Ascaris lumbricoides: A Review of Its Epidemiology and Relationship to Other Infections

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Key Words

Ascaris lumbricoides • Epidemiology, infection • Water scarcity • Cross-transmission • Immunology • Infections, concurrent

Abstract

This review highlights advances made since 2004 in understanding the epidemiology of infection and the interactions between *Ascaris lumbricoides* and other concurrent infections. As water scarcity increases, untreated wastewater is increasingly used to irrigate crops, thus increasing the risk of transmission. New methods to detect and inactivate *Ascaris* eggs in water, soil and food are described. The association between pig ownership and *Ascaris* infection in humans may represent cross-transmission as hybridization among the pig and human ascarids occurs more frequently than previously believed. Geospatial analyses have successfully predicted infection levels both at a regional level (based on vegetation indices, temperature and humidity) and within communities (based on social and environmental factors). The interpretation of antibody and cytokine responses to *Ascaris* is becoming clearer, as researchers recognize the role of antigen type, age, the history of *Ascaris* and other infections. The considerable interest emerging on the interactions between *Ascaris* and other infections (helminths, malaria, HIV, tuberculosis) and allergy is explored. The impact of concurrent infection on the design of control strategies is discussed including the benefits arising from combination therapies and the evi-

dence that intestinal nematodes impair the efficacy of childhood vaccines. Finally, recommended areas for future research are identified.

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Introduction

Over the past century, advances in our understanding of virtually all aspects of parasitic disease have contributed to reducing the prevalence of parasitic infection in many populations. Regrettably, however, these infections still remain embedded within the daily life of the majority of impoverished populations living in the tropical and subtropical regions of the world. At center stage are the three common soil-transmitted, intestinal nematodes, *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm, that are estimated to infect 1.4, 1 and 1.2 billion people, respectively, representing about 25% of the world's population [1]. Perhaps more important than the numbers infected are the estimates of the resulting disease burden of 10.5, 6.4 and 22.1 million disability adjusted life years, respectively [2].

This review focuses on *A. lumbricoides* (referred to henceforth by the generic designation of *Ascaris*), and provides an overview of the major research developments since 2004. It also demonstrates the need to consider parasitic infections in a much more holistic manner than is traditional in both the research and clinical setting by ar-

guing that one person may well be concurrently infected with a vast array of other helminth, protozoan, bacterial, and viral infections, and that these organisms interact with each other and their host in ways that critically affect the design of prevention and control interventions.

Life History and Epidemiology

Life Cycle and Pathology

Ascaris is transmitted by the ingestion of eggs [for reviews, see 1, 3]. These eggs hatch, larvae penetrate through the intestine and migrate through the portal vessels to the liver and lungs where they are coughed up and swallowed, a process that takes several weeks. After the worms return to the intestine, they mature as adult male and female worms, typically measuring about 20 and 30 cm in length, respectively.

The migratory phase is responsible for inflammatory and hypersensitivity reactions in the lung, including pneumonitis and pulmonary eosinophilia. Pathology induced by the adult worms includes malabsorption, intestinal obstruction, and invasion of the bile duct or appendix, leading to acute pancreatitis and appendicitis. *Ascaris* has also been associated with impaired cognitive function. Although much of the early evidence is quite weak [4], Ezeamama et al. [5] found that Filipino children (7–18 years old) with moderate and high intensity *Ascaris* infection had lower responses in cognitive tests of memory compared with uninfected children, after controlling for nutritional status, socioeconomic indicators and other helminth infections.

The period from ingestion of eggs to their detection in feces ranges from 10 to 11 weeks, and adult worms live for 1–2 years. During this time, adults mate and *Ascaris* eggs are passed in the feces. Fecundity estimates vary considerably among geographical regions, ranging from 10 to 220 eggs per female worm per gram of feces [6]. *Ascaris* eggs are more resistant to desiccation than hookworm or *Trichuris* eggs. Given favorable environmental conditions, they have been reported to survive for up to 15 years [3]. They are also very sticky [7], and attach easily to fruit, vegetables, soil and dust particles, children's toys, currency notes, flies and cockroaches [3, 8–10].

Risk Factors Associated with Transmission

Transmission of *Ascaris* eggs is typically associated with accidental ingestion of soil [11], but deliberate ingestion of soil and ingestion of contaminated vegetables, greens and fruit are also important. Purposeful ingestion

of soil, 'pica', is a significant risk factor for *Ascaris* in children [12], and in pregnant and lactating women in some populations. In western Kenya, 45.7% of pregnant women surveyed were geophagous, consuming an average of 45.4 g of earth/day during mid-term pregnancy and 25.5 g/day at 6 months postpartum [13]. Following mebendazole treatment at 28–32 weeks of gestation, *Ascaris* prevalence (percent infected) was significantly higher in geophagous women at 3 and 6 months postpartum compared with those not consuming earth [14], indicating more rapid re-infection among those eating soil. Interestingly, prevalence increased more rapidly in women who preferred termite mound earth that is typically found around the home, compared with those consuming other soft earths that are normally purchased and thus less likely to be contaminated with *Ascaris* eggs [14]. Ingestion of termite mound earth has also been reported as a risk factor for *Ascaris* among grade 3 children in South Africa [12].

Evidence from several studies suggests that the risk of transmission due to contaminated food may be increasing as pressures to minimize the use of artificial fertilizers and to conserve water indirectly promote re-use of wastewater as an organic fertilizer and for irrigation of field crops and hothouse gardens [15]. In Morocco, *Ascaris* prevalence was significantly higher in children living in a peri-urban area where urban wastewater was used for irrigation (13.3%), compared with children of similar living standards but where well water was used for irrigation (1.7%) [16]. A survey of vegetables from urban markets in Ghana revealed *Ascaris* eggs on 60% of lettuce, 55% of cabbage and 65% of spring onion samples, with mean helminth egg numbers (including *Ascaris*, *Trichuris*, hookworm and *Schistosoma*) of 1.1, 0.4 and 2.7/g wet weight of lettuce, cabbage and spring onions, respectively [17]. Thus, despite recommendations to the contrary, in situations of water scarcity, where the use of untreated wastewater for irrigation is seen as the only alternative to ensure continuing food production [17], the risk of *Ascaris* transmission from food sources may be expected to increase.

