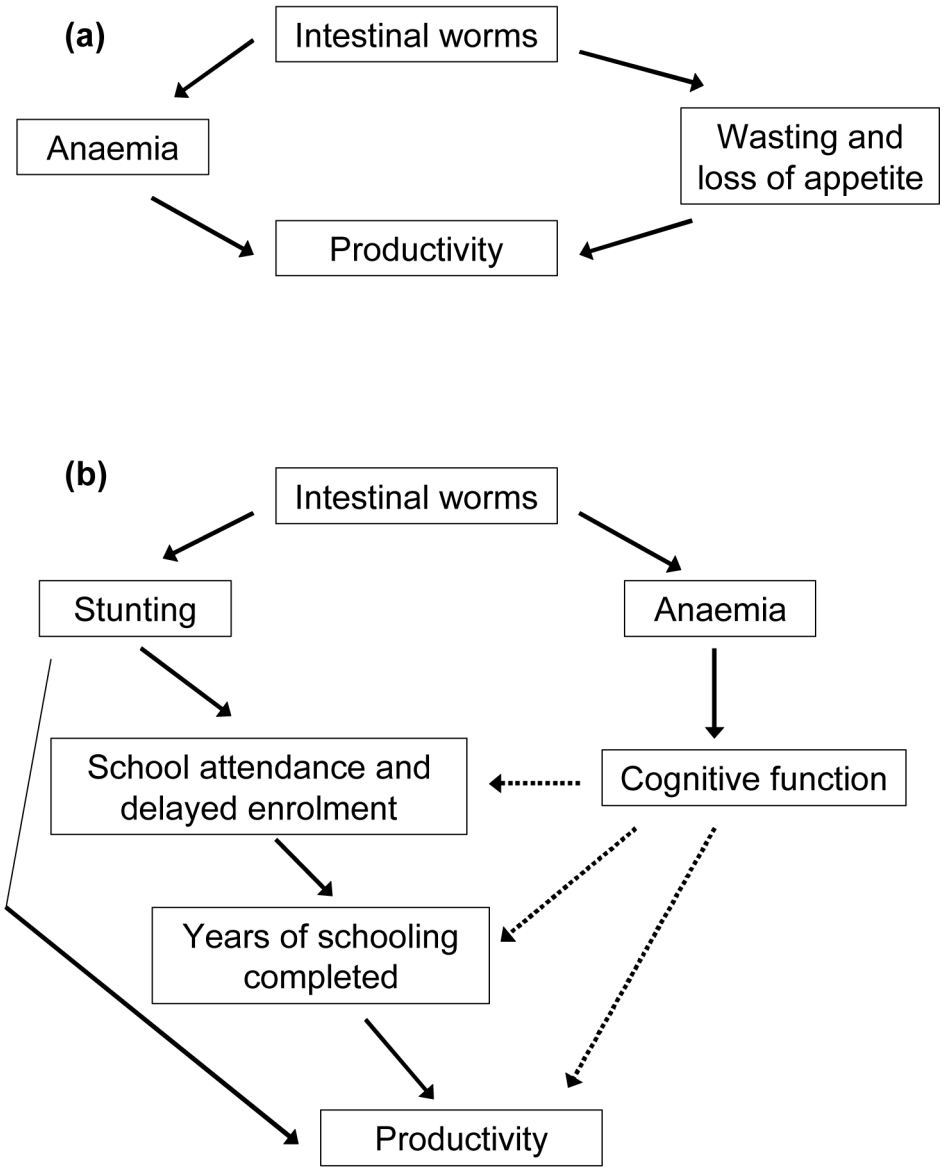

Figure 6.
The ways which intestinal nematodes may affect either (a) current productivity through causing anaemia and undernutrition or (b) future potential productivity mediated anaemia and undernutrition and their effect on cognitive function and school performance. The link between cognitive function and productivity is less clear (as indicated by dashed line). Taken from Guyatt (2000).

