Trichuris and Ascaris Infections

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Abstract Trichuriasis and Ascariasis are neglected tropical diseases caused by the gastrointestinal dwelling nematodes *Trichuris trichiura* and *Ascaris lumbricoides* respectively. In both cases, infection is initiated by ingestion of infective eggs, with eggs hatching in the intestine. Thereafter however the similarity ends: *Trichuris* sp. larvae go through a succession of moults within intestinal epithelial cells, with adult worms subsequently taking up a partially intracellular residency in the large intestine. By contrast, *Ascaris* sp. larvae leave the gut, penetrating the mucosa, and migrate round the body passing through the liver and lungs before finally arriving back in the intestine to become a luminal dwelling small intestinal adult. Both parasites are staggeringly prevalent and are associated with significant morbidity, with type 2 anti-parasite immunity evidenced in both humans and animal models. Whilst diagnosis, screening and prevention strategies for *Trichuris* sp. and *Ascaris* sp. share many commonalities, the effectiveness of drug treatment is strikingly different. Thus, whilst all current drugs recommended by the WHO achieve cure rates for *Ascaris* sp. approaching 100%, *Trichuris* sp. is curiously difficult to treat with cure rates as low as 23% reported. Novel anthelmintic drug discovery therefore needs expediting in conjunction with vaccine development, with advances in the control of both parasites also requiring improved water, hygiene, education, and tools for diagnosis and assessment of parasite control in the field.

[H1] Introduction

Whipworms are large-intestinal nematode parasites of mammals. The generic name for whipworm is *Trichuris* meaning “hair tail”; a name applied by Johann Georg Roederer in 1761, mistaking the thin front end as the tail. Over 70 species of *Trichuris* are recognised, including the medically important human species *T. trichiura* and the pig whipworm *T. suis*. Whipworms have been associated with man for over eight thousand years, as evidenced by the presence of *Trichuris* eggs in coprolites found in both Old and New World archaeological sites[1-3]. *Ascaris lumbricoides* (first described by Carl Linnaeus in 1758), commonly known as the human roundworm, is also an intestinal nematode and is the causative agent of the disease ascariasis. In contrast to whipworms, roundworms dwell in the small intestine. *Ascaris* also differs from *Trichuris* in that only one other species of *Ascaris* has been described, *Ascaris suum*, a ubiquitous infection of pigs. After considerable debate as to whether these two ascarids are in fact distinct species, current opinion is that the two species are closely related at the phylogenetic level but reproductively isolated[4]. Like *Trichuris*, *Ascaris* has had a long association with its human host with infections detected in embalming material from over 7000 years ago[5] (Figure 1).

Both *T. trichiura* and *A. lumbricoides* are highly prevalent infections[6,7]. The infections occur by ingestion of embryonated eggs through contaminated soil and food. Both parasites contribute to chronic, long-term nutritional morbidity and less well supported impacts on cognitive development. Acute complications such as intestinal obstruction and biliary ascariasis are associated with heavy *Ascaris* infection, whereas for *Trichuris* these include dysenteric syndrome and rectal prolapse. The main approach to control is large-scale provision of anthelmintic treatment to children, and girls and women of reproductive age with accompanying improvements in access to clean water and sanitation with an aim to reduce worm burden-associated morbidity[8]. Whilst largely effective against *Ascaris*, mass drug administration programmes have been significantly less impressive against *Trichuris* particularly in Sub-Saharan Africa[9].

This Primer provides a current view of both *Ascaris* and *Trichuris* epidemiology, disease mechanisms, diagnosis, screening and prevention. We also review current management strategies and consider key research areas, which, in the future may move us towards improved control of these two important neglected tropical diseases. Further, we take the opportunity to compare and contrast *Ascaris* and *Trichuris* infections, which despite sharing several parasitic traits, differ in important areas, with important consequences for control strategies.

[H1] Epidemiology

T. trichiura and *A. lumbricoides* infections are highly prevalent worldwide, infecting an estimated 465 and 819 million humans, respectively[6,10]. Both infections often occur together in children and are generally overlooked being associated with non-specific gastrointestinal symptoms. Morbidity is most likely to occur among children with moderate to heavy infection intensities and is attributed to chronic effects on nutrition and growth. Heavily infected children with *T. trichiura* may present to health facilities with failure to thrive and diarrhoea, which may be bloody, and occasionally with rectal prolapse. Adults with heavy infestations may present with chronic iron-deficiency anaemia and colitis. Heavy infections with ascariasis are a common cause of surgical emergencies in endemic regions causing intestinal obstruction in children and biliary and pancreatic disease in adults. There are limited data to quantify the frequency of these complications but current estimates for deaths attributable to ascariasis were 3,205 worldwide in 2017 while no deaths were considered attributable to trichuriasis[11].

[H2] Trichuris trichiura

T. trichiura infects humans most frequently in warm and moist conditions in tropical and sub-tropical regions. Although zoonotic infections in humans have been reported with other species of *Trichiura* such as *T. suis* (from pigs) and *T. vulpis* (from dogs) these generally cause attenuated infections and rarely develop to sexual maturity in humans. The geographical distribution of *T. trichiura* - estimated using geographical information systems tools that allow predictions of regions permissive for transmission based on spatial information for temperature, humidity, and population density - overlaps largely with that of *A. lumbricoides* with which it shares similar epidemiological characteristics (Figure 2). Human trichuriasis is a classic infection of poverty, where a lack of education and access to sanitation and clean water within an ecologically permissive environment, determines opportunities for transmission. In such environments, community prevalence of infections can be in excess of 90%, particularly affecting children aged 5 to 15 years among whom parasite burdens are greatest[12]. Age-prevalence profiles are concave with peak prevalence occurring at an earlier age in areas of more intense transmission and likely relates to exposure risk of ingestion of eggs from a faecally-contaminated environment. An age-dependent decline in prevalence is often seen in older children and adults relating to reduced exposure and possible age-acquired immunity. Transmission requires embryonation of *T. trichiura* eggs in the environment, and whilst eggs will survive temperatures below freezing, they will not embryonate in freezing conditions or where temperatures exceed 37°C[13,14].

The risk of *T. trichiura* infection is not uniform within endemic populations: A small proportion of infected individuals (typically less than 10% in high prevalence populations), generally small children, harbour most adult worms while the remaining infected children and adults harbour few adult worms[15]. Such aggregated distributions of adult worms (which may survive for 1-8 years in the human intestine[16]) within endemic communities are typical of soil-transmitted helminths (STHs). There is evidence from some but not all epidemiological studies for an increased susceptibility to *T. trichiura* infection among some groups of individuals – infected individuals are more likely become re-infected after chemotherapy[17]. Individual susceptibility may be determined by one or more of behavioural, environmental, or genetic factors and immunological factors[17]. Further, heavily infected individuals tend to be those who reacquire the heaviest parasite burdens following treatment[14,17,18]. *T. trichiura* has been shown to cluster within families in rural China[19] and linkage analysis in Nepal identified two quantitative trait loci on chromosomes 9 and 18, respectively, associated with susceptibility to infection[20] although the contributing genes at these loci remain unknown.

Further, a recent study in Brazil showed susceptibility to *T. trichiura* infection to be associated with polymorphisms in the TGF-B1 gene[21]

Treatment of school age children is considered a cost-effective strategy for the control of *T. trichiura* in endemic communities by cutting infections in the primary infection reservoir[15], thus reducing transmission within communities. Temporal increases in economic and environmental conditions coupled with increased access to periodic chemotherapy of school-age children have led to substantial declines in prevalence and intensity of infection in Asia over the last decade, particularly in China, Korea, and Indonesia[6].Similar declines have not been seen in Latin American and Sub-Saharan African regions [6,22]. However, declines in the numbers of children with moderate to heavy infection intensities, the group most at risk of severe disease, have been observed in almost all populations where school-children have received repeated preventive chemotherapy[23]. Overall, prevalence of ascariasis was estimated to decline by 10% between 2005 and 2015 while trichuriasis declined by only 2%[10].

Under experimental conditions, humans can become infected with the pig whipworm T. suis[13] but these infections appear at least in some cases to only establish temporarily[24]; equally *T. trichiura* can be established in pigs but they do not persist (Beer 1976). Futher, Ghai et al present data indicating that the taxonomic, population and phylogenetic structure of *T. trichiura* is complex[25]. Thus these data suggest that *T. trichiura is* not a single multi-host species but a series of lineages some of which are able to infect multiple host species within the Order primates.

[H2] Ascaris lumbricoides

Globally, *A. lumbricoides* is estimated to infect 819 million humans[6,26] following the same geographical distribution in tropical and subtropical areas as observed in trichuriasis (Figure 2). While the route of infection (oral-fecal transmission) is the same for both parasites, the geographical distribution of the parasitism does not perfectly overlap although no evidences were described to explain specific areas for each disease. However, in the endemic areas with overlap of geographical distribution of both parasites, the coinfection might occur and it results in exacerbation of morbidity and high intensity infections[27-31]. Ascariasis is also associated with poverty and hence the lack of proper sanitary infrastructure and poor socio-economic conditions favours the transmission of the parasite[32,33]. An over-dispersed frequency distribution[34] is overall observed, with most individuals harbouring a low to moderate parasite infection and few heavily infected hosts, possibly due to the chronic

exposure to the parasite that might lead to protection despite the morbidity, as evidenced in experimental infection[35] .

Predisposition (reinfection with similar or higher worm burdens to those before treatment) is also an epidemiological phenomenon observed for ascariasis in mice[36] in a similar way observed for *T. trichiura* infection. While the mechanisms that determine predisposition are not fully elucidated, exposure to infection and host susceptibility are likely to be important. Socio-economic circumstances such as poor housing infrastructure[37] and deficiency in hygiene practices[38] are factors that influence the intensity of infection. The difference of worm burden in adults (which often present lower intensity of infection than children)[36] might suggest a behavioral-mediated reduction of exposure or acquired immunity after continuous exposure to the parasite. While experimental data in mice demonstrate the reduction of parasite burden after repeated exposure to *Ascaris* sp. infection[35], the over-dispersed worm frequency distribution in humans is recorded in all age classes, indicating that neither age nor immunity are the primary determinants of variability in infection intensity. Environmental and behavioural features[39], as well as hosts genetics and immunity[40-44], are important determinants of infection status[45].