The set of environmental, social and behavioral predictors of elevated *Ascaris* egg output are not always consistent across studies, but crowding [18–20], poor education of mothers [18, 20–22], open defecation [20, 22], inadequate water supply [18–20], poverty [18, 20, 22–25], poor nutritional status [24, 26, 27], use of human biosolids for fertilizers and irrigation [16], geophagy [12], not washing hands before eating [25], pig ownership or livestock breeding [20, 25, 28], and consumption of raw pork and raw water plants [25] are often reported, depending on the population under consideration (preschool chil-

dren, schoolchildren, migrant women, street vendors, entire communities).

As one example, Traub et al. [20] surveyed households on tea plantations in India and identified six significant predictors of *Ascaris* prevalence: age (children), household crowding (more than 6 people per household), employment (tea plantation workers had a higher prevalence than plantation staff), outdoor defecation, ownership of pigs, and low level of education. Three of these (age, crowding and pig ownership) as well as being Hindu were also associated with elevated *Ascaris* intensity, measured as eggs per gram feces (epg). However, neither employment nor outdoor defecation entered the final model for intensity, likely because they were highly correlated with household crowding and pig ownership, respectively.

The association with pig ownership is intriguing, given the continuing interest in the zoonotic potential of transmission of *Ascaris suum* from pigs to humans. Pigs may act as mechanical disseminators of *Ascaris* eggs, as unembryonated *A. suum* eggs that pass through the pig intestine retain infectivity to pigs, and pigs are coprophagous [29]. Although molecular epidemiology indicates that cross-infection is uncommon [30], evidence for hybridization between sympatric pig and human worms [31] has recently been reported. Criscione et al. [31] examined 23 microsatellite loci from 129 *Ascaris* collected from pigs and humans in China and Guatemala, and from humans in Nepal. Using Bayesian clustering methods to detect host colonization patterns and to identify hybrid worms, their analysis indicated that 4% of the worms from Guatemala and 7% of the worms from China were hybrids and they interpreted this to mean that cross-transmission between pigs and humans may occur more often than previously detected [31].

Age Profile

The epidemiological pattern of *Ascaris* infection in a community is well documented, and follows a similar pattern in all regions where it is endemic. In large part, the pattern results from the continual ingestion of eggs, leading to superimposition of existing worms and new incoming worms, and to rapid re-infection following drug treatment. Thus, in areas where *Ascaris* is endemic, individuals are likely to be infected with *Ascaris* for a large portion of their life.

Infection has been reported in children as young as 5 months of age [32] and both the prevalence and intensity increase rapidly with age. Among Zanzibari infants, the prevalence of *Ascaris* increased from approximately 7% in the 5- to 9-month-old children to about 20% in infants

aged 10–11 months [32]. The prevalence and intensity typically peak in the 6- to 10-year-old age group [33].

Although prevalence usually remains elevated even in the adult population, intensity typically declines in adults [33]. This has been interpreted to result from a combination of reduced contact with eggs, and an accumulated immunological resistance to incoming infection [34]. Recently, a third alternative has been proposed by Galvani [35] who suggests that slow development of resistance with age could result from the antigenic diversity of circulating parasite strains. Interestingly, there are also reports of elevated infection in the elderly [36], and this may be more common than reported, given that most community-based surveys do not identify the elderly as a separate age group in the data analysis. The elderly population is growing in many countries where *Ascaris* is endemic, and their weakening health and immunocompetence may put them at risk of elevated infection and associated morbidity.

Large Scale Spatial Pattern

Whereas epidemiological studies in the past have focused primarily on age-related patterns of infection, and occasionally on temporal changes in infection, more recently, interest has shifted to spatial patterns of infection both at the small village-level scale and the large, country-wide scale. Such research has benefited substantially from methodologies in geographic systems analysis, the large spatial databases that are now available, and the ease of use of hand-held geopositioning systems [37, 38]. Vegetation indices are strongly associated with information on temperature and rainfall, both of which explain pockets or entire regions within a country where *Ascaris* prevalence may be very high or unusually low. For example, a normalized difference vegetation index based on satellite imaging at a 1-km resolution was a significant predictor of both *Ascaris* infection and re-infection intensity [12]. Brooker et al. [39] demonstrated that land surface temperature and rainfall were important determinants of *Ascaris* in Uganda. Prevalence was higher in regions where maximum land surface temperatures were less than about 38°C and was considerably lower at higher land surface temperatures. In Sri Lanka, Gunawardena et al. [40] found that the total number of days per month with rain (wet days) provided a better correlation with *Ascaris* intensity and rates of re-infection than total rainfall per month or mean monthly temperature. Prevalence typically does not exceed 10% unless annual rainfall exceeds 1,000–1,400 mm, depending on the local soil conditions [39]. Interestingly, in an overview of climatic factors affecting the species diversity of human pathogens,

Guenier et al. [41] found that ranges in monthly temperature and maximum range in precipitation were better predictors of latitudinal differences in human helminth parasite assemblages than mean values.

Considerable differences in prevalence and intensity can also exist within a much finer spatial scale. For example, *Ascaris* prevalence among 9–12 year olds in 43 schools in the Forest Zone of Pernambuco, Brazil, ranged from 6.7 to 55% [42]. In Cameroon, the average *Ascaris* epg in 4- to 11-year-old children was almost twice as high in one village compared with another village only 6 km away, despite the apparent similarity between the villages [43]. At this spatial scale, environmental and lifestyle factors are likely to play a larger role than rainfall and temperature [39]. For example, researchers in Brazil have developed a geospatial model that used data on mother's education, family income, use of filtered water, and number of people per room to effectively describe the regions at risk of *Ascaris* infection in children aged 1–9 years at a resolution of 30 m [18].

Aggregation within Communities

In addition to the typical age-prevalence and age-intensity profiles, *Ascaris* infection is aggregated within the population, such that the majority of worms are found in a small proportion of the host population [33]. This aggregation is believed to result from heterogeneity among hosts in a variety of factors that influence rates of exposure to eggs in the environment and the establishment and survival of the parasites.

Given that so many of the risk factors for *Ascaris* transmission, establishment and survival are common within the family or household, it is not surprising that *Ascaris* is also aggregated at the household level. Household clustering accounted for 21 and 39% of the variance in heavy infection (defined as >10,000 epg), respectively, in urban and rural regions of the municipality of Americaninhas, Brazil [44]. In southwest Uganda, 51% of total variance in the 90th percentile of epg was explained by household in a community-wide survey [45], and in China household accounted for 31.7% of the risk of *Ascaris* infection [46].

