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TOPICS EXPLORING LOA-LOA, ONCHOCERCIASIS, HOOKWORM, ASCARIS, TRICHURIS (A SANCHEZ AND R FUJIWARA, SECTION EDITORS)

Human Ascariasis: Diagnostics Update

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Abstract Soil-transmitted helminths (STHs) infect over one billion people worldwide. Ascariasis may mimic a number of conditions, and individual clinical diagnosis often requires a thorough work-up. Kato-Katz thick smears are the standard detection method for *Ascaris* and, despite low sensitivity, are often used for mapping and monitoring and evaluation of national control programmes. Although increased sampling (number of stools) and diagnostic (number of examinations per stool) efforts can improve sensitivity, Kato-Katz is less sensitive than other microscopy methods such as FLOTAC®. Antibody-based diagnostics may be a sensitive diagnostic tool; however, their usefulness is limited to assessing transmission in areas aiming for elimination. Molecular diagnostics are highly sensitive and specific, but high costs limit their use to individual diagnosis, drug - efficacy studies and identification of *Ascaris suum*. Increased investments in research on *Ascaris* and other STHs are urgently required for the development of diagnostic assays to support efforts to reduce human suffering caused by these infections.

Keywords Ascariasis · *Ascaris* · Diagnosis · Microscopy · Immunology · PCR

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Introduction

Soil-transmitted helminths (STHs) infect over 1.45 billion people worldwide, with an estimated 819 million individuals infected with *Ascaris lumbricoides*, 465 million with *Trichuris trichiura* and 439 million with hookworm (*Necator americanus* and/or *Ancylostoma duodenale*) [1]. Single- and multi-species infections cause human disease ranging from mild to severe and even fatal cases, as well as increased school absenteeism, although this might not be detectable at a community level [2]. Most of such neglected tropical diseases (NTDs) occur in areas with poor sanitation and hygiene; however, increased travel and migration have made STH infections more common also in non-endemic areas.

The World Health Assembly, together with endemic countries, donors and drug-donating pharmaceutical companies, have set ambitious goals for the control of STH-related morbidity by 2020, aiming to treat at least 75 % of school-age children and high-risk groups, with mass drug administration (MDA) of albendazole or mebendazole [3, 4]. Sensitive, specific, user-friendly and cost-effective diagnostic tests are imperative for individual diagnosis and for planning, monitoring and evaluation (M&E) of mass 'preventative chemotherapy' programmes, and novel tools are needed, especially for measuring decreased infection intensities and drug efficacy [3]. With the scale-up of national STH - control programmes, the associated scientific opportunities and known limitations of the currently recommended techniques, research on *Ascaris* diagnostics is needed more than ever.

We review the available literature for the diagnosis of *A. lumbricoides* infection and discuss the research and field trials that inform current and potential future diagnostic assays. We cover scenarios ranging from clinical settings to large-scale control programmes, and emphasise the need for integration of diagnosis of multi-species infections.

Methods

We searched the databases PubMed, Google Scholar, Web of Science and EMBASE for all publications on diagnostic techniques of *Ascaris* using combinations of *Ascaris*/Ascariasis/*A. lumbricoides*/soil-transmitted helminths/STH/helminth and diagnostics/diagnosis/sensitivity/specificity/Kato-Katz/FLOTAC/ethyl/midi/ether/antigen/immunology/immunoglobulins/LAMP/loop/polymerase chain reaction/PCR/FECPACK/2010/2011/2012/2013/2014/2015, and searched for individual publications by title and/or authors when necessary. Three hundred and sixty-eight papers were retained based on titles, 146 articles were read after screening of abstracts, and the final number of references was limited according to the publisher's guidelines.

Clinical Presentation

A. lumbricoides is a parasitic nematode that causes two main forms of pathology: immune-mediated reaction to migrating larvae and nutrient depletion and/or obstruction due to physical presence of adult worms in the gastrointestinal tract [5] (Fig. 1). Infection is often asymptomatic and may occur alongside other diseases. Ascariasis may present as a differential diagnosis to a wide range of conditions (Table 1).

Similar to a number of parasite infections, individual diagnosis of ascariasis often depends on a thorough investigation that may include travel history or origin from endemic countries (when presenting in non-endemic areas) and clinical and laboratory examinations, including potentially serological, molecular and image-based diagnostics. Recent findings suggest that ascariasis should be suspected in patients with relevant symptoms even without travel to *A. lumbricoides* endemic areas, as *Ascaris suum*, a species that commonly infects pigs, may also infect and cause pathology in humans [6].

Migrating *Ascaris* Larvae. Löffler syndrome, or eosinophilic pneumonitis, is an immune-mediated type I hypersensitivity reaction to larvae migrating through the pulmonary tissue and typically occurs in initial or intermittent infections [7]. Following an incubation period of 4 to 16 days, patients present with fever, cough and dyspnoea. Clinical findings may include urticaria or other rash, abnormal breath sounds by auscultation and tender hepatomegaly. The leukocyte differential count typically reveals eosinophilia, and the chest X-ray may show pulmonary infiltrates. Serology can aid the diagnosis, especially if egg excretion has not yet started, although cross-reactivity with other parasites is common. The syndrome may last up to 3 weeks and can ultimately be fatal. Rarely, *Ascaris* larvae migrate to ectopic sites, and associated eosinophilia may cause complications [8].