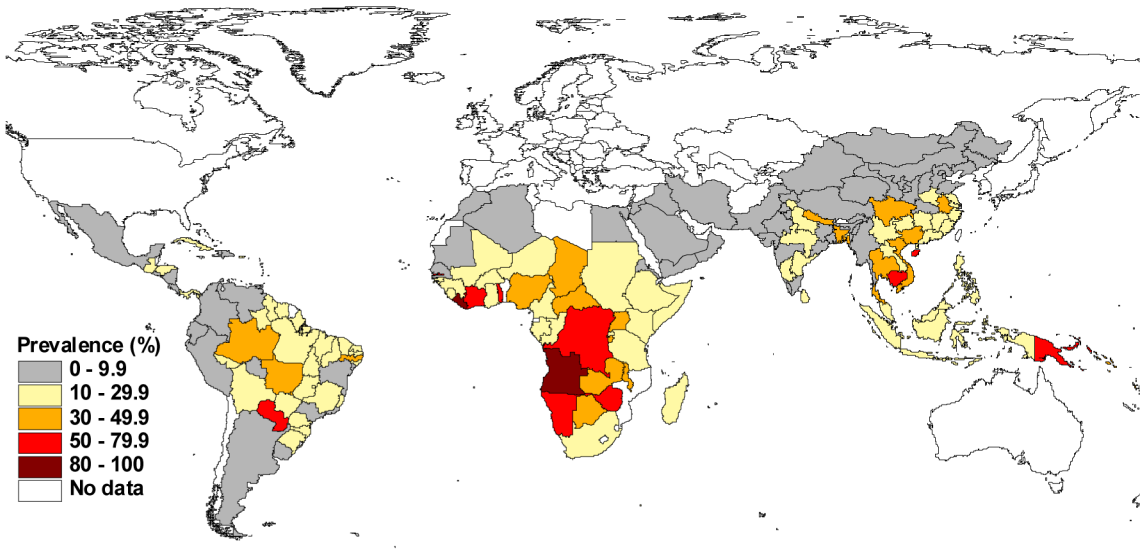

Figure 7.
Map showing the global distribution of hookworm. Taken from a global WHO update by de Silva *et al.* (2003), which included 494 publications from 1990 onward reporting community-based studies incorporating data from 112 countries.