Experimental and molecular evidence of possible cross-transmission indicated that humans can be infected by *A. suum*[46-48] and, similarly, swines can harbor *A. lumbricoides*[48] . These data suggest that pigs might act as a potential reservoir of infection for humans and, more importantly, might point out a possible role of zoonotic infection by *A. suum* in humans[49]. **The zoonotic potential of both *A. suum*, and *T. suis*, has been reviewed Nejsum et al[50].**

[H1] Mechanisms/pathophysiology

Studies on immunity to, and the pathology of, human whipworm and roundworm infections have generated interesting correlates with resistance to reinfection, however it is through the use of animal models, and particularly the laboratory mouse, that mechanistic insights have been gained. The information below is divided up into current knowledge for the human infection, followed by insights from animal models, and includes, where possible, reflections on how findings in animal models fit with the human disease.

[H2] Trichuris species

Whilst different species of *Trichuris* are very host-specific, they all follow a similar life cycle pattern (Figure 3). After the ingestion of embryonated eggs on contaminated food or in water, eggs from the soil hatch in the large intestine (caecum/proximal colon); in the mouse,

hatching is triggered by the presence of bacteria and it is likely that similar bacterial cues are applicable to egg-hatching in other *Trichuris* species[51]. First stage (L1) larvae are released and these penetrate the epithelial cells at the crypt base, taking up an intracellular niche within a multicellular epithelial "tunnel" the biology of which is unknown[52]. There they grow and moult through the L2 to L4 and adult stages with timings of these moults defined in the mouse model[53]. The pre-patent period, the time from infection to egg production - is defined in mice at around 33-35 days. However the equivalent timings in humans are unclear. By the L3 stage the parasite is no longer fully intracellular with its posterior end loose in the gut lumen whilst its long thin anterior end, containing the stichosome, a modified oesophagus comprised of multiple cells called stichocytes that duct into the oesophageal lumen, remains embedded within a syncytial tunnel of modified host epithelial cells, without significantly compromising gut barrier integrity. Adult male and female stages of *T. muris* emerge around 32 days after infection, with fertilized adult females releasing 2,000 to 8,000 eggs per day[54]. Eggs of *Trichuris spp* pass out with host faeces in an uninfective state, taking two weeks to one month, according to environmental conditions to become infective[13] by which time the L1 larva has developed within the egg and the egg is now described as "embryonated". The life cycle of *T. trichiura* is similar to that of *T. muris*, although the timings of moults may differ. Thus, in humans, patent infections from ingestion of eggs to the development of mature adult females takes 2-3 months and adults, measuring 3 to 5 cm, may survive for 1-8 years in the human intestine[16]. Novel imaging tools are beginning to provide unique insights into both host pathology and parasite behaviour[55,56]. Throughout the life cycle in both the murine, porcine and human host, whipworms are known to excrete and secrete a variety of parasite derived molecules that interact with their environment and host. Some are known to be antigenic and some have been shown to be immunomodulatory[57-59], but the functions of most are still to be determined. A better understanding of the host-parasite relationship will likely support the development to new therapeutics (see Outlook).

[H3] Human trichuriasis: the evidence for Type 2 acquired immunity to infection

The concept of T helper cell subsets which emerged in the late 1980s from laboratory mouse models[60], revolutionised our understanding of resistance and susceptibility to infection. Whilst the original T helper 1 and T helper 2 framework has been superseded by a much more complex model embracing other T cell subsets (for example T regulatory cells, Th17 cells) and cell subsets within the innate immune system, the original paradigm remains sound. Studying immunity to human trichuriasis is fraught with difficulty, with challenges including genetic heterogeneity, undefined infection history and exposure, and polyparasitism. Nevertheless, comprehensive cross-sectional serological field studies point clearly to a positive correlation between anti-Trichuris IgE levels and decreasing infection

levels[61], with IgE representing an antibody isotype controlled by Type 2 responses. Analyses of Type 1 and Type 2 cytokines in supernatants from re-stimulated peripheral blood leukocytes from humans infected solely with *T. trichiura* are lacking, given that polyparasitism is usual in endemic populations. However, important data sets from polyparasitised populations infected with gastro-intestinal nematodes including *T. trichiura* strongly support the view that these infections induce Type 2 and regulatory responses[62] and that acquired immunity requires Type 2 protective immune responses that develop slowly after years, if not decades, of exposure[63]. More recently single-subject self-infection studies have contributed to our understanding of how *Trichuris* modulates human immunity: a longitudinal analysis of T cell subsets in mucosal biopsy samples and peripheral blood revealed a mixed local T cell response (T helper Type 1, 2, 17 and T regulatory) whilst circulating mononuclear cells became predominantly Type 2 (Ref[64]). A second such study revealed an amelioration of the symptoms of colitis following *T. trichuria* infection likely through improved Th2 and IL-22 mediated barrier function[65].

[H3] Insights from animal models

Preclinical models have been able to delve more deeply into both the underlying cellular regulatory mechanisms that control resistance and susceptibility to infection and the effector mechanisms that eliminate the parasite. Although we focus on the *T. muris* mouse model of human trichuriasis in the following section, *T. suis* in pigs has also generated important data which reveal commonalities between mouse, human and pig in Type 2 immunity[66].

Trichuris muris is the natural whipworm of mice, and is genetically and antigenically similar to *T. trichiura*, with *muris* and *trichiura* also showing similar epidemiological patterns in their respective hosts. The importance of Type 2 immunity in resistance to infection has been unequivocally demonstrated by many different research laboratories[67-70] and research now focuses on untangling the contributions of other cellular subsets[68,71,72]. An emerging concept from these studies is that the relevance of different cell types in promoting Type 2 immunity is context dependent; thus, essential cellular contributions in one strain of mouse become redundant in a different strain of mouse or when the cytokine balance is artificially manipulated[73,74] with important implications for translation to man. One of the burning questions is how do protective Type 2 responses develop; with answers to this question likely to inform smart vaccine development in the future. For a summary of our current knowledge in this context see Box 1.

[H3] Type 2 controlled effector mechanism: how are whipworms expelled?

In addition to the wealth of evidence supporting the importance of Type 2 immune responses in protective immunity to trichuriasis, mouse models continue to provide data addressing exactly how CD4+ Type 2 cells bring about worm expulsion. Arguably the most persuasive

effector mechanism described is the role established for goblet cells and mucus. Through the use of mucin deficient mouse strains, muc 2 and muc5ac[75,76] have been shown to be important in resistance to *T. muris*, likely via direct interactions with the parasite in the gut. The presence of muc 2-degrading enzymes in the *Trichuris* genome also supports an anti-helminth role[77]. Complementing a mucus-based effectore mechanism, Type 2 cyokines have also been shown to stimulate intestinal muscle contraction in the context of *T.muris*, and this enhanced contractility is associated with an acceleration of worm clearance[78]. While increases in mucus production and changes in the contraction of gut muscles may be common host responses to most gastro-intestinal helminths, regulation of epithelial cell turnover may be an effector mechanism specific for *Trichuris* through effects on its intracellular habitat. Here the Type 2 cytokine IL-13 has been shown to increase the rate of epithelial turnover thus displacing the parasite from its niche[79]. Though likely, whether these effector mechanisms also apply to human trichuriasis is difficult to establish. Gastro-intestinal helminth infections of mouse and man drive strong IgE responses, much of which is non-specific[80]. As mentioned above, human *Trichuris*-specific IgE antibody levels negatively correlate with worm burden. Thus, the older age cohorts which harbour lower *Trichuris* infection burdens have significantly higher *Trichuris*-specific IgE. A direct role for IgE in host protection has been difficult to establish and instead of having a functional role, parasite-specific IgE levels in man may represent a useful biomarker of a Type 2 immune response. Animal models have certainly revealed B cells to be important, though not essential, in resistance to *T. muris* infection.[73,81] However, exactly how the B cell contributes to the protective immune response is unclear and may not be related to its role in antibody production. Thus, the B cell can also act as an antigen presenting cell[82] and a cytokine-producing regulatory cell[83,84], making it well placed to influence the development of either Type1 or Type 2 immune responses and thus worm expulsion.

In chronic trichuriasis, as seen in humans, and mice infected with low numbers of eggs, regulation of gut pathology in the context of a large burrowing parasitic nematode is critical in the maintenance of gut barrier function and prevention of sepsis. Regulation of pathology has been dissected in some detail in the mouse model, and a considerable literature places IL-10 centre stage as the regulatory cytokine vital in regulating IFN-g mediated intestinal pathology and host protection[85,86]. Interestingly, in human trichuriasis, one of the QTLs on chromosome 9, mentioned above, contains genes that can influence IL-10 levels[20]. The cellular source of IL-10 is still debated with FoxP3+ T regulatory cells and other CD4+ T cell populations likely contributing[68].

[H3] Trichuris and its relationship with the microbiota

The close relationship of whipworms with the microbiota in the intestinal niche, extends beyond the trigger for egg hatching[51] and provides a fascinating and evolving story. It is clear that the presence of *Trichuris* infection alters the microbiome in terms of both numbers and composition, and this has been reported for *T. muris* in the mouse[87,88], *T. suis* in pigs[89,90] and in some, but not all, human studies[91,92] . Studies using *T. muris* in the mouse have revealed that parasite fitness requires that the parasite acquires its own distinct microbiota from the host. The parasite microbiome of *T. muris* is dominated by Bacteroidetes and Firmicutes, with a significant rise in the proportion of Proteobacteria that is not seen in the infected host microbiota[93]. Further, successful infections require the presence of host microbiota, and, remarkably, the *T. muris*-induced changes in the host microbiota may limit the success of subsequent infections. In the case of the latter, parasite numbers are controlled, thus providing a mechanism to limit host pathology and support chronicity of infection[93].

Moving forward, further dissection of mechanisms of resistance and pathophysiology in animal models must embrace more physiologically relevant dosing regimens (low-dose infection, repeated low-dose (trickle) infections[94]). It is also vital that the sorts of mechanistic studies that mouse models enable embrace the importance of context in order to better model human trichuriasis. This should include a consideration of the array of intrinsic and extrinsic factors such as genetics, age, gender, microbiome (to include viruses, fungi and gut protozoa), coinfections, nutrition and reproductive state. Complex environmental factors will combine to impact on immune variation and this can be modelled for example, using wild mouse populations[95] and semi-wild systems[96] both of which embrace environmental variation.