Adult *Ascaris* Worms Light infections are frequently asymptomatic, whereas heavy infections commonly lead to acute abdominal pain and ileus from conditions such as mechanical small bowel obstruction, volvulus and intussusception, especially in children [5, 9]. In endemic countries, intestinal ascariasis is also a common cause of hepatic, biliary and pancreatic disease, including acute pancreatitis and cholecystitis [10]. Ultrasonography, abdominal X-ray, computed tomography and magnetic resonance imaging scans may identify the cause [11–13]. Endoscopic retrograde cholangiopancreatography may be both diagnostic and therapeutic, and capsule endoscopy can be considered, even in individuals with negative conventional gastrointestinal endoscopy [14, 15].

In endemic countries, *Ascaris* infection is a common cause of malabsorption, and undernutrition and micronutrient deficiencies may lead to growth failure and cognitive impairment, as well as defective immune regulation and increased risk of other parasitic infections [16, 17].

Coprological Diagnosis

Quantifying the worm burden of *A. lumbricoides* in stool following treatment is time-consuming and cumbersome, and detection of eggs by light microscopy remains the mainstay for diagnosis. The various microscopy-based techniques are also commonly used for other intestinal parasites.

Kato-Katz thick smear [18] is currently the recommended method by the World Health Organization (WHO) for detection of STH infections in endemic areas [3]. For intensity of infection, measured as number of eggs per gram of stool (EPG) [3], Kato-Katz correlates well with worm burden [19]. Kato-Katz slides are relatively cheap and simple to prepare, produce few false positives and allow detection of several co-endemic intestinal parasite species [20•]. However, the high variance of EPG from repeated Kato-Katz sampling, with non-random egg distribution (within the same stool) and daily fluctuations in egg detection (from different stools from the same person, and potentially from mislabelled stools from different people), is an important limitation of this technique [19]. Due to the high variance, probably exacerbated by the small fixed volume of stool used (normally 41.7 mg), Kato-Katz has limited sensitivity at lower intensities of infection [21, 22•]. In addition, the number of eggs recorded in each smear is multiplied to calculate EPG, but the volume-to-weight ratio is affected by stool density, and actual weights vary considerably [23]. Finally, the diagnostic accuracy of Kato-Katz depends on sufficiently well-trained laboratory technicians.

Increased sampling from one to multiple slides from stools collected on consecutive days greatly improves the sensitivity of Kato-Katz, often resulting in higher prevalence estimates [24–26]. Three days of two Kato-Katz slides per stool is sufficient to reach ≤1 % false negative diagnostics for

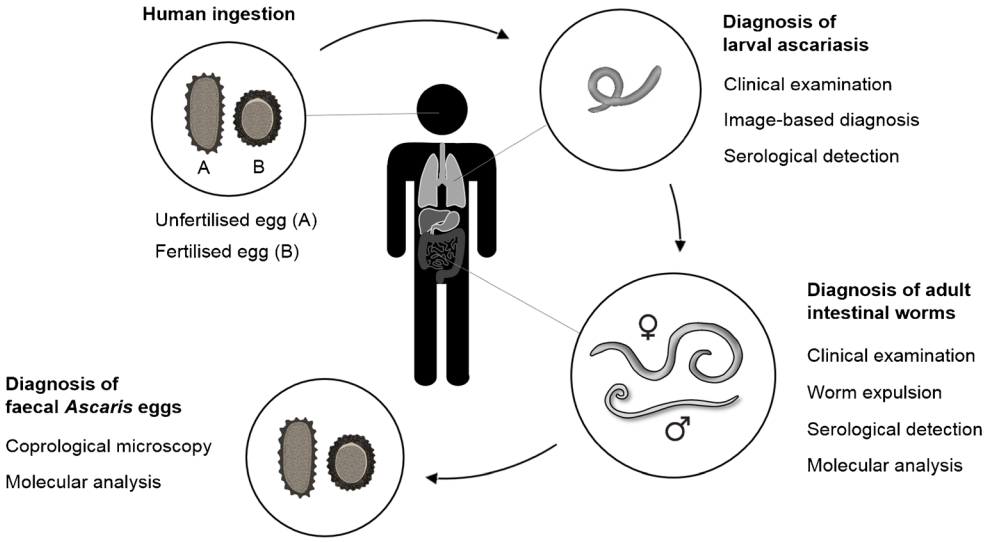

Fig. 1 *A. lumbricoides* life cycle and diagnostic markers of infection. After being swallowed, an *A. lumbricoides* larva hatches from the infective egg*, migrates into the vascular system and is transported through the portal veins and right side of the heart to the pulmonary circulation. Unable to cross the capillary network, the parasite penetrates the walls of the alveoli, migrates to the larynx and is swallowed, ending up as an adult worm in the small intestines. The female parasite lays tens of thousands of eggs daily that, through stool excretion, enter the environment and may infect other human hosts. The time from egg ingestion to larval migration takes 10 to 14 days, with egg production starting from 2 to 3 months. Adult worms can live in humans for 1–2 years [5]. *Only fertilised eggs may become infective

A. lumbricoides in a moderate prevalence setting, in comparison with up to 20 smears required for *T. trichiura* [24]. On the other hand, multiple smears do not always improve sensitivity, may bias results through age-related non-compliance [27] and require increased human and financial resources.