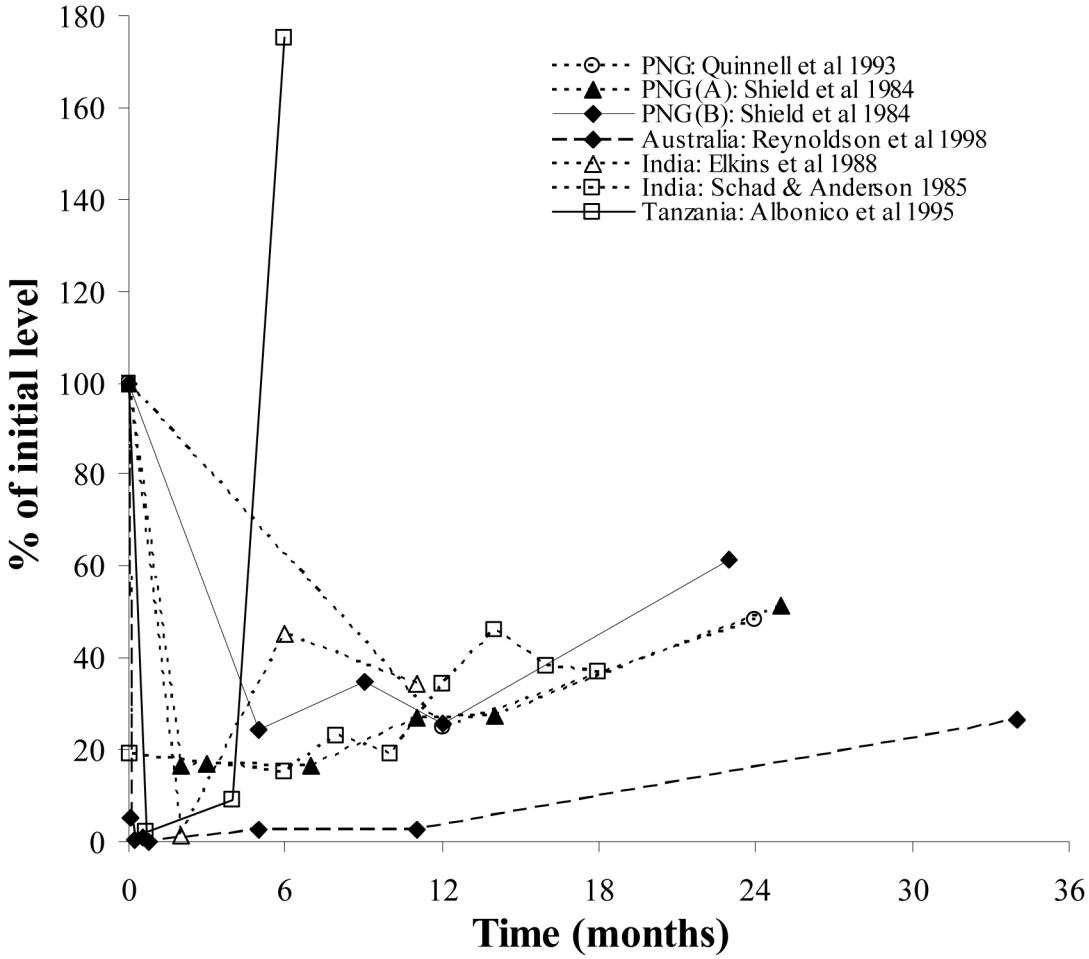

Figure 8.
Intensity of hookworm infection at multiple sampling rounds following initial treatment.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Table 1

Biological characteristics of *N. americanus* and *A. duodenale* in the human host (adapted from Hoagland and Schad (1978); Schad and Banwell (1984); Bundy (1990); Anderson and May (1991); Crompton (2000))

Characteristic	***N. americanus***	***A. duodenale***
Male adult size (mm)	7-9	8-11
Female adult size (mm)	9-11	10-13
Rate of egg production	3000-6000	10,000-20,000
Maturation delay in humans (days)	40-50	28-50
Life expectancy of infective larvae (days)	3-5	1
Life expectancy of adult worm (years)	3-10	1-3
Blood loss (ml). worm/day, mean (range)	0.03 (0.01-0.04)	0.15 (0.14-0.30)
Lactogenic transmission	No	Yes
Oral transmission	No	Yes
Arrested development	No	Yes

Table 2

Macromolecules from Hookworms of Presumed Importance in the Host Parasite Relationship[1]

Molecule & Description	Function	Species	Stage	Reference
MTP-1 Metalloprotease (astacin-type)	Larval invasion	A + N	L3	Zhan *et al.* (2002a) Hotez *et al.* (2003)
ASP-1 & 2 Ancylostoma secreted protein	Immunomodulation † Larval invasion	A + N	L3	Hawdon *et al.* (1996, 1999); Goud *et al.* (2004)
ASP-3,4,5, & 6 Ancylostoma secreted protein	Immunomodulation †	A + N	Adult	Zhan *et al.* (2003)
NAP c2 Nematode anticoagulant protein	VIIa/Tissue factor inhibitor Anticoagulant	A	Adult	Lee and Vlasuk (2003)
NAP 5 & 6 Nematode anticoagulant protein	Factor Xa inhibitor Anticoagulant	A	Adult	Stannssens *et al.* (1996)
HPI Platelet inhibitor	Platelet inhibitor	A	Adult	Del Valle *et al.* (2003)
KI and KPI Kunitz-type serine protease inhibitor	Protease inhibition	A	Adult	Milstone *et al.* (2000) Hawdon *et al.* (2003)
NIF Neutrophil inhibitory factor	Immunomodulation	A	Adult	Muchowski *et al.* (1994); Moyle *et al.* (1994)
FAR-1 Fatty acid Retinol binding protein	Retinol scavenging Immunomodulation †	A	Adult	Basavaraju *et al.* (2003)
CBP Collagen-binding protein	Unknown	N	Adult	Viaene *et al.* (2001)
CTL-2 C-type lectin	Immunomodulation †	N	Adult	Loukas *et al.* (2002)
TMP Tissue inhibitor of metalloprotease	Immunomodulation † Protease inhibitor?	A	Adult	Zhan *et al.* (2002b)
Calreticulin Calreticulin	Complement-binder Immunomodulation †	N	Adult	Kasper *et al.* (2001)
SOD Cu/Zn superoxide dismutase	Antioxidant	N	Adult	Taiwo *et al.* (1999)
GST Glutathione S transferase	Immune defense? Heme detoxification?	A + N	Adult	Brophy *et al.* (1995)
ACHe Acetylcholinesterase	Unknown	N	Adult	Brown and Pritchard (1993)
APR-1 Aspartic protease (Cathepsin D)	Hemoglobinase Tissue degradation	A + N	Both	Williamson *et al.* (2003c)
APR-2 nemepsin	Hemoglobinase Tissue degradation	N	Adult	Williamson *et al.* (2003b)
CP-1 and 2 Cysteine protease (Cathepsin B)	Hemoglobinase Tissue degradation	A	Adult	Harrop *et al.* (1995)
MEP-1 Metalloendopeptidase (neprilysin-type)	Eotaxin-degradation? Tissue degradation	A + N	Adult	Jones and Hotez (2002) Culley *et al.* (2000)