[H2] Ascaris species

Ascaris eggs are very robust due to their outer corticated coat and can survive in the environment for long periods of time. Estimates include up to 6 years in Germany and 14 years in Russia; although it is likely that the majority of eggs die on shedding[97] Indeed, in the context of tropical soils, evidence exists that *Ascaris* eggs, and those of other geohelminths including *Trichuris,* may be depleted within two months if no further contamination occurs[98]. The life cycle of Ascaris has proved difficult to precisely define.

An early and extensive study in pigs[99] described how after egg hatching, larvae within the sheath of the first molt, are released in the small intestine and such L2 larvae migrate to the caecum and proximal colon and then penetrate the mucosa. However, more recently Fagerholme et al[100] reported that both the first and second ecdysis occur in the egg, such retention of two moults being a feature favourable to parasite development. (Figure 3). The larvae then undergo what is known as a hepato-tracheal migration, a phenomenon that distinguishes *Ascaris* from *Trichuris* infection. Larvae migrate via the portal blood vessels to the liver. In the liver, the L2 cuticle is shed and some larval growth occurs. Subsequently, L3 larvae leave the liver and advance to the lungs, via the bloodstream to the heart and then

the pulmonary vasculature[97], penetrate the alveolar spaces and then migrate up the airway tree to the pharynx where they are coughed up and swallowed. On their return to the small intestine, L4 larvae undergo a final moult (L5) and then develop to adulthood and sexually mature male and female worms , within the small intestine[101]. Male and female adult worms measure 15 to 25 cm and 20 to 35 cm respectively. The life expectancy of an adult worm has been estimated to be 1-2 years[102]. Adult worms produce unembryonated eggs that are shed in the faeces where they develop to infectivity under appropriate conditions of temperature and moisture. The speed with which eggs embryonate varies considerably according to the environmental conditions. For example at 30 degrees centigrade embryonation takes around 10-14 days; however at 17 degrees centigrade embryonation can take 45-55 days[103]. Eggs that fail to embryonate are uninfective and cannot lead to infection. The explanation for this undoubtedly arduous and risky migration is unclear although some authors have argued that migration confers fitness benefits on the parasite including enhanced growth[104]. What is undoubtedly clear is that larval migration of *Ascaris* contributes to both liver and lung-associated pathology[105,106]. Furthermore, the role of the liver in resistance to ascariasis is important but significantly understudied.

[H3] Human ascariasis – pathophysiology/immunology

Ascaris is an excellent example of a chronic infection that contributes to chronic morbidity, particularly impacts upon child growth via anorexia, malabsorption of nutrients and jejunal mucosal abnormalities, and less well established impacts upon cognitive development. The mechanisms underlying cognitive defects are not well understood but are most likely nutrionally mediated, although the impact of inflammation should not be disregarded. Due to its large size, *A. lumbricoides* can also cause acute effects including intestinal and biliary tract obstruction with related complications.

The relationship between humoral immune responses and *Ascaris* infection in humans has been explored in a variety of different contexts[107,108]. Several studies have established a clear association between parasite-specific IgE and *Ascaris* infection. For example, a study of Nigerian children predisposed to heavy or light *Ascaris* infection and utilising a defined protein allergen, Ascaris-ABA-1, provided evidence for a significant relationship between raised levels of parasite-specific IgE to this antigen and putative immunity in children[109]. Thus, children with higher IgE titres are less predisposed to heavy infection, in keeping with the association seen in trichuriasis between elevated levels of parasite specific IgE and reduced worm burdens in adults. Furthermore, higher levels of inflammatory markers such as C-reactive protein were also detected in the same group of children[109] . By contrast, a study by King et. al found no relationship between humoral immune responses and current or re-infection with *Ascaris*[110]. *Ascaris* infection was also found to be associated with a highly polarised Th2 response with IL-4 and IL-5 responses predominating[111]. Two important studies in Cameroonian children and adults provided further evidence for the role of Th2

cytokines during *Ascaris* infection including IL-5, IL-9, IL-10, IL-13 (Refs[63,112]). However, the authors did report differential responses with age and speculated that these age and related differences in host responses might have implications for treatment success[112]. Thus, the authors suggested that heterogeneity in cytokine responses may operate differently depending upon the geographical location of the study. This may be due to differences in transmission patterns or even historical differences in parasite dynamics. Cooper and colleagues reported enhanced Th2 cytokine production among children who had been repeatedly treated for *A. lumbricoides* infection providing evidence that long-term treatment may enhance Th2 anti-parasite immunity[113].

[H3] Insights from animal models

The immunology of ascariasis is much less well understood than that of trichuriasis. One reason for this relates to the fact that there is no rodent model of ascariasis that allows for the completion of the entire life-cycle[114]. However, mouse models do provide insights into the factors that influence early infection and larval migration[114].

The rodent model enables an assessment of pathophysiological alterations under different parasitic burdens[115,116], genetic backgrounds[116-119], host ages[120], egg infectivities[120], and repeated parasite exposure[35]. The acute, early stages of infection are well established[114,120] and demonstrate the physiological changes elicited by larval migration in the host, especially in the liver and lung tissues. During larval migration in the liver, an intense inflammatory response is observed, particularly in resistant strains of mice [118]. Of note, proteomic analysis of hepatic tissues from resistant (CBA/Ca) and susceptible (C57BL/6J) mice strains infected with *A. suum* demonstrates intrinsic differences between the two strains, suggesting that resistance might be associated with oxidative phosphorylation pathway and reactive oxygen species (ROS) production[119] and differential expression of components of the complement system[116].

In primary infections with *Ascaris* spp, larval migration in the lungs promotes a local Type 2 inflammatory response, marked by early production of IL-5, followed by increased levels of IL-4, IL-5, IL-6, IL-33, CCL-11 (eotaxin), CCL-2 (MCP-1), CXCL-10 (IP-10), and an eosinophilia[120-122]. Interestingly, this elevated Type 2 immune response associates with a marked increase in IL-13 production by both Type 2 and innate lymphoid cell subset, ILC2 and this response was able to bestow protection against the rodent hookworm *Nippostrongylus brasiliensis*[123].This robust Type 2 inflammatory response is associated with lung pathology, characterized by persistent airway hyper-responsiveness resembling an extreme form of allergic airway disease[121]. The severe impairment in respiratory function is aggravated during multiple exposures to the parasite despite the significant reduction of

parasitic burden[35], which presents as a reduction in larval migration in the liver and lungs. The inflammatory influx of cells in both the lung parenchyma and bronchoalveolar fluid is initially dominated by neutrophils, correlating with IL-6 production in lung tissue[35,120,122]. As the infection progresses, mononuclear cells accumulate at the inflammatory site, associated with TNF-alpha production induced by larval migration[35,120], ultimately differentiating into M2 macrophages in the Type 2 environment[122]. Interestingly, parasite antigens can modulate macrophage differentiation and dendritic cell maturation[124-126] with further evidence of parasite-induced immunomodulation observed in experimental models of LPS-induced inflammation[127], autoimmune hepatitis[128] and heterologous immune response[129] and viral coinfection[130].

The protective inflammatory response observed in the rodent model of ascariasis may not be parasite-specific given that pre-sensitization with unrelated allergens (house dust mite) induces protection to a subsequent *A. suum* infection[122]. Conversely, pre-sensitization with *Ascaris* antigens accelerates mite-specific IgE response upon mite antigen inhalation[131]. These data indicate the possible cross-reactivity between the *Ascaris* and arthropod antigens.

Anther important animal model for ascariasis is the *A. suum* pig model. Pigs are costly to maintain and inbred and knockout porcine strains are currently unavailable. Nevertheless, given the economic impact of *Ascaris* infection on the food industry and the fact that pigs are natural hosts for *Ascaris* infection, understanding the pathophysiology of *Ascaris* infection in the swine model, particularly in the gastrointestinal phase of infection, is highly significant. Of note, the use of the pig model enabled an understanding of both parasite-host interactions during establishment, and the mechanisms of intestinal expulsion [132,133]. Although the mechanisms by which Ascaris parasites are expelled from the gut are less well defined than for *Trichuris*, evidence suggests that elimination from the gut involves the “weep and sweep” mechanism, embracing an increase in muscle contractility and fluid secretion[133], mechanisms also likely to contribute to elimination of *Trichuris.* Further, there is some evidence in pigs naturally exposed to A. suum infection, that continual exposure to infective larvae emerging from the egg may inhibit larval migration from the intestine[134]. Profound changes in the gut microbiome during *Ascaris* infection occurs, especially in the proximity of the initial site of larval infection were demonstrated using the pig model[135]. Thus, *Ascaris* infection leads to a significant reduction in the gut microbial diversity, which is not related to worm burden. Moreover, the infection impacts the abundance of specific microbial genera, particularly in the proximal colon. The relevance of microbial composition alterations due to *Ascaris* infection remains unknown.

The initial phase of *A. suum* infection in pigs is very similar to the parasite migration seen in humans, and induces both liver and lung pathology[136-138]. As observed in *Ascaris* infections of humans and mice, production of IL-5, IL-13, eotaxin, and an intense eosinophilia are observed[133,139]. Blood basophilia and intestinal mastocytosis are also common[139-141] and may contribute to Type 2 immunity induced by infection.

Pathophysiological changes similar to those described to humans, mice and pigs have also been observed in other animal models including calves[142], guinea pigs[143], rabbits[144], gerbils[145] and non-human primates[146-148].

[H1] Diagnosis, screening and prevention

[H2] Clinical presentation

[H3] Trichuriasis.