Efforts continue to determine the role of genetic factors in susceptibility to *Ascaris* infection, especially following a study in Nepal indicating that 30–50% of the variability in *Ascaris* worm burden could be attributed to genetic pedigree [47]. Follow-up genome scans in the same population revealed genes on chromosomes 1 and 13 that were significantly linked with *Ascaris* epg [48], at least one of which may be involved with B cell activation. They also detected significant heritability of cytokines

IL-4, IL-5, IL-10, IFN- γ , and of the ratios of IL-4/IFN- γ and of IL-10/IFN- γ , indicators of T-regulatory processes [49]. Ellis et al. [46], however, were unable to detect a significant genetic component to risk of *Ascaris* infection in their study of familial aggregation of helminth infections in China, though the use of prevalence (not intensity) data may be an explanation. Genetic differences in the STAT6 gene, which is involved in Th2 immune signaling, have also been linked with *Ascaris* intensity [50].

Aggregation has important consequences as those few heavily infected individuals are more likely to suffer the clinical consequences of the infection. Even though egg production per female worm declines as worm burdens increase [6], this density-dependent process is not sufficient to reduce net egg output from heavily infected individuals below that of lightly infected individuals, and thus the aggregated dispersion of *Ascaris* results in the few heavily infected individuals playing a key role in contaminating the environment with eggs. Also, high levels of aggregation are predicted to increase the stability of the host–parasite association, making the parasite population more resilient to external forces, such as drug control, with the predicted consequence that highly aggregated infections will be more difficult to eradicate [51].

Finally, many of the factors that make an individual at risk of heavy infection do not change over a lifetime, and as a consequence certain individuals are predisposed to heavy infection [52]. This means that, at a community level, those individuals with a high egg output prior to drug treatment are likely to rapidly regain large numbers of worms following treatment, whereas those with low epg are likely only to regain a few worms.

Immunology

Immune Responses to Ascaris

Ascaris induces a strong humoral response characterized by elevated IL-4, IL-5, eosinophilia and *Ascaris*-specific IgE, hallmarks of a Th2 immune response [53; for review, see 54]. Although correlations between antibody titers and egg production are not always detected [55], there is evidence that individuals with high *Ascaris*-specific antibody titers and Th2 cytokines have lower epg, suggesting that continuing exposure to *Ascaris* eggs from the environment acts as an ongoing booster to maintain elevated protective immune responses, and that individuals with low responses tend to have higher egg output, indicating a weakness in their ability to control the infection [56–58].

Fig. 1. Relationship between antibody responses to recombinant *Ascaris* allergen (rABA-1A) and age-adjusted *Ascaris* eggs per gram (epg) in 4–11 year olds (**a, b**) and 12–36 year olds (**c, d**) from Cameroon. Data on IgG4 responses (**a, c**) and IgE responses (**b, d**) are shown. Whereas IgG4 titers are elevated as infection intensity increases in the 4- to 11-year-old age group, IgE titers decline with increasing intensity among the 12–36 year olds. Spearman's rho (r) and p values are reported. * $p < 0.05$. Reprinted with permission from Turner et al. [34].

It appears that at least three factors account for the differing results among studies: type of antigen, isotype of antibody response, and age [34, 59]. There appears to be no association between infection intensity and either IgE or IgG4 responses to crude antigens [34]. However, the IgE-specific response to a recombinant form of an *Ascaris* allergen (rABA-1A) is associated with resistance to *Ascaris*. IgE titers to rABA-1A decreased with increasing *Ascaris* intensity in 12- to 36-year-old participants from Cameroon [34], as shown in figure 1, and elevated IgE responses to *Ascaris* glycolipids were also detected in lightly infected children from Cameroon, compared with heavily infected children and uninfected European controls [59]. In contrast, IgG4 titers to rABA-1A increased with increasing *Ascaris* intensity in 4- to 11-year-old children (fig. 1) [34] as did the IgG4 response to proteins in the Indonesian children [59]. Perhaps of most interest is the observation that the ratio of IgG4 to IgE in response to rABA-1A increased with *Ascaris* epg [34], indicating that IgG4 may either block the IgE response which is necessary for resistance to *Ascaris* or interfere with class switching of IgG4 to IgE production. Turner et al. [34] suggested that these responses may be modulated by the regulatory processes involving IL-10.

In the same population, Jackson et al. [57, 58] examined cytokine responses prior to and 8–9 months after deworming. Using a principal components analysis which enabled them to collapse large amounts of data into a few principal variables, they were able to demonstrate that general susceptibility to either *Ascaris* or *Trichuris* was negatively associated with Th2-dominated responses (driven by IL-13) in 4- to 13-year-old children as shown in figure 2, but not in the 14- to 57-year age group [57]. They also showed that resistance to re-infection was positively associated with a Th2-dominated response, driven primarily by IL-5 [57]. When they analyzed the data separately for the two parasites [58], it became evident that the cytokine expression patterns were species specific. For *Ascaris*, a negative association was detected between Th2-dominated responses (driven by IL-13) and epg before treatment but not after treatment. In contrast, *Trichuris* epg was negatively associated with Th2-dominated responses only after treatment, and involved both IL-13 and IL-5, as shown in figure 3. The studies by Williams-Blangero et al. [47–49] provide clear evidence that at least some variability in immune responsiveness has a genetic component (see Aggregation within Communities, p. 10).

It is important to note that nematodes also have a suppressive effect on the host immune response. *A. suum* re-

Fig. 2. Relationship between cytokine responses and susceptibility to *Ascaris* and *Trichuris* infection in 4- to 13-year-old children from Cameroon based on principal components analysis. The x axis captures a set of data reflecting general susceptibility to one or the other of the nematodes, based on fecal egg output. The y axis shows the average IL-13 (a) and IL-5 (b) standardized data based on the ex vivo response to 3 parasite-derived antigens (*Ascaris lumbricoides*, *Trichuris muris* and *T. muris* excretory-secretory antigen). IL-13 declines with increasing general susceptibility score ($F_{1,60} = 5.80$, $p = 0.019$) and the IL-5 response shows a similar but nonsignificant trend ($F_{1,58} = 3.70$, $p = 0.059$). Reprinted with permission from Jackson et al. [57].

leases potent immunosuppressive molecules that interfere with antigen presentation of dendritic cells [60]. The purified proteins maintain their ability to inhibit T-cell-dependent antibody production and to suppress both Th1 and Th2 responses through modulation of IL-4 and IL-10 [61]. Similar processes are likely to occur in human *Ascaris* infections as well. Thus, variability in antibody titers and cytokine levels may be, in part, related to the responsiveness of the host to such immunosuppressive molecules.