[1] Only molecules for which the protein has been isolated and characterized or a cDNA cloned are included. Molecules identified exclusively from EST databases are not included. Emphasis on molecules either secreted by the parasite or attached to the adult hookworm alimentary canal.

† These molecules have been found to be immunomodulatory in other mammal systems and yet to be defined as such in hookworm.

Europe PMC Funders Author Manuscripts

Table 3

Global 2003 estimates of prevalence and the number of cases of hookworm (for *A. duodenale* and *N. americanus* combined) by region and age group[a] (Taken from de Silva *et al.*, 2003)

Region	Population (in millions)		Infection prevalence	Estimated number of infections (millions)				
				Age group				Total
	Total	At risk		0 - 4 y	5 - 9 y	10-14 y	> =15 y	
LAC	530	346	10%	1	3	5	41	50
SSA	683	646	29%	9	18	29	142	198
MENA	313	73	3%	0	1	1	8	10
SAS	363	188	16%	2	5	8	44	59
India	1,027	534	7%	2	5	8	56	71
EAP	564	512	26%	4	9	16	120	149
China	1,295	897	16%	3	9	18	173	203
Total	4,775	3,195	15%	21	50	85	584	740

[a]Following the approach used by Chan *et al.* (1994b), these estimates are based on prevalence rates reported from 5 regions: Latin America and the Caribbean (LAC); sub-Saharan Africa (SSA); Middle East and North Africa (MENA); South Asia (SAS); East Asia and the Pacific Islands (EAP).

Table 4

The World's Tropical Communicable Disease Burdens (WHO, 2002)

Disease	DALYs (000)	% total
World's total DALYs	1,467, 257	100
Infectious diseases	359, 377	24.5
HIV/AIDS	88,429	6.0
Malaria	42,280	2.9
Tuberculosis	36,040	2.6
Measles	26,495	1.8
Lymphatic filariasis	5,644	0.4
Trachoma	3,997	0.3
Leishmaniasis	2,357	0.2
Hookworm disease	1,825	0.1
Schistosomiasis	1,760	0.1
Trichuriasis	1,649	0.1
African typanosomiasis	1,598	0.1
Ascariasis	1,181	0.1
Onchoceriasis	987	0.1
Japanese encephalitis	767	0.1
Dengue	653	<0.1
Chagas disease	649	<0.1
Leprosy	177	<0.1

Europe PMC Funders Author Manuscripts

Adv Parasitol. Author manuscript; available in PMC 2008 March 18.

Table 5

Population attributable fractions of anaemia associated with hookworm in school-aged populations in East Africa (unweighted estimates with 95% confidence intervals in parenthesis, where reported)

Area	Age	N	Anaemia[1]	Hookworm		Source
			%	%	AF	
Lindi District, Tanzania	6-14	727	13.0	13.0	0	Tatala *et al.* (1998)
Magu District, Tanzania	6-20	6897	31.2	37.0	1.2 (0-5.6)	Lwambo *et al.* (2000)
Tanga Region, Tanzania	8-12	466	54.1	61.2	6.4 (0-17)	Guyatt *et al.* (2001)
Kwale District, Kenya[3]	6-17	392	65.7	93.9	15.3 (0-66)	Stephenson *et al.* (1985)
Zanzibar, Tanzania[2]	7-13	3428	94.0	93.7	25 (15-35)	Stoltzfus *et al.* (1997c)

[1] Haemoglobin concentration <110 g/L