Clinical disease is caused largely by inflammation of the caecum and large intestine due to the presence of adult worms inducing a local inflammatory response and blood loss from bleeding and oozing of 'insertion' sites caused by adults as they forage across the mucosa (Figure 4a). Clinical disease in *T. trichiura* infection is related to parasite burden. Most inhabitants (children and adults) of endemic areas are infected with relatively few worms (i.e. <15 adults worms[149]) and such infections are often free of significant symptoms. Eosinophilia, if present, tends to be mild. *T. trichiura* is an infection of poverty and those infected are likely to be infected with other enteric parasites and exposed to a range of environmental hazards. Non-specific symptoms of urticaria, anorexia, abdominal pain, and other gastrointestinal symptoms are difficult to attribute to any single cause although have been associated with *T. trichiura*[150]. However, heavy infections with several hundred or even thousands of worms[151,152] are often associated with significant illness that may present as chronic iron-deficiency anaemia in adults[151] while children may present with short stature with or without symptoms of colitis or a severe illness. *Trichuris* dysentery syndrome (TDS), also known as massive infantile trichuriasis, is a severe illness associated with iron-deficiency anaemia, chronic mucoid diarrhea, rectal bleeding, rectal prolapse, and finger clubbing[149,153]. The exact pathogenesis of clubbing, a non-specific manifestation of many chronic diseases, is unknown but may relate to increased platelet derived growth factor in the nail beds[154]. The triad of finger clubbing, rectal prolapse, and chronic diarrhoea in children used to be pathognomic of trichuriasis in endemic areas: 3-5% of children aged 6 months to 6 years were estimated to have recurrent rectal prolapse in a region of the Carribean[155]. However, with improvements in environmental hygiene and access to

anthelmintics, TDS and rectal prolapse, the latter a consequence of increased straining and or peristalsis, are now seen infrequently. TDS has more recently been recognised as a problem in adults presenting with severe iron deficiency anaemia[151] and likely reflects poor clinical recognition of trichuriasis in adults living in conditions of severe poverty and who are not included in anthelmintic treatment programmes. Heavy infections may be associated with increases in intestinal permeability and the induction of a chronic inflammatory response, reflected in elevated circulating levels of the pro-inflammatory cytokine TNF-α[156]

T. trichiura may be a chance finding in individuals undergoing colonoscopy for abdominal pain and altered bowel habits[157,158]. During heavy infections, colonoscopy shows numerous motile worms tethered in the intestinal mucosa by their anterior ends[151,158]. Histopathology of the large intestine in patients with trichuriasis often shows only mild changes with increased inflammatory cells in the lamina propria, particularly in adults[151,159], while children may show a range of histological changes from mild inflammation to localized cryptitis at infection sites to a highly inflamed intestinal mucosa that is oedematous, eroded, and friable[64,152]. In heavy infections, adult worms may be found from the caecum to the rectum and the mucosa is studded with bleeding points representing previous mucosal entry points of foraging adults[151,159]. Blood loss in trichuriasis has been estimated at of 0.005 ml per worm per day[160]. Risk of anaemia is significant among those with heavy infections (defined as 800 or more worms[160] or >5,000 eggs per gram of stool[161]) or those co-infected with hookworm[162,163]. Mucosal bleeding and inflammation occurring over prolonged periods affect the nutritional state of children, particularly those on marginal diets (i.e. low in iron and other essential nutrients)[161]. Further, the presence of adult worms may also affect nutrient absorption through mucosal damage or disruption of intestinal microbiota although evidence for the latter effect is limited[92,164]. Damaged mucosa may be more susceptible to infections with other intestinal pathogens with which trichuriasis has been associated such as *Entamoeba* histolytica[165]. Indeed, *T. trichiura* infection has been shown to correlate with both the presence of *A. lumbricoides* and *Campylobacter* spp. Whether multiple intestinal infections are simply coincidental or whether they influence each other's pathogenicity in humans is unclear[166] although exacerbated disease and pathology has been reported in pigs coinfected with *T. suis and Campylobacter jejuni*[167]. Even mild trichuriasis may be accompanied by growth retardation in children[14] while TDS may be associated with severe malnutrition and growth stunting[14,159]. Curative chemotherapy and treatment with iron in children with TDS can have dramatic effects on linear growth velocities[149] . The benefits of deworming programmes for children has generated considerable controversy given negative findings of meta-analyses[168]. However, these studies were done using data that include uninfected children, thus diluting likely benefits among the sub-group of children with significant parasite

burdens. *T. trichiura* infection may impair developmental and cognitive abilities in children, although the benefits of treatment in reversing such deficits is hotly debated[152,168-170].

The potential immune regulatory effects of *Trichuris* on inflammation in the large intestine[65] has formed the basis of clinical trials using the pig whipworm *T. suis* that causes an infection that generally does not persist beyond 6 weeks in the human intestine, to treat inflammatory diseases such as inflammatory bowel disease (IBD). To date, trials in which humans have ingested orally *T. suis* ova have shown no statistical benefits in IBD patients[171-173]. Therapy with *T. suis* ova have also been evaluated in clinical trials for a number of other inflammatory diseases including rheumatoid arthritis, multiple sclerosis, psoriasis and food allergy but none have shown clear clinical benefit[174,175].

[H3] Ascariasis.

In endemic areas, the majority of *Ascaris* sp. infections are asymptomatic or produce mild symptoms. Clinical disease is restricted to a small percentage of individuals who present heavy parasite burden as most individuals harbour only a few worms[176,177], although there are no up to date figures on the actual percentage of clinical cases. The clinical features of the disease are directly related to the parasite life cycle (due to larval migration during the initial phases of infection or establishment of adult parasites in the final habitat) and are dependent on the infection intensity. During the larvae migration (10-14 days after infection), classical respiratory alterations including lung infiltration in the chest X ray, intense eosinophilia, cough and wheeze are observed, reported as the Loeffler's syndrome[178]. Urticaria, cough, dyspnoea, and haemoptysis, and abnormal auscultatory breath sounds are also non pathognomonic signs associated with larval migration through pulmonary tissue. After the establishment of adult parasites, according to the burden of infection, the presence of the parasites may lead to gastrointestinal outcomes including upper gastrointestinal bleeding, small bowel obstruction (Figure 3b and 3c), volvulus, intussusception, peritonitis, hemorrhagic infarction of the bowel, and perforation[179,180]. Following the dispersion of the adult worm to extra intestinal sites, hepatobiliary and pancreatic ascariasis may occur and lead to biliary colic, acute cholecystitis, acute pancreatitis, acute cholangitis, and hepatic abscess[181]. Peritoneal (patients with fatal peritonitis)[182] and appendicular ascariasis[183] are clinical diseases observed in severe infections in endemic areas.

Asthenia, lack of appetite, abdominal pain, distention, nausea, diarrhoea and weight loss are common in children with severe intestinal ascariasis in endemic areas[181]. Moderate to heavy infections in children has been extensively associated to impairment in physical and mental development[184] and also contribute to the malnutrition[185] and vitamin A and C deficiency[186].

[H2] Diagnosis of Ascariasis and Trichuriasis

The laboratory diagnosis of ascariasis and trichuriasis, as for any other soil transmitted helminths, relies on the examination of a limited sample of stool to determine the presence and, whenever it is possible, the amount of parasite eggs. Currently, the WHO recommends the use of the Kato-Katz method[187], assessing two slides per sample[188]. Other parasitological methods include direct microscopy, formol-ether concentration, McMaster, FLOTAC, and Mini-FLOTAC, which present variable sensitivity according to the intensity of infection[189]. New parasitological methods, such as mobile phone microscopy[190] and FECPAKG2 (Ref[191]) have been developed but require extensive evaluation.

Considering the reduced sensitivity of parasitological methods, molecular assays have been developed to diagnose ascariasis and trichuriasis, aiming to improve sensitivity and specificity when compared to microscopic techniques. The development of molecular diagnosis for helminthic infection is hampered due to the relative higher cost and requirement for specific equipment, and the lengthy DNA extraction procedure of the stool samples, both of which may limit the application of molecular diagnostic assays. However, the reported sensitivities of molecular methods are significant and higher than observed for conventional microscopy for the diagnosis of both ascariasis[192-195] and trichuriasis[194,195], despite the lack of an adequate gold standard[196]. Of note, mostly molecular assays have been developed as multiplexed[197-199] or multi-parallel assays[193,200,201] for simultaneous detection of different parasites. A colorimetric isothermal assay, embracing a one-step DNA amplification method, was also developed for the diagnosis of ascariasis and trichuriasis, combining high sensitivity and high tolerance to inhibitors present in fecal samples[202], such as complex polysaccharides, salts, lipids, urate, among others[203], which might be a promising tool for diagnosis in the field.

The fecal examination by conventional (microscopy) or molecular methods are important tools for determination of infection but are only effective after infections have become patent (i.e. adult females have been fertilized and start producing eggs). Microscopic methods present very limited sensitivity for of low intensity[189] withintensity of *Ascaris* and *Trichuris* infection estimated as EPG (eggs per gram of feces) and classified into light (1–4999 EPG and 1-1000 EPG, respectively), moderate (5000–49,999 EPG and 1001-9999 EPG, respectively) and heavy (≥50,000 EPG and ≥10,000 EPG, respectively), according to WHO classification[204]. Under such circumstances, molecular-based assays, although more expensive and of limited field-applicability offer potential advantages, for example, to detect low intensity infections where anthelmintic control programmes have reduced prevalence and intensity to very low levels and where local or regional elimination strategies are being considered. Mothers living in endemic communities attribute considerable illness in their

children to the presence of parasites so the demand for clinical diagnosis in poor communities should not be under-estimated. Further, the demand for community diagnosis using approaches such as qPCR, which offer greater sensitivity, is growing, particularly under scenarios where elimination of transmission might be considered (i.e. very low prevalence levels and the need to detect low-level infections among the few who remain infected). The use of more sensitive assays such as qPCR at central laboratories might be justified under such circumstances despite the extra cost and need for sophisticated equipment and trained personnel. Low cost field applicable assays are presently not available such as lateral flow assays to detect specific antigen in stool but would enhance considerably the effectiveness of control programmes where decisions have to be made about the frequency of anthelmintic treatment and population groups to be targeted for treatment. The use of more sensitive assays such as qPCR at central laboratories might be justified under such circumstances despite the extra cost and need for sophisticated equipment and trained personnel. Low cost field applicable assays are presently not available such as lateral flow assays to detect specific antigen in stool but would enhance considerably the effectiveness of control programmes where decisions have to be made about the frequency of anthelmintic treatment and population groups to be targeted for treatment. The development of serological tools to improve the detection of pre-patent infections – such as during earlier phases of infection (for example, hepatic or pulmonary burden during *Ascaris* sp. larval migration) – could improve the effectiveness of surveillance during elimination programmes. The high-throughput assessment expected for serological assays indicates the suitability of these tools in epidemiological surveillance. However the development of serological assays is largely hampered by the lack of specificity due to cross-reactivity observed among helminth infections[205-208], and even with arthropods such as mosquitoes and ticks[209,210], and the inability to discriminate between past and current infections. While serological assays are available for the diagnosis of animal infection[211-214], the development of serological assays are still very limited for the detection of human infection and restricted to detection of *A. suum*[215] in humans. Of note, the development of anti-*Ascaris suum* IgY antibodies in the immunodiagnosis of human ascariasis allowed the detection of immune complexes during human infection and showed diagnostic values of 80% sensitivity and 90% specificity[216]. While the cross-reactivity would reduce the discrimination among helminth infections, the use of cross-reactive or conserved epitopes among different helminth parasites would be useful for the control of helminth infections, particularly in the application and assessment of parasite control achieved using mass drug administration (MDA) (see Outlook).