Fig. 3. Relationship between Th2-dominated cytokine responses of 4- to 13-year-old children from Cameroon and *Ascaris lumbricoides* log egg prior to treatment or *Trichuris trichiura* log egg 8–9 months after treatment, based on a principal components analysis. The y axis captures a set of data predominantly reflective of Th2 cytokines. *Ascaris* egg was negatively associated with the Th2-dominated response prior to drug treatment ($F_{1,59} = 5.95$, $p = 0.0018$) but not after re-infection. In contrast, *Trichuris* egg was not associated with the Th2-dominated response prior to treatment, but was negatively associated after treatment ($F_{1,45} = 6.38$, $p = 0.015$). Reprinted with permission from Jackson et al. [58].

Ascaris and Allergy

There is considerable recent evidence that the Th2 environment induced by *Ascaris* infection is beneficial in preventing and/or controlling a range of atopic and autoimmune conditions where an aggressive Th1 response is pathogenic [50]. This is believed to result either from cross-regulation between Th1 and Th2 response phenotypes or from the influence of T-regulatory cytokines [62], and may depend on the specific STAT6 haplotype involved in Th2 immune signaling [50]. Atopy refers to clinical manifestations of IgE-mediated hypersensitivity, including allergic rhinitis (hay fever), eczema, asthma and various food allergies, and is defined operationally as the presence of either a positive skin test to extracts of inhaled allergens or the presence of allergen-specific IgE in serum.

The interaction between *Ascaris* and atopic diseases differs depending on whether current infection or history of infection with *Ascaris* is considered [63]. There is also evidence that the interaction is modified by the presence of other infections including active tuberculosis

[64]. Based on a recent study in South Africa where the prevalence of *Ascaris* in 6- to 14-year-old children was only 14.8%, elevated *Ascaris*-specific IgE (but not *Ascaris* eggs in stool) increased the risk of positive skin tests to a wide range of aeroallergens as well as to atopic rhinitis and asthma, but this effect was not detectable in children who had active tuberculosis [64]. In Ecuador, researchers have shown a strong negative association between allergen skin test reactivity and *Ascaris* infection [65]. In mice, acute *A. suum* infection was found to exacerbate allergic symptoms whereas a chronic infection was protective [66]. A similar distinction has been observed in human studies. A meta-analysis of data on eggs in stool [67] revealed that current infection was associated with an increased risk of asthma, whereas a retrospective cohort study in East Germany demonstrated that children with a history of intestinal worm infections (*Ascaris* and/or pinworms) had reduced atopic and non-atopic eczema, as well as a reduced risk of allergic sensitization to common aeroallergens [62].

One intriguing recent observation [68] may provide an alternative explanation for the association between *Ascaris* and many allergic responses, because *Ascaris* shares a common antigen with several environmental allergens. Arruda and Santos [68] found that tropomyosin occurs in a wide range of invertebrates, including *Ascaris*, and that IgE against this molecule is found in over 50% of individuals living in regions where *Ascaris* is endemic.

***Ascaris*, Growth and Malnutrition**

Malnutrition impairs immune responses which in turn can increase susceptibility to infection [for review, see 69, 70]. The negative effects of *Ascaris* on the absorption of vitamin A and fat, protein, and certain sugars, together with anorexia induced by infection, account for the poor growth of *Ascaris*-infected children, and the improved growth that is evident following deworming [70, 71]. In fact, parents report that deworming increases the activity and appetite of their children [72]. In a recent study in Panama, rates of re-infection with *Ascaris* were reduced by providing vitamin A supplements to preschool children [27]. This conclusion is consistent with observations in Mexico where *Ascaris*-infected children less than 2 years of age who received vitamin A supplementation had higher fecal concentrations of IL-4 than infected children who received a placebo [73]. These studies are encouraging as they demonstrate the scope of health benefits resulting from nutritional interventions.

Ascaris infection in infants is understudied. Nevertheless, evidence is emerging to suggest that infants as young as 5 months of age [32] are infected and that infection in these infants is important. Stoltzfus et al. [74] recently demonstrated that a reduction in *Ascaris* prevalence and intensity by treating with mebendazole resulted in more dramatic improvement in growth of children aged 6–30 months than in children from 30 to 71 months of age, despite the lower baseline infection in the younger children. In regions where infection is very common, it is probable that children will become infected before they are able to rely on their own immune system. Also, the presence of a few worms in infants may be more clinically significant than the same number of worms in older children.

In many developing countries, a new form of malnutrition is emerging – obesity. Among schoolchildren living on Pacific Islands where *Ascaris*, *Trichuris* and hookworm are endemic, helminth-infected children had a significantly lower risk of elevated body mass index, an indicator of overweight [19]. This survey of 27 schools found 6 schools where more than 10% of children were overweight, and 5 other schools where more than 10% of children were stunted. Public health initiatives now must fight both undernutrition and overnutrition. There is a critical need to understand interactions between overnutrition and parasitic infection, and to consider the most appropriate mix of interventions in populations where undernutrition and overnutrition co-occur.

Diagnosis

Eggs in Stool

Among the variety of techniques for detecting *Ascaris* infection, observation of eggs in stool samples is most commonly used. Studies that require an estimate of intensity typically use the Kato Katz technique. It is important to note that unfertilized eggs are often missed when using the Kato Katz technique [32], and thus a concentration technique may be more appropriate in infants who may have single sex infections or for monitoring re-infection shortly after deworming programs. Also, in areas where hookworm is present, samples must be examined within a few hours after preparation if results on hookworm are to be considered reliable. Alternatively replacing malachite green with a nigrosin/eosin solution has been recommended as this allows hookworm eggs to remain visible on the Kato Katz slides [75].

Whereas epidemiological studies in the past often provided data only on the prevalence of infection, there has

been increasing appreciation of the importance of data on the intensity of infection. Researchers have responded by more consistently providing this information. However, many report intensity according to the WHO categories of light (<5,000 epg), moderate (5,000–50,000 epg) or heavy (>50,000 epg) [6]. Based on the review by Hall and Holland [6] that demonstrated considerable geographic variability in egg production per female *Ascaris*, it is not clear that these WHO categories are appropriate in all regions, as there is a risk that high worm burdens may be misrepresented as moderate or even low intensity infections.

Immunodiagnosis

When information on the history of exposure to *Ascaris* is more relevant than the presence of current infection [63], *Ascaris*-specific IgE may be a useful marker of previous exposure, and more relevant than eggs in stool samples in studies concerned with the effect of *Ascaris* on immunocompetence [76] (see *Ascaris* and HIV, p. 16).

Eggs in Wastewater

The development of new diagnostic approaches has focused on real-time PCR methods of detecting eggs in the environment. Pecson et al. [77] have had good success using quantitative PCR of an internally transcribed spacer region of ribosomal DNA that provides results comparable to traditional microscopy in assessing the success of heat, UV and ammonia treatment in removing *Ascaris* eggs from sludge.

Control Strategies

The goal of most control programs [for review, see 78] is to reduce the intensity of infection because it is argued that the few heavily infected children are not only at higher risk of infection-induced morbidity, but also that they are the major contributors to transmission because they pass such a large number of eggs in their feces. Interestingly a recent theoretical study, that makes more explicit account of the dispersion of worms among individuals, suggests that reducing intensity may not have as dramatic an effect on reducing transmission as is commonly understood [51], due to density-dependent mechanisms within the parasite population that make the relationship between worm burden and egg production highly nonlinear.

Anthelmintics

By far the most common method for controlling *Ascaris* is treatment with anthelmintic drugs. A single oral

dose of albendazole reduces *Ascaris* epg by more than 95% and is safe for use in mass treatment campaigns [79]. Furthermore it also reduces *Trichuris* and hookworm epg, though not as effectively as for *Ascaris* [80, 81]. The chief disadvantage of drug treatment is rapid re-infection that necessitates repeated drug treatment to achieve lasting benefit.

Community-based control programs most frequently focus on delivery through the school system, as school-aged children tend to have the highest intensity of infection, and delivery through the schools is more efficient than community-based or household distribution. In the Republic of Korea [82], nationwide screening of all school-children followed by twice yearly anthelmintic treatment of all infected children between 1969 and 1995 reduced the nationwide *Ascaris* prevalence from 55.4 to 0.02%. This remarkable reduction coincided with investment in infrastructure and a dramatic increase in the annual per capita GNP from USD 210 in 1969 to USD 10,315 in 1997, both of which would also have contributed to reduced transmission of *Ascaris*.

Although reports of drug resistance to human anthelmintics are rare, evidence of emerging resistance does exist. In Madagascar, treatment with levamisole every 2 months over 16 months successfully reduced *Ascaris* prevalence and intensity over the first 8 months [83], but both prevalence and egg output then increased, despite continued drug treatment every 2 months.

The cost of mass treatment programs can be reduced by using locally manufactured generic drugs, but quality control is needed. A comparison between albendazole from GlaxoSmithKline and two generics produced in Nepal showed that all three products were equally effective in reducing *Ascaris* epg but that cure rates were lower for one of the generics [84]. Reduced efficacy will not only hinder the effectiveness of the program, but it may also promote emergence of drug resistance.

Other cost-saving measures have been explored. In Vietnam, the cost per child of school-based treatment was reduced from USD 0.77 to 0.03 by improving efficiencies in distribution and teacher training, and also by eliminating the baseline survey and monitoring activities [71]. Although it is understandable that countries need to reduce the cost of these programs, the monitoring and evaluation side are essential, if changes in the frequency of drug treatment, in the preferred drug, or the need for alternative or additional interventions are incorporated in a timely manner. Without a monitoring program, poor efficacy of generics and emerging drug resistance may not be detected in a timely manner.

Sanitation, Hygiene and Health Education

Remarkably, use of a latrine does not always reduce the prevalence or intensity of *Ascaris*. In tea plantations in Sri Lanka, the high fecal contamination of the environment due to indiscriminate defecation may override any benefit of using latrines [85]. Furthermore, eco-san latrines designed to produce a safe biosolid for application in agriculture can also be a source of contamination. In El Salvador, households that buried biosolids from eco-san latrines were 8.3 times more likely to be infected with *Ascaris* than those using pit latrines where biosolids were untouched [28].

In Uzbekistani households, promotion of hygienic behaviors (hand washing, safe disposal of feces and boiling of drinking water) through participatory methodologies was successful in reducing re-infection rates with intestinal parasites (including *Ascaris*) by 30% after a year, compared with treated children not benefiting from the educational intervention [86]. The risk of *Ascaris*, *Trichuris* and/or hookworm infection was elevated by 4.1 times in Pacific Island schools that did not have a water supply, regardless of water quality [19], highlighting the importance of access to water in schools. Interestingly, however, although regular washing of hands before eating reduced the prevalence of *Ascaris* among 176 subjects aged 2–50 years old from tea plantations in Sri Lanka, *Ascaris* intensity was unaffected by the source of water for drinking or bathing, by boiling drinking water, or by washing hands before eating and after defecation [85].

Vaccines

No vaccine against *Ascaris* is available, and it is likely to be many years before one is developed. However, in light of evidence that antibody responses to a recombinant form of a human *Ascaris* allergen (rABA-1A) are associated with reduced *Ascaris* epg (fig. 2, 3) [34], and the promising results following experimental mucosal vaccination of pigs against *A. suum* [87], scientists remain hopeful.

Removal of *Ascaris* Eggs from Water, Soil and Food

A recent survey of Iranian water treatment plants revealed that wastewater treatment reduced the number of eggs to less than 1 helminth egg/liter [88], a level considered acceptable by WHO for irrigation purposes. Anaerobic digestion under thermophilic conditions for 30 min at 53–55°C has been shown to reduce *A. suum* egg numbers by threefold [89], but UV radiation has little effect [90]. Liming is commonly used, and several studies have considered the conditions needed for effective control. Capizzi-Banas et al. [91] were able to inactivate *Ascaris* eggs

through liming for 75 min at 55°C or for 8 min at 60°C. More recently, Bean et al. [92] showed that the process of liming whereby *Ascaris* eggs are exposed to high pH (12 then 11.5) for up to 72 h at room temperature had no effect on viability. (Incidentally, they observed that this liming procedure reduced bacterial, viral and *Giardia* infectivity, but increased that of *Cryptosporidium* oocysts!) One factor often not considered in assessing conditions for inactivation is the ammonia concentration, as ammonia can reduce both the pH and temperature required to inactivate *Ascaris* eggs in sludge samples [93]. High temperature treatment can also generate manure safe for use as fertilizer; use of a biodrying compost system with forced aeration and temperatures that exceeded 55°C for 4 days prevented embryonation of *Ascaris* eggs [94].