[H2] Prevention of Ascariasis and Trichuriasis. The prevention of ascariasis and trichuriasis, as in any other STH infections, relies on the combination of several conventional approaches that reduce prevalence. Among them, the WHO guidelines on so called preventive chemotherapy based on MDA in endemic areas aim to reduce the morbidity in pre-school-aged and school-aged children by lowering the prevalence of moderate- to heavy-intensity infections[217]. Preventive chemotherapy has been proved as an important tool for reduction of prevalence and morbidity of both ascariasis and trichuriasis, with a reduction of up to 80% in the overall parasite burden and prevalence in endemic areas[218-220]. There is consensus that the drugs applied in preventive chemotherapy programs are safe and effective. However, there has been a public debate ("worm wars") on the impacts on health, including short-run impacts on weight and long term educational and economic impacts. While no benefit was identified in randomized clinical trials, contradictory findings were observed in the clinical literature[221]. For example, a meta-analysis estimated that the average weight gain per dollar expenditure from twice annual preventive chemotherapy is more than 35 times than that from school feeding programs[222]. Moreover, males who received deworming drugs a decade ago in Kenya worked 17% more hours per week and had higher living standards and girls were one quarter more likely to have attended secondary school[223]. Based on this debate, in 2017 a WHO Guideline Review Committee revisited the earlier preventive chemotherapy guidelines providing updated global, evidence-informed recommendations on preventive chemotherapy[224] in areas endemic for STH but it represents a short-term strategy for control of helminth infection as reinfection often occurs in endemic areas in the absence of clean water, sanitation and hygiene[225]. A comprehensive programme consisting of improved water, sanitation, and hygiene (WASH) includes improvements in water access (water quality, water quantity, and distance to water), sanitation access (as access to latrines and their proper maintenance, as well as faecal sludge management), and finally, the use of hygiene practices and changes in behaviour related to environment and family hygiene[226,227]. Lower odds of *A. lumbricoides* and *T. trichiura* infection are associated with treated water, access to sanitation and hygiene procedures (handwashing before eating and after defaecation and use of soap)[228], however there is an urgent need to gather stronger evidence to support the role that WASH programmes play in the control of STHs[229].

An additional measure for control of ascariasis and trichuriasis would be the use of vaccines, which might reduce the parasite burden and, consequently, the morbidity and transmission of infection (see Outlook). Evidence from experimental murine models indicated that continuous exposure to *A. suum* eggs, (three subsequent infections with 2,500 eggs), led to up to 98% of protection, determined by larval reduction in the host tissues[35,230]. For *T. muris*, the immunization with adult worm extract or excreted-secreted proteins induced a high

degree of protection (up to 100% of larval reduction)[231,232]. Over the past years, the development of vaccines using defined antigens against ascariasis and trichuriasis has been pursued, but it is still restricted to experimental models and no vaccines against *Ascaris* sp. or *T. trichiura* are currently being assessed in clinical trials. The selection of new vaccine candidates and the understanding of protective mechanisms induced by immunization might open new perspectives for the control of these infections in endemic areas, as individual or combined ('pan-helminth') vaccines[233].

[H1] Management

As described above for the prevention of these diseases, the control and treatment of ascariasis and trichuriasis, like other STHs, can be achieved through a number of strategies which include environmental sanitation and hygiene, health education and the use of anthelmintic drugs. Environmental sanitation and hygiene (by provision of safe and adequate potable water and safe disposal of human excreta) are effective but take very long to bring about appreciable reduction in prevalence and intensity. Indeed, it is difficult to evidence the effects of Water, Sanitation and Hygiene Interventions (WASH) in control programmes[229], with a better understanding of how we assess levels of environmental exposure to STHs and how we measure WASH uptake and usage important in understanding the role of WASH as an adjunct to deworming programmes. The use of effective and safe anthelminthic drugs on the other hand, has been shown to be more effective and rapid in reducing prevalence, intensity and morbidity. Treatment of ascariasis and trichuriasis includes management of diagnosed patients, aiming for cure of patients as well as large-scale administration of anthelminthic drugs to populations in endemic areas to reduce the burden of disease (preventive chemotherapy). In contrast to most regimens for individual patient management, preventive chemotherapy programs, advocated since 2001 by the WHO rely on single dose treatment (Table 2). Preventive chemotherapy involves periodic administration of a single dose of oral albendazole or mebendazole to pre-school-aged children, school-aged children, women of reproductive age (including pregnant women in the second and third trimesters and lactating mothers) and adult groups particularly exposed to STH infections, such as for example tea pickers. The recommended treatment schedule of once or twice annual administration is determined by the initial prevalence of STH infection[234,235]. The goal wasto achieve a minimum coverage of 75% of the most affected groups by 2020. In 2017, over 598 million children were treated in endemic countries corresponding to 69% of all children at risk[229]. Given the achievement of the 2020 targets, recently new targets and indicators were set by WHO[236] namely i) to achieve and maintain elimination of STH morbidity in pre-schoolers and school-aged children by 2030 (defined as prevalence of moderate and heavy infections below 2%) ii) to reduce the number of tablets needed in PC for STH iii.) to

increase domestic financial support to PC for STHs iv) to establish an efficient STH control programme in adolescent, pregnant and lactating women, v.) to establish an efficient strongyloidiasis control programme in SAC and vi) to ensure universal access to at least basic sanitation and hygiene by 2030 in STH-endemic areas.

The current drugs recommended by the WHO for the treatment of STH Infections are albendazole, mebendazole, levamisole and pyrantel pamoate[234,237]. Albendazole and mebendazole are the two benzimidazoles that have been used most widely for decades against STHs in the treatment of individual patients and in MDA programmes. For MDA programmes, millions of tablets are donated each year. Albendazole, a benzimidazole carbamate, is supplied in tablets and as suspension. It is administered orally to both adults and children above 2 years of age. Mebendazole, same as albendazole, kills the worms in the intestine leading to their expulsion within 24 hours of drug administration. Mebendazole is available in oral tablets and in suspension.

[H2] Treatment of Trichuriasis

When used at single oral doses, the treatment schedule compatible with preventive chemotherapy programmes, none of the recommended monotherapies shows acceptable efficacy (egg reduction rates above 90% based on the target product profile for drugs used for STHs)[238] against *T. trichiura* infections (Table 2). The efficacy is higher when the drugs are used in the recommended dosing schedules. For example, a double-blind clinical study on Pemba island showed that mebendazole given to school-aged children twice a day for three days achieved considerable higher cure and egg reduction rates against *T. trichiura* infections when compared to single dose treatment (cure rate of 6.8% *versus* 42.9% and egg reduction rate of 71.7% *versus* 98.1% [239]. Why *T. trichiura* infections are less affected by the drugs is not known but the location of the parasite (as discussed in the Outlook section) might have a role. To date evidence of resistance against benzimidazoles in human medicine has not yet been established [240]. However, drug selection pressure that led to widespread anthelminthic resistance in veterinary helminths is now similar for human STHs given the large scale use of preventive chemotherapy. The reasons for the little knowledge on human anthelmintic resistance include the variable drug efficacy, lacking validated phenotypic or genotypic tests for resistance as well as working with difficult samples matrices (i.e. stool). Efforts are ongoing to develop molecular and genomic screens of human STH populations for mutations likely to be associated with benzimidazole resistance based on the understanding on resistance in veterinary helminths. It is important to monitor the presence of resistance-associated single nucleotide polymorphism (SNPs) in human soil-transmitted helminthiasis before resistance becomes clinically established.

Given the low efficacy of the standard treatments at monotherapy against *T. trichiura* infections, monodose combination chemotherapy has been widely advocated in the past years, embracing the advantages of a single administration with drug combination therapy. Albendazole combined with ivermectin is since 2017 on the essential medicine list of the WHO for the treatment of soil-transmitted helminthiasis and strongyloidiasis [241]. This drug combination was classified as high priority combination given that the treatment is already widely used for lymphatic filariasis [242]. Despite its large scale use the available efficacy data for soil-transmitted helminthiasis is limited [239] and a multi-country randomized controlled double-blind trial has therefore been launched to provide strong results on the efficacy and safety of co-administration of ivermectin and albendazole [243].

Given the recent registration of moxidectin for onchocerciasis at the Food and Drug Administration (US FDA) [244] albendazole-moxidectin might serve as an alternative drug combination to albendazole-ivermectin. Moxidectin combined with albendazole, used at the recommended dosages, was shown safe and effective against *T. trichiura* infections[245]. The use of higher dosages showed no benefit. Large scale trials to establish the effectiveness are necessary for moxidectin-albendazole as currently under way for ivermectin-albendazole [245].

In contrast to the recommended treatments (Table 2), ivermectin [246] or moxidectin [245], oxantel pamoate has excellent trichuricidal properties [247]. To compensate for its lack of efficacy against *A. lumbricoides* and hookworm, it was combined with pyrantel pamoate (e.g. Quantrel®). In the past years, several clinical trials have successfully demonstrated that a combination of albendazole-oxantel pamoate is safe and efficacious [248]. Moser and colleagues calculated a cure rate of 88.7% and an egg reduction rate of 96.7% by means of network meta-analysis for this combination using a single dose [242]. Efforts are ongoing to determine if any existing data on oxantel pamoate (from veterinary medicine, where the drug is widely available or the countries where it is registered as human drug, e.g. the Philippines) can be utilized to support EMA/FDA registration with the ultimate goal that oxantel pamoate could be used as partner drug in treatment campaigns.