Removing *Ascaris* eggs from food presents considerable challenges though recent work has shown that application of high hydrostatic pressure (<241 MPa for 60 s) prevented embryonation of *A. suum* eggs [95]. This opens a new possibility for killing eggs that have already contaminated food items.

Concurrent Infections

Ascaris and Other Helminths

Ascaris is rarely the only parasite in a population. Among pregnant women enrolled in a study in Uganda, 15% were HIV-positive, 15% had malaria, 38% had hookworm, 13% had *Trichuris*, 6% had *Ascaris*, 15% had *Strongyloides*, and 22% had *Mansonella perstans* [96]. In addition, *Ascaris* is rarely the only parasite in an individual. As just one example, stool examinations in a community-wide survey of 1,240 people in Americaninhas, Brazil, revealed that over half the population had multiple intestinal helminths [44].

Concurrent infections within a single host may arise by chance. A survey of 1,370 children (6–11 years old) in Palestine [97] revealed that the two most common intestinal parasites, *Ascaris* (12.8% prevalence) and *Giardia* (8.0% prevalence), occurred together in 1.02% of children, exactly the percentage that would be expected by chance ($0.128 \times 0.08 = 0.0102$). In many cases, however, concurrent infection cannot be explained by chance events. Based on a comprehensive set of data from 335 households in Brazil [98], triple infection with *Ascaris*, *Schistosoma mansoni* and hookworm occurred significantly more frequently than expected, as did dual infections with *Ascaris* and hookworm, and with *Ascaris* and *S. mansoni*. These dual combinations remained more

Table 1. Comparison between observed and predicted frequency of single and multiple helminth infection among 1,332 individuals in Americaninhas, Minas Gerais, Brazil, in 2004 and odds ratio (adjusted by age group, sex, presence of other infections, and clustering by household) that the first listed parasite is associated with the second

Species present	Percentage of population		χ^2	Prob > p	Adjusted odds ratio (95% CI)	Prob > p
	observed	expected				
<i>Ascaris</i> only	6.1	7.8	3.1	0.080		
Hookworm only	11.4	20.6	41.6	<0.0001		
<i>Schistosoma mansoni</i> only	4.6	8.0	13.4	<0.0001		
<i>Ascaris</i> with hookworm	19.9	16.7	4.6	0.03	3.65 (2.71–4.91)	<0.001
<i>Ascaris</i> with <i>S. mansoni</i>	3.8	6.5	9.9	0.001	0.99 (0.75–1.29)	0.917
Hookworm with <i>S. mansoni</i>	17.9	17.1	0.3	0.610	2.95 (2.19–3.98)	<0.001
<i>Ascaris</i> , hookworm and <i>S. mansoni</i>	19.0	13.8	13.0	0.0003		

Adapted from Fleming et al. [98], with permission.

common than expected even after adjustment for age, sex, household clustering and the presence of other infections as shown in table 1. Although the joint occurrence of *Ascaris* and *S. mansoni* was found more often than expected, fecal egg counts were lower for both species than when each species was present as a single infection, suggesting an antagonistic association [98]. Antagonistic associations have also been reported in pigs concurrently infected with *A. suum* and *Trichinella spiralis* [99].

Concurrent infections may arise from a common mode of transmission or from common household or genetic factors. The positive association between *Ascaris* and *Trichuris* observed by Ellis et al. [46] in China was explained by household factors that accounted for 32.7% of the risk of concurrent infection and by genetic makeup that explained 16.3% of the risk of multiple helminth infection (*Ascaris* and/or *Trichuris* and/or *Schistosoma japonicum*). Positive correlations between egg counts for all paired combinations of the soil-transmitted nematodes, *Ascaris*, *Trichuris*, and hookworm were reported in an Indian fishing village [21] and for *Ascaris* and *Trichuris* in Cameroon [43]. However, the positive association between *Ascaris* and *Onchocerca* in a village highly endemic for *Ascaris* [43] cannot be explained by a common route of transmission as *Onchocerca* is transmitted by the bite of a black fly.

Ascaris and Malaria

Since the 1970s there has been debate regarding the relationship between *Ascaris* infection and clinical malaria. Several, though not all [45, 100], recent studies provide evidence that *Ascaris* increases the risk of clinical malaria [100–103]. Children admitted to hospital in Senegal for severe clinical malaria had a significantly higher prevalence

of *Ascaris* than age-matched controls from the community at large [102]. However, in southwest Uganda, where malaria is less common and where *Ascaris* prevalence and intensity are also relatively low, a community-wide survey revealed no evidence that infection with *Ascaris* (or other intestinal nematodes) influenced the occurrence of clinical malaria [45]. Most recently, a randomized trial in Madagascar examined the effect of repeated levamisole on *Plasmodium falciparum* density [83]. Levamisole treatment every 2 months significantly reduced *Ascaris* infection but increased *P. falciparum* density in blood smears in subjects 5 years of age and older. The authors suggested that the presence of *Ascaris* was beneficial in keeping malaria parasitemia down. A wide variety of factors differed among these studies, but nevertheless, the data suggest that further research is needed on the interaction between *Ascaris* and malaria, particularly as large scale anthelmintic campaigns are ongoing in many regions of the world.