Emodepside, a veterinary anthelmintic licensed under the name of Profender® and Procox® is the only advanced drug in the depleted drug development pipeline. Emodepside is a cyclooctadepsipeptide, targeting the evolutionary conserved calcium-activated potassium channel slowpoke 1 (SLO-1) and the latrophilin receptors LAT-1/LAT-2 (Ref[249]) targeting nematode neuromuscular function. The drug is currently undergoing clinical testing against onchocerciasis. In laboratory models of soil-transmitted helminthiases emodepside showed a broad spectrum of activity against the major soil-transmitted helminths [250]. Emodepside should therefore also be considered for the development of soil-transmitted helminth infections. Its disadvantage is its high production costs since it is a semi-synthetic compound

whose precursor is a metabolite of the fungus *Mycelia sterilia.* Testing of SLO-1 inhibitors is therefore currently ongoing.

[H2] Treatment of Ascariasis

Clinical disease resulting from ascariasis in children and adults includes intestinal obstruction, a common occurrence in children in endemic areas; peritoneal ascariasis due to the migration of *Ascaris* larvae into the peritoneum and appendicular ascariasis due to worms entering the appendix lumen. Other complications due to ascariasis include hepatobiliary and pancreatic ascariasis (HPA) which commonly occurs in adults.

A number of anthelminthics have been developed to effectively manage ascariasis including albendazole, mebendazole, levamisole, pyrantel pamoate and ivermectin, although their long term effectiveness remains a concern and new approaches such as crystal toxins from. Bacillus thuringiensis are being explored ((Hu et al., https://www.ncbi.nlm.nih.gov/pubmed/29772478). In HPA, endotherapy is recommended to remove worms from the ductal systems if the worms fail to move out of the ductal lumen by 3 weeks post anthelmintic treatment. Conservative treatment is the mainstay of treating hepatobiliary and pancreatic ascariasis. This involves appropriate treatment for clinical syndromes such as bowel rest, intravenous fluids, analgesic-antispasmodics and antibiotics followed by mebendazole once acute symptoms subside[251]. However, if this treatment option fails, Endoscopic Retrograde Cholangio-Pancreatograph (ECRP), involving endoscopic examination of bile and pancreatic ducts and the extraction of worms without sphincterotomy (enlargement of the bile duct opening) or surgery are used[252]. Intestinal obstruction, which rarely occurs in children, is treated through surgery. However, when perforation of the intestine occurs, the type of surgery depends on the findings during laparotomy and is tailored to individual needs[253].

Albendazole, mebendazole, levamisole and pyrantel pamoate have high efficacy against *A. lumbricoides* both in terms of cure rates and egg reduction rates (Table 2) following a single dose[254]. Several other marketed anthelminthics, such as ivermectin (Table 2), moxidectin or tribendimidine have also been shown to be highly effective against *A. lumbricoides*[254].

[H1] Quality of life

[H2] Trichuriasis

Estimates of the effects of trichuriasis on quality of life in populations where the parasite is endemic is complicated by unsure estimates of prevalence and parasite burdens and imprecision in estimates of impact on quality of life indices. Quality of life is most likely to be affected during chronic and/or high-burden infections. Death is thought to be an unusual

outcome of infection although no reliable estimates of mortality exist[255]. Measures used to determine quality-of-life effects include those of economic, educational, social, health, environmental and other aspects of the well-being of individuals. Trichuriasis likely has direct effects on a number of these domains such as economic productivity, educational performance, and ill-health although there are limited data measuring such effects. Trichuriasis has been shown to affect cognition[256], school performance[257], and school absenteeism rates[258] and thus likely has direct effects on educational achievement and economic potential of individuals. Health effects such as those associated with anaemia and poor growth will likely affect physical fitness[259] and economic productivity[260], as well as having effects on the quality of social interactions and well-being. Anaemia can be severe in vulnerable groups such as pregnant women whose iron reserves are most depleted, although not as pronounced as for hookworm[151,163]. The various health consequences of infection can be summarized crudely using a widely-used metric, disability-adjusted life years (DALYs), that estimates the number of years of 'healthy life' lost attributable to a specific infection using both morbidity and mortality data. For trichuriasis, estimated DALYs are highly variable between studies but were estimated at 0.213 million in 2017 (Ref[261]) with the greatest burden in the populous countries of Asia (~60% of DALYs). This represents a decline of 23% since 2007 largely due to reductions in poverty and improved access to anthelmintic drugs among high risk groups. These estimates were based on disability weights for 'symptomatic infection', 'wasting, and 'mild abdominopelvic problems' with no attributed mortality. Recently, girls and women of reproductive age have been included as a high-risk group for anthelmintic treatment programmes, based partly on the epidemiological links between *T. trichiura* infection and risk of anaemia in this group[262]. *T. trichiura* is an infection of poverty, most common among those living in tropical regions in conditions of extreme poverty (i.e. on less than US$1.90/day). Many of the factors that feed extreme poverty are linked to risk of *T. trichiura* infection (i.e. poor sanitation, education, etc.) which itself contributes to the underlying causes of poverty. The effective control of *T. trichiura* would be expected to reduce poverty through the improvements in health, educational achievement, and economic productivity.

[H2] Ascariasis

In keeping with trichuriasis, the burden of ascariasis is associated with the chronic and insidious impact this disease has on the health and quality of life of infected individuals. *Ascaris,* like *Trichuris,* has been shown to have a significant role in childhood protein energy malnutrition and reduced food intake leading to growth retardation, poor cognitive development, school-absenteeism and poor academic performance. Collectively these

impacts combine to affect an individual's productivity thus limiting the economic prospects of countries where Ascaris is endemic[263-265].

The unique hepatic migration of *Ascaris* can contribute to liver inflammation. An extensive prospective study of Indian hospital patients revealed that 14.5% of patients with liver abscess had biliary *Ascaris* as the cause and eleven patients had intact *Ascaris* larvae within the liver abscess[105]. In the early stages of Ascaris infection, individuals may suffer cough and high fever[266]. Loeffler[267] described a transient or seasonal syndrome of pulmonary infiltrates, mild to marked respiratory symptoms and peripheral eosinophilia that he subsequently attributed to *Ascaris* in the lungs and termed "Loeffler's syndrome"[178]. Later in infection, and in contrast to trichuriasis, high adult worm burdens can be life-threatening for both adults and children where intestinal obstruction and biliary complication predominate[268]. In children, intestinal obstruction due to *Ascaris lumbricoides* infection accounted for 1.8% of the 902 cases of acute abdominal surgery, as reviewed at the University of Benin Teaching Hospital, Nigeria over a five-year period[269] and may be caused by heavy worm burden in the range of 60 or more parasites[270]. Airway obstruction, a potential life-threatening event arising from *Ascaris* infection has also been reported[271],[272,273] however, this condition rarely occurs and there is no available data regarding its prevalence. The global disability-adjusted life year estimates for ascariasis are 0.861 million in 2017 (Ref[261]). In comparison to 2007, ascariasis presented the largest decrease in DALYs among all intestinal nematode infections, possibly due to deworming and socioeconomic development, although it could also be accounted for by follow-up studies in areas where control programmes have been previously conducted[7]. Further, a recent co-morbidity study has indicated that patients with chronic pancreatitis with concomitant ascariasis have a significantly lower level of quality of life score than individuals with chronic pancreatitis not associated with ascariasis [274]. Ascariasis can also cause allergy and immunopathology in infected people, and non-infected people who have inhaled antigens from *Ascaris* life cycle stages. Such allergic immune responses can present as cough, bronchial asthma, eosinophilia, gastrointestinal disorders and urticaria[275].

Nutritional and cognitive impacts of soil-transmitted helminth infections. Cross-sectional and prospective observational studies from 20 or more years ago have indicated significant long-term impacts of soil-transmitted helminth infections on a number of nutritional induces such as stunting and also on childhood cognitive development[276]. Randomized controlled trials have been more equivocal in showing effects of STH infections on nutritional and cognitive indices and more recent systematic reviews of intervention studies have been

able to demonstrate only negligible effects on growth and nutritional parameters, cognition, and mortality[168,277,278]. A meta-analysis of observational and randomized treatment studies showed no overall effect on cognitive parameters in children in treatment trials but infection-related deficits in some parameters for observational studies, although the latter effects were considered to be highly vulnerable to bias[170]. A systematic review of nutritional supplementation (e.g. Iron) as a benefit in addition to anthelmintic treatment, highlighted the fact that the evidence base was so weak that no recommendation nutritional supplementation could be recommended[279]. Criticisms of systematic reviews have focused largely around the dilutional effects on impact measures by including uninfected children or children with low parasite burdens, the fact that study populations may be infected with a variety of different helminth species making it impossible to attribute species-specific effects, and that school absenteeism related to the most affected children could bias results towards no effect. A recent critical appraisal noted the need for new studies designed and powered to overcome these limitations in order to measure morbid effects of STH[276] . Certainly, observational studies of heavily infected children have shown dramatic effects of treatment on catch-up growth post-treatment, particularly for severe triuchuriasis[151,280,281], but the frequencies of children at risk has declined markedly in line with worldwide reductions in poverty rates[282,283].

[H1] Outlook

[H2] The development of new drugs

The long-term effectiveness of the drugs currently available to treat *Ascaris* and *Trichuris* (levamisole, pyrantel pamoate, albendazole and mebendazole) is a major concern and underpins the need for novel drug discovery. . Encouragingly however, new mechanism of action drugs are being discovered, for example, the pore-forming protein Cry5B produced by the soil bacterium Bacillus thuringiensis (Bt) is effective against hookworm in preclinical models[284]. Further, access to the genomes of these[77,285] and many other parasites[286] offers the prospect of enhanced target-based screening for new anthelmintics. A chemo-genomics approach (which takes the most promising of druggable targets in parasite genomes and

exploring their drug repurposing prospects using the ChEMBL database) is underway searching for compounds targeting the most druggable of whipworm candidate targets. For whipworm, 40 priority targets were associated with 720 drug-like compounds (181 of which reached phase III/IV clinical trials[286]). For *Ascaris*, new targets with their variety of inhibitors may also offer new routes to drug discovery[285].

Phenotypic screening, using live, *ex vivo* nematodes, has resulted in the discovery of most currently available anthelmintics [287] and this is likely to remain an important approach in the future. New platforms encompassing automated phenotyping that are suited to high-throughput chemical screening for motility and growth impairment in *C. elegans* and parasitic nematodes are available[288]. Such platforms facilitate putative drugs to be tested across different parasite species with the aspiration of discovering moieties with activity against trematodes and nematodes. Access to the wealth of behavioural data on mutants of *C. elegans* is also a resource in the search for new candidate drug targets[289]. Chemistries active on parasites and *C. elegans* will facilitate genetic approaches to target identification. By this means new classes of chemistry with anthelmintic properties are emerging[290] including some with activity against both adult and egg stages, which may enable a break in the life cycle[291]. This could be important as both whipworm and *Ascaris* eggs can remain viable in soil for extended periods [292].

The use of advanced imaging technologies may enhance our understanding of parasite-host biology and facilitate the development of novel drugs against soil transmitted helminths in general (Figure 5). One such example is X-ray computer tomography, which provides re-constructed 3D images of parasites in situ and over time[55]. This can highlight in detail parasite interactions with host tissue. For example, the attachment site of *Trichuris*, the epithelial tunnel, remains poorly understood. To date the tunnel has only been viewed by scanning electron microscopy[293], looking down on to the surface from the gut lumen, and by conventional histology, which provides a 2D view[294]. 3D imaging offers the potential to view the attachment site in a more holistic way and has already begun to show the complexity of whipworm interactions with intestinal cells, which may present particular challenges for worm clearance[55]. Further, acknowledging and addressing important differences in the biology of *Ascaris* and *Trichuris* will facilitate the development of bespoke strategies to reduce prevalence and control morbidity. Anthelmintic drug resistance mechanisms can involve pharmacokinetics, detoxification and target-site modifications, which can shorten the life of valuable chemistry, so discovering ways to circumvent this will be important in the future. Arguably the few compounds currently in use may increase the chances of resistance developing[295]. Enhancing the pipeline of new chemistry will be important, as will rotating or

combining drug treatments. Resistance may be under-reported if we only score known resistance-associated polymorphisms. Improved molecular markers[296] are needed to better understand resistance, especially when planning large-scale deworming programmes worldwide.

[H2] Targeting liver immunity

Stimulating host immunity may offer a therapeutic avenue. There is emerging evidence for the role of the liver in immunity to ascariasis[114,297]. A mouse model of ascariasis has been used to explore the liver proteome in two inbred mouse strains, susceptible and resistant to *Ascaris* infection[117]. Higher levels of mitochondrial proteins involved in oxidative phosphorylation were observed in the resistant strain (both intrinsically and under infection), when compared to the susceptible strain. Thus an intrinsic difference in reactive oxygen species (ROS) in the liver could give the resistant strain an advantage in contending with the parasite[119]. In another study, a lower larval burden of *Ascaris* was observed in the lungs of reinfected mice, and lesions caused by hepatocyte necrosis and infiltration of eosinophils and neutrophils were more pronounced in the reinfected group. The more pronounced hepatic immune response in the reinfected group results in a lower lung larval burden[35]. Novel therapies targeting the liver could conceivably stop larval migration in its tracks, reducing tissue damage and impairing development of adult worms.

[H2] Drug treatment and parasitological monitoring

Significant challenges remain if soil-transmitted helminths such as *Ascaris* and *Trichuris* are to be eliminated. These challenges are complex and multifaceted and include the sustainability of preventative chemotherapy, the choice of at risk groups (for example at present adult males are currently excluded from MDA), the possible emergence of anthelmintic resistance and the fact that a pan STH vaccine[233] is an ambitious endeavour. Furthermore, the data emerging on the impact of WASH[229] suggests that while STH infection remains high, MDA will still be required and the impact of WASH will be longer term. Certainly the funding of such initiatives as the deWorm3 project[298] represents a welcome endeavor that will test the feasibility of interrupting STH transmission using biannual mass drug administration targeting all age groups coupled with large scale application of PCR for monitoring drug- treatment. We urgently require well designed, long-term quantitative epidemiological data in order to plan the future for elimination including the provision of data for appropriate mathematical modelling. In this context, parasitological monitoring[301] is a key component required to enhance our understanding of the efficacy of control strategies, in tandem with the development of appropriate mathematical modelling approaches. This paper suggests that methodology needs to be developed to enable the

measurement of prevalence of soil-transmitted helminth infection in Preschool children (PSAC), school-age children (SAC) and women of reproductive age (WRA) and other risk groups, providing a more complete picture of the burden of soil-transmitted helminthiasis in the entire community. In this context, the most urgent need is for better estimates of key parameters can be fitted to mathematical models in order to assess the impact of treatment to key at risk groups such as density dependence in fecundity, observed as a reduction in egg production with increasing worm burdens, parasite life expectancy, egg survival and age-specific force of infection, which describes the per capita rate at which susceptible individuals acquire infection [302].

Part of the WHO strategy to control soil-transmitted helminths is the periodic administration of benzimidazoles such as Albendazole and Mebendazole. However, such extensive use could foster the emergence of anthelmintic resistance. Presently, large scale monitoring for resistance is absent and detection has relied on microscopic methods such as the insensitive egg reduction rate[303]. In a recent viewpoint[304], the authors highlighted a number of initiatives including the STOP, deWorm3 and the Starworms projects that are focusing on the assessment of drug efficacy and the development of molecular methods for the detection of anthelmintic resistance.

One argument that is gaining momentum is the need to move away from an emphasis on the treatment of school-age children only to a community-wide approach especially in the context of high transmission areas[299]. A recent, large-scale randomised trial in Kenya that compared 3 treatment strategies (including the current focus on children aged 2-14 years) concluded that annual or bi-annual community treatment was more effective against the prevalence and intensity of hookworm than school-based treatment of children only but also raised the argument that this approach needed to be explored in the context of *Ascaris* and *Trichuris*[300]. A recent study in Myanmar identified adult males (who are not the focus of the current WHO strategy) with significant burdens of both hookworm and *Trichuris*[305].

[H2] The development of vaccines

However, concern remains that MDA alone will not be sufficient to eliminate soil-transmitted helminths such as *Ascaris* and *Trichuris*. Explanations include rapid-reinfection in environments where long-lived and resistant eggs survive, the lack of drug efficacy particularly for *Trichuris*, the possibility of drug resistance and a lack of access to clean water and adequate sanitation. Thus, vaccination will be a continued focus for the future. However, in contrast to the efforts made to develop an anti-hookworm vaccine, progress with respect

to *Ascaris* and *Trichuris* has been slow. Pigs, exposed to UV-irradiated eggs of *A. suum*, demonstrated reduced numbers of migrating larvae and adult worms in the intestine[306] in response to both humoral and cellular acquired immunity[139]. However, crude antigen sources carry a risk of inducing allergic responses due to their allergenic properties. Several chemically defined antigens have been expressed and 6 antigens have been targeted for further investigation[233] including As14, an antigen found in both larval and adult *Ascaris* worms that has a 64% level of protective immunity in mice[307] and As16 (Ref[308]). In contrast to *A. suum*, *T. muris* has not been studied as extensively with respect to the development of recombinant antigens[233]. Antigens derived from the stichosome have induced significant reductions in worm burdens in a mouse model[232]. More recently, however, the *T. muris* whey acidic protein (r*Tm*-WAP49), secreted from the parasite's stichosome and tentatively ascribed pore-forming activity, has been proposed as a promising vaccine candidate[309], suggesting that the evaluation of *T. muris* recombinant proteins as immunogenic entities is gathering pace. Thus rTm-WAP49 achieved a 48% reduction in worm burden in mice and showed high sequence conservation with the *T. trichiura* WAP proteins[309].

[H2] Final words

Soil transmitted helminths are complex pathogens and their control presents complex challenges. Further, these challenges differ according to context making it impossible to be prescriptive. Never the less, it is clear that a holistic approach embracing MDA, education and sanitation is critical, working hand in hand with basic biological research. Enabling countries to take ownership of control programmes, thus moving towards self-sustainability in both drug administration and drug procurement is a key goal. In this context, exciting new targets and indicators have been set by WHO[236]. For example, countries deworming by domestic funds is scheduled to increase from 5 in 2023 to 25 in 2030 and improved sanitation is a major goal with targets to decrease to open defaecation to 0 by 2030. Just as enabling countries to take ownership of control programmes is important, so is building critical mass in basic biological research in countries where helminth infections are endemic. Multiple unmet needs exist in the area of basic biology of infection, including the need to develop affordable, sensitive tools to monitor parasite prevalence, and innovation in vaccine research. Despite these unmet needs, the current pace of technological advances in biological research combined with the growth of multi-disciplinary approaches gives optimism that living with helminth infections will one day not be the norm.

Table 1: Diagnostic methods for whipworm and roundworm infections.

Test	**Procedure**	**Output**	**Sensitivity**		**Specificity**		**Advantages**	**Limitations**
			Ascaris	Trichuris	Ascaris	Trichuris		
Microscopy based techniques (Kato-Katz, Direct microscopy, formol-ether concentration, FLOTAC, Mini-FLOTAC, McMaster)[189,310,311]	Identification of parasite eggs in fecal samples by microscopy	Egg detection or egg quantification	56.9-79.7	62.8-91.0	99.6	97.5	Relative low cost. Possible to determine burden of infection.	Overall low sensitivity (especially at low infection intensities). Need of qualified microscopist.
Molecular diagnostic techniques (qPCR, LAMP assay, conventional PCR)[312,196]	Amplification and identification of specific parasite sequences	Identification or quantification of DNA from roundworm or whipworm	85.7-100	100	100	100	Possible to detect multiple infections by multiplexed assays. High specificity.	Risk of low sensitivity due the presence of inhibitors in the fecal sample. Decreased sensitivity if formalin fixation of samples. Requires specialized equipment and has restricted used in the field.