Ascaris and HIV

Ascaris and other helminth infections are often present in HIV-seropositive individuals. In Honduras, for example, among 133 HIV-positive adults, 24% were infected with *Ascaris* [104]. In Zambia, of 297 seropositive, asymptomatic adults, 13.1% were infected with *Ascaris* and 24.9% were infected with at least one helminth [105]. There is growing evidence that the *Ascaris*-induced Th2 environment is permissive for the establishment and proliferation of other infections, including HIV. For example, HIV-seropositive South Africans were more likely to recall having *Ascaris* infection as a child or to recall prior deworming than the seronegative comparison group [76]. However, no detectable association between *Ascaris* eggs

Table 2. Impact of latrine type on prevalence of parasitic infection among 107 households in El Salvador

Type of latrine (number of homes)	n	<i>Ascaris lumbricoides</i> ¹	<i>Trichuris trichiura</i> ²	Hookworm ²	<i>Giardia lamblia</i> ³	<i>Entamoeba histolytica</i> ⁴
Double-vault desiccating (31)	127	15.5 (3.3–74.8)	7.1 (3.0–17.1)	0.5 (0.2–1.3)	0.4 (0.2–1.1)	0.5 (0.2–1.4)
Solar (20)	79	0.7 (0.1–8.2)	0.7 (0.2–1.9)	0.4 (0.1–1.3)	0.3 (0.1–1.1)	1.4 (0.5–4.2)
Pit (31)	141	0.9 (0.1–6.0)	0.6 (0.1–1.5)	1.4 (0.5–3.5)	0.5 (0.2–1.3)	0.8 (0.4–1.8)

Adjusted odds ratio (95% CI) indicating the risk of infection relative to those using no latrine (25 homes). Significant odds ratios are shown in bold. Adapted from Corrales et al. [28], with permission.

¹ Adjusted for age, anthelmintic in previous 3 months, having a dirt floor, owning pigs.

² Adjusted for age, anthelmintic in previous 3 months, having a dirt floor, type of water source.

³ Adjusted for having a dirt floor, socioeconomic status, type of water source.

⁴ Adjusted for overt signs of malnutrition and type of water source.

in stool and HIV seropositivity was detected among 907 adults from a villages in Tanzania [106]. These studies indicate that the interaction between *Ascaris* and HIV may be more related to a history of *Ascaris* infection than to current infection. Adams et al. [76] highlighted the importance of not relying only on *Ascaris* eggs in stool (current infection) when attempting to understand co-infection dynamics; this perspective was also shared by Schäfer et al. [62] and Fincham et al. [63] with regard to *Ascaris*, co-infection and allergy. They suggest that circulating antibody responses or other immunological conditions indicative of a history of exposure to *Ascaris* may be more relevant than current infection in explaining the occurrence (or absence) of other infections.

Helminths affect the immune response to HIV [107]. Of particular interest was the improvement in immune function following anthelmintic treatment of HIV-infected Ethiopians who migrated to Israel [108]. Despite tremendous advances in our understanding of immune responses to pathogens, it is clear that much more research is needed with regard to how pathogens respond to the variety of immunodominant situations generated by the presence of other pathogens.

Impact of Concurrent Infections on Control Approaches

Recommended Type of Latrine

New types of latrine are now available, and a recent study conducted in El Salvador has compared infection levels among households with no latrine to those using the standard pit latrine, or a solar desiccating latrine, or a double-vault desiccating latrine [28]. What is most striking about this research is that no one type of latrine

was beneficial against both nematode and protozoan parasites (table 2) and, moreover, households using the double-vault desiccating latrine had a significantly higher prevalence of both *Ascaris* and *Trichuris* than those using no latrine. In addition, households that used biosolids from either of the desiccating latrines had elevated risk of infection with *Trichuris*, *Giardia*, and *Entamoeba histolytica* compared with those who buried the biosolids. Despite the evidence presented above that improvements in sanitation and hygiene may not always result in a detectable reduction in *Ascaris* infection in the short-term [28, 85], such improvements tied with health education are considered essential for long-term control, and it is important that latrine design be optimized to reduce transmission of all fecally transmitted pathogens.

Drug Interventions

The widespread occurrence of concurrent infections has led policy makers to recommend drug interventions that target multiple parasitic infections at the same time. Recognizing that soil-transmitted helminths frequently co-occur with filarial infections and/or schistosomiasis, the Global Programme to Eliminate Lymphatic Filariasis combines albendazole with either ivermectin or diethylcarbamazine (DEC) if intestinal nematodes are present, and the Schistosomiasis and Soil-Transmitted Helminthiasis Control Programmes use praziquantel together with either albendazole or mebendazole [79]. Surprisingly few studies have examined the pharmacokinetics and side effects of co-administration of these drug combinations, but a review of the studies available to date indicates no reason for concern in the use of several of the 2-drug combinations [79].

Combination therapy provides many benefits, both in treating multiple infection and in reducing drug delivery

costs. Drug combinations that are effective against *Ascaris* can improve compliance with treatments against other infections such as filariasis [109], because of the immediate expulsion of *Ascaris* worms in the feces. People see the expelled *Ascaris*, perceive removal of intestinal worms as a benefit, and thus are more inclined to comply with other treatments where obvious immediate benefits are not evident. The expulsion of *Ascaris* may also have a more direct benefit in controlling filarial infection. Sahoo et al. [110] hypothesize that the presence of intestinal worms acts to regulate filarial infection. They observed that albendazole treatment of subjects infected with both intestinal worms and filarial parasites significantly reduced levels of circulating filarial antigen, but that this effect was not observed in subjects who did not have intestinal worms at the time of treatment.

Oqueka et al. [111] followed the pattern of *Ascaris* infection status over 2 years of combined, yearly albendazole and DEC treatment. As expected, the prevalence of *Ascaris* dropped from 43.8 to 26.5% between 2002 and 2004, demonstrating that infection had not returned to pre-intervention levels between treatments. Given the well-described tendency for predisposition, it would be expected that most of those who were infected with *Ascaris* in 2004 would also have been infected in 2002. However, when data on individuals were examined, more than half of those who were infected in 2004 had been uninfected in 2002. Although the authors do not report intensity data, it would be interesting to determine if, compared with use of albendazole alone, the combination of DEC and albendazole reduced the tendency for heavily infected individuals to become heavily re-infected.

As with any intervention, unexpected consequences need to be considered. In China, despite the decline in the prevalence of *Ascaris*, *Trichuris* and hookworm following a decade of control, a dramatic increase in *Clonorchis* and *Taenia* prevalence has occurred in some regions [112]. The reduced prevalence and intensity of *Ascaris* in the Republic of Korea [82] was associated with increased numbers of people infected with *Clonorchis sinensis* between 1997 and 2004, and the reemergence of vivax malaria over the past 15 years is also of some concern [82]. It is not clear whether the emergence of these infections was caused in some way by the reduction in nematode infections, but these observations highlight the need for adaptive management of such programs.