Table 2: recommended treatment regimens and efficacy of anti-helminth drugs

Recommended treatment	Mechanism of action	*T. trichiuria* infections				*A. lumbricoides* infection			
		Individual patient management[a]	Preventive chemotherapy[b]	Cure rate[d]	Egg reduction rate[d]	Individual patient management[a]	Preventive chemotherapy[b]	Cure rate[d]	Egg reduction rate[d]
Albendazole	B-tubulin binding	once a day for three days	once	32.1	64.3	once	once	96.5	99.7
Mebendazole	B-tubulin binding	twice daily for three days	once	44.4	80.7	twice daily for three days or once (depending on the strength of the available formulation)	once	96.8	99.5
Levamisole	L-subtype nAChR agonist	NA	Once	23.4	41.8	NA	Once	93.0	97.0
Pyrantel pamoate	L-subtype nAChR agonist	NA	Once	28.5	62.3	Once	Once	97.5	91.7
Ivermectin	GABA-gated chloride and potassium channel agonist	Once daily for three days	(once)	32.1	78.9	once	once	97.3	>99.9
Albendazole-ivermectin	NA	NA	once	60.0	95.5	NA	once	96.7	99.9

Based on References a[313] and b [237]. Treatments in brackets indicate drugs that are not recommended for treatment but that have a (suboptimal) effect against the disease

NA not applicable, c: available for this indication in several countries (e.g. Cobantril®) but not listed in Reference a; d: after single dose administration, based on Reference [242].

FIGURE LEGENDS

Figure 1. Soil-transmitted helminth infections

Panel A shows the two major Phyla, the Nematoda and the Platyhelminthes within which the human multicellular endoparasites fall. A third Phyla also exists, the Acanthocephala however humans are very rarely infected, serving only, on rare occasions, as accidental hosts. The Trematoda and Cestoda are Classes of Platyhelminth, with the term Helminth an umbrella term covering the Nematoda and the Platyhelminths. Examples of genera found within each Phylum are included. The so-called Soil Transmitted Helminths are found within the Nematoda.

Panel B summarizes the main similarities and differences of Trichuris and Ascaris parasites

Figure 2: Prevalence of *Trichuris trichiura* and *Ascaris lumbricoides* infections in 2010. (A) *Trichuris trichiura* infection and **(B)** A*scaris lumbricoides* infection; based on geostatistical models for sub-Saharan Africa and available empirical information for all other regions. *T. trichiura* infections may also occur in populations in high-income countries living in conditions of poverty such as in aboriginal populations in Australia[314] or among migrants[7]. In the case of the latter, most infections are acquired elsewhere given the limited opportunities for transmission because of adequate hygiene and sanitation in most high income country settings. Adapted with permission from Pullan et al Parasit Vectors. 2014: 7, 37

Figure 3: Life cycles of Ascaris and Trichuris species.

Trichuris: infection with Trichuris is initiated by the oral ingestion of infective embryonated eggs. Eggs hatch in the large intestine after receiving signals to do so from bacteria. The first stage L1 larvae burrow in to epithelial cells lining the crypts and in this intracellular niche grow and moult through to the adult stage. Thus unlike Ascaris, Trichuris is an entirely enteric parasites and does not undergo any migratory phase. From the L3 onwards not all of the nematode body is found inside the gut epithelial cells, with the posterior end protruding in to the gut lumen. Sexually mature adult parasites are found in the large intestine, contrasting with Ascaris, and here they mate, and the females release unembryonated eggs which pass out with the faeces, becoming embryonated and thus infective after a period of time in the external environment.
Ascaris: after ingestion of embryonated eggs, eggs hatch and release L3 larvae, covered by the L2 cuticle. Although the site of egg hatching has been a topic of some discussion, the current evidence points to the larvae hatching in the large intestine. L3 larvae penetrate the caecal and proximal colon mucosa and undergo what is known as a hepato-trachael migration, a phenomenon that sets *Ascaris* apart from the other soil-transmitted helminths, including *Trichuris*. Larvae migrate via the portal blood vessels to the liver. In the liver, the L2 cuticle is shed and some larval growth occurs. Subsequently, larvae advance to the lungs, penetrate the alveolar spaces, move to the pharynx where they are coughed up and swallowed. On their return to the small intestine, the now L4 larvae undergo a final moult (L5) and develop to adulthood with sexually mature male and female worms within the lumen

of the small intestine. Adult worms produce unembryonated eggs that are shed in the faeces where they develop to infectivity under appropriate conditions of temperature and moisture.

Images of Trichuris eggs and adult stage parasite courtesy of Ruth Forman; images of Ascaris larvae and larvae in lung courtesy of Celia Holland; Ascaris larva in liver reproduced with permission from PLOS Neglected Diseases when this paper was published - Deslyper, G., Colgan, T., Cooper, A., Holland, C.V. and Carolan, J. (2016). A proteomic investigation of hepatic resistance to Ascaris in a murine model. PLOS Neglected Diseases 10(8): e0004837. and at http://www.bpod.mrc.ac.uk/archive/2016/9/13 Image by Dr Christina Dold and Professor Celia Holland; Ascaris egg courtesy of Gwendoline Deslyper

Figure 4: The anti-parasite effector mechanisms operating in the protective immune response to Ascaris and Trichuris

a) In mice resistant to Ascaris elimination of parasites from the gut involves the "weep and sweep" mechanism, embracing an increase in muscle contractility and fluid secretion[133]. Lung stage immunity lack mechanistic clarity, but likely involve Type 2 controlled effector mechanisms. Both neutrophils and eosinophils feature in the lung infiltrating cells. Even less is understood about liver stage immunity although reactive oxygen species have been implicated in the mechanism of resistance.

b) In strains of mice resistant to *T.* muris, the Type 2 cytokine IL-13 has been shown to increase the rate of epithelial turnover thus displacing the parasite from its niche[79]. Resistance to infection also correlates with and expansion of goblet cells. Through the use of mucin deficient mouse strains, muc 2 and muc5ac[75,76] have been shown to be important in resistance to *T. muris*, likely via direct interactions with the parasite in the gut. Changes to gut physiology, increased muscle contractility and fluid secretion are also thought to contribute to parasite expulsion

Although likely, it is not known if similar effector mechanisms also operate in man.

Figure 5: Clinical complications of trichuriasis and ascariasis

a) Colonoscopic image of Trichuris dysentry syndrome (TDS). Note petechial lesions and mucosal haemorrhages (taken from Khuroo et al (2010) Gastrointestinal endoscopy, 71 (1), 200-204)
b) Abdominal X-ray demonstrating "tramline" appearance caused by a heavy intestinal infestation by *Ascaris lumbricoides*. The duodenum is packed with worms, presenting as a tangled mass of black within the white of the contrast medium (reproduced from https://en.wikipedia.org/wiki/Ascariasis#/media/File:Ascaris_infection_in_X-ray_image-_Duedenal_worms_in_the_first_portion_of_the_bowel_after_the_stomach_(South_Africa)_(16238958958).jpg)
c) Small bowel obstruction by *Ascaris lumbricoides*. The image shows a piece of intestine, blocked by *Ascaris lumbricoides* which has been surgically removed from a 3-year-old boy in South Africa. Reproduced from SuSanA Secretariat https://www.flickr.com/photos/gtzecosan/16424898321/, CC BY 2.0, https://commons.wikimedia.org/w/index.php?curid=38219947

Figure 6: Outlook for development of novel drugs for STH infections.

(**a)** Chemogenomics approaches will help identify new candidate anthelmintic drugs targeting *Ascaris* spp. and *Trichuris* spp. Targets common to all soil transmitted helminths will be of particular interest. A greater understanding of the worm life cycle, host-parasite interactions and host immunity to infection **(b)** may assist in adding context to omics-based discoveries, and this too may highlight additional candidate targets as well as challenges in developing new therapies. **(c)** Advanced, automated phenotypic screening platforms will emerge. Images courtesy of James O'Sullivan and Hannah Smith.

Box 1: **How do Type 2 immune responses develop?**

Although several cell types (e.g. Innate Lymphoid Cells, B cells, macrophages) possess MHC II and so can present antigen to CD4+ T cells, their *in vivo* contribution in the context of murine trichuriasis is not fully defined. In contrast, the dendritic cell represents a potent antigen presenting cell known to play a key role in *Trichuris* infections in the mouse. Different subsets of dendritic cells (DCs) exist with the IRF4+ CD11c+ CD11b+ DC being the potent driver of Type 2 immunity post *Trichuris* infection and IRF8+ CD103+ DC associated with Type 1 immunity and thus chronic infection. Exactly how these subsets have compartmentalised roles is unclear but mechanisms are likely to embrace both cell intrinsic factors and external signals. For example, if the cellular phosphatase SHIP-1 is deleted specifically from DCs, *T.muris* expulsion is impaired. Further, different DC subsets may express different levels of cytokine receptors and so be educated differently towards a Type 2 promoting phenotype by the family of alarmin cytokines (IL-25, IL-33, TSLP). Indeed raising IL-25 or IL-33 levels in normally susceptible mice promotes resistance to *Trichuris*[315] and blocking TSLP signalling in normally resistant mice delays worm expulsion[316]. Other evidence implicating the dendritic cell as a key player in the development of Type 2 immunity comes from circadian studies. Here, the effect of time-of-day on the outcome of *Trichuris* infection was shown to be, at least in part, dependent upon the dendritic cell clock[317]. Thus, mice infected in the morning are more resistant to infection than mice infected at night. Transgenic mice created such that dendritic cells lack a core clock gene lose this time-of –day dependency in resistance to infection, with the mechanism hypothesised to be due to circadian regulation of levels of Type 1 promoting cytokines.

Box 2. **An Economic Perspective**

Potential cost-effectiveness of treating soil-transmitted helminths has been reviewed but relatively few studies have provided data for individual STH parasites[318]. In the case of ascariasis, such studies have indicated that with school-targeted control of high prevalence communities, that a DALY can be averted at a cost of US$8[319], that enhancing coverage is more cost-effective than increasing the frequency of treatments[320], and that MDA is more cost-effective in high-transmission areas with longer rather than shorter intervals between treatments[321]. Studies estimating productivity loss of working adults measured significant losses among STH-infected compared to uninfected agricultural workers, generally attributed to the effects of anaemia, although attribution to specific STH parasites is problematic[260].

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