Vaccination

Given the endemic, chronic nature of *Ascaris* that begins at a very young age, many people in *Ascaris*-endem-

ic areas are likely to be infected throughout much of their lives. There is mounting evidence that the Th2-biased immune environment induced by *Ascaris* (and other helminth infections) may influence their ability to respond appropriately to vaccinations. For example, albendazole treatment in a group of pregnant Ugandan women infected with hookworms and other helminths significantly reduced the IFN- γ response following BCG vaccination of their infants [95], indicating that hookworm infection of mothers may suppress immune responses to vaccines administered to their infants. *Ascaris* infection was very low in this population (prevalence of only 6%), so the study was unable to make conclusions on whether *Ascaris* might have similar effects to hookworm. However, a recent vaccination study in pigs demonstrated that the presence of *A. suum* reduced the efficacy of vaccination against *Mycoplasma hyopneumoniae* [113]. Given the important implications for the success of vaccination programs, it is remarkable that more research has not been conducted on the potentially critical role of *Ascaris* and other helminth infections on development of immune responses following vaccination.

More Comprehensive Intervention Packages

Interventions that target bacterial infections together with helminths have received recent attention through the efforts of Hotez et al. [2] who argue that there is a complex of 'neglected tropical diseases' that can be effectively controlled as a group through combined administration of anthelmintic drugs, albendazole, praziquantel and ivermectin, along with the antibiotic azithromycin. It is their premise that control of these neglected infections (among which they include *Ascaris*) will be beneficial in efforts to manage malaria, tuberculosis and HIV/AIDS, particularly because of the evidence that co-infection with one or more of the 'neglected' diseases alters the outcome of the 'big three'.

Micronutrient supplementation is another intervention that is frequently combined with deworming. This combination attacks the infection-malnutrition cycle from two angles, with the intent of removing existing parasites and also improving the ability of the child to resist future infections by boosting the immune response [27, 73–74]. Other forms of comprehensive interventions include conditional transfer programs whereby poor families receive cash supplements or food vouchers in return for ensuring that their children are dewormed regularly, that they are vaccinated and that they attend school [114; a detailed review of the interactions between multiple nutritional deficiencies and infections is beyond the scope of this review, but see 69, 70].

As more is understood about the wide range of interactions among infectious agents, nutritional and immunological status, and broader environmental and social determinants of health, it will become clearer that integrated health programs are desperately needed.

Research Priorities and Conclusion

Based on this review of the recent literature, there is an urgent need for further research in the following areas.

Water Resource Management and Latrines

The provision of safe drinking water to resource-poor communities is becoming more difficult as water scarcity is increasing. Simple, cost-effective methods for the treatment of wastewater are urgently needed if we are to prevent the rates of transmission of *Ascaris* and other fecal pathogens that may be transferred onto crops from increasing, not decreasing. Improvements in the design of latrines that prevent transmission of the range of fecal pathogens is still needed as some of the currently tested latrines may increase the risk of transmission of certain infections [28, 85].

Role of Pigs

Given the recent report that cross-transmission of *Ascaris* from pigs to humans occurs more frequently than previously believed [31], further molecular studies on larger samples of sympatric pig and human ascarid populations are needed to confirm the suspected high rate of hybridization. Much more attention is needed to prevent human contamination with pig feces.

Geospatial Patterns and Modeling Spatial Dynamics

The recent advances involving geographic information systems and remote sensing provide a tremendous opportunity to provide both regional and local scale monitoring and predictions. This will allow a combination of temporal and spatial analyses that can provide important insights into the changing pattern of concurrent infections – something that would have been relatively intractable just a decade ago.

Interaction between Immune Responses and Molecular Genetics of the Host

The cascade of immunological and inflammatory markers that are induced in response to *Ascaris* infection in humans remains poorly understood. We have barely begun to understand the role that host genetics may play

in explaining why some individuals are more susceptible to infection than others. We know very little about how an infant responds to the first *Ascaris* infection and how these responses vary over the continuing cycle of repeated re-infection through childhood. We know virtually nothing about the response of the elderly to *Ascaris*. Without an understanding of the link between current infection intensity, history of infection, and immune responses, it will be difficult to tease apart the effect of *Ascaris* (or removal of *Ascaris*) on susceptibility to other infections or to efficacy of vaccines.

Concurrent Infections and Their Interactions – A New Research Agenda

It is becoming very clear that a complex dynamic interplay exists among the various pathogens that infect an individual and the host response to these pathogens, and that a pathogen-specific focus may be insufficient. However, expanding to a more holistic view of the set of pathogens in an individual will place considerable demands on the research community. It will require large interdisciplinary teams who have experience in HIV, malaria, tuberculosis, intestinal nematodes, diarrheal disease, filarial infections, schistosomiasis, food-borne digenaeans, skin diseases, respiratory infections, and allergy, to name just a few. The research team should also include epidemiologists, immunologists, nutritionists, statisticians and modelers if sense is to be made of the data. Such research will also require a different funding regime from that usually available, because of the wide range of assays and expertise required.

A Systems Level Approach for Design of Integrated Control Strategies

Upon recognizing the community of interacting parasites within an individual, it becomes obvious that programs designed to control one infection at a time should be discouraged. Rather, comprehensive attention to several infections and to environmental, biophysical and social factors that put individuals at risk of infection should be the norm. The design of such programs requires research that captures not only the wide scope of potential benefits to the health and well-being of the community, but also the potential negative consequences that may emerge when trying to solve one set of problems. It also requires integration of expertise of environmental biologists, and social and cognitive scientists who are able to work with the communities and the biomedical scientists to ensure that the planning, monitoring and adaptive management of the interventions is optimized.

Concluding Thoughts

Ascaris remains the most common parasitic infection in the world. Although it is not a major cause of mortality, relative to other infections, its impact on the health of children has been well known for decades. However, evidence now exists that *Ascaris* infection modifies the immuno-

logical environment within an individual host to be protective against certain conditions, but permissive to others, with implications not only for the health of children but also of adults. Furthermore, the presence of *Ascaris* may reduce the efficacy of vaccines. Thus, this review highlights the fact that control of *Ascaris* may have a greater public health significance than previously understood.

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