FLOTAC® is more sensitive than a single [21, 28] or multiple [22•, 29, 30] Kato-Katz slide/s, possibly due to the larger volume of processed stool (1 g). FLOTAC® could therefore be a useful tool for mapping and monitoring integrated control programmes and for surveillance in low-endemic areas. However, FLOTAC® requires a centrifuge, lacks 100 % sensitivity and often results in reduced egg counts [22•, 28, 29, 31].

The Mini-FLOTAC is a simpler test which does not require expensive equipment or an energy source and has been found to be at least as sensitive as Kato-Katz for determining STH infection intensities across a number of different settings [22•, 32–34]. The choice of flotation solution (FS) for both FLOTAC® and mini-FLOTAC affects species-specific diagnosis, with FS2 recommended for hookworm [32], FS7 for *Schistosoma mansoni* and *A. lumbricoides* [32, 33, 35, 36] and FS4 for all STHs [37].

Mini-FLOTAC has been found to be more expensive [34] yet quicker [33] than Kato-Katz in low-intensity infections following treatment. However, cost per detected case increases as prevalence decreases [34]. Although FLOTAC® is more expensive than Kato-Katz [37], a single FLOTAC® is cheaper, and more sensitive, than triplicate Kato-Katz slides [31], and may be the most suitable coprological technique for accurate prevalence diagnostics in the field.

The McMaster egg counting technique provides accurate estimates of EPG [21], is very easy to use [23] (1-day training) and provides the most reliable estimates of drug efficacies (see below) [38••]; however, it is not as sensitive as FLOTAC [21].

Other diagnostic techniques have shown promising results for *A. lumbricoides*, including the TF-Test® [39], Baermann-Moraes [39], Paratest [40], formalin-ethyl acetate sedimentation [41], sodium acetate formalin (SAF) [42], Hoffman-Pons-Janer [39, 40] and the spontaneous sedimentation in tube technique (SSTT) [43]. In contrast, other methods have shown less promise, such as the Midi Parasep® [41]. Further studies are needed to determine the diagnostic value of these tests for *Ascaris* and other intestinal parasite infections.

Diagnosis in Infants. Kato-Katz has low sensitivity for detection of *A. lumbricoides* in breastfed infants, who have more liquid stools and, if infected, lower EPGs than older children [44]. Modified Wisconsin floatation and simple gravity sedimentation are more sensitive for infants than Kato-Katz, formal-ethyl acetate sedimentation or modified formal-ethyl acetate sedimentation [44]. The gravity sedimentation method is labour-intensive but can distinguish fertilised from unfertilised *Ascaris* ova and is unaffected by diarrhoeal stool, unlike the Wisconsin method [44].

Drug Efficacy. Statistical simulations indicate that McMaster and Kato-Katz provide reliable estimates of drug efficacy and are suitable for M&E of control programmes [23]. FLOTAC® has also been shown to be more sensitive than Kato-Katz post-treatment for the detection of all three main STHs, particularly when performed on preserved samples [21]; however, this is

Table 1 Differential diagnoses to ascariasis morbidity in humans, grouped by larval and intestinal stages of infection

Findings	Differential diagnoses
Larval ascariasis[a]	
Urticarial, other rash	*Allergy*, *drug reactions*, *infections,* including other parasites, *environmental causes*, *dermatological conditions*
Tender hepatomegaly	*Infections*, including intestinal ascariasis (see below); other parasitic infections, including malaria, amoebiasis, echinococcosis; bacterial infections, including enteropathogenic bacterial abscesses, typhoid and paratyphoid fever; viral infections, including EBV, CMV, HIV and hepatitis; fungal infections *Tumours*, including metastatic or (less common) primary hepatocellular carcinoma; haemangioma; polycystic disease; lymphoma *Vascular causes*, including congestive heart failure; haemolytic disorders; Budd-Chiari syndrome *Toxicity*, including alcoholism and other toxic substances *Metabolic*, including congenital deficiencies such as haemochromatosis, glycogen storage disease; amyloidosis
Cough, dypnoea	*Pulmonary infections* and/or *inflammation*, including infections with other parasites, pneumonia, lung abscess, bronchiectasis, asthma, allergy, COPD, cystic fibrosis, sarcoidosis *Tumours*, including primary or metastatic neoplastic tumours *Vascular causes*, including congestive heart failure, pulmonary embolism, coronary artery syndrome, anaemia *Mechanical causes*, including pneumothorax
Eosinophilia	*Other parasitic infections*, *allergy*, *drug reactions*, rare *congenital* or *malignant diseases*
Increased IgE titres	*Other parasitic infections*, *allergy*, *drug reactions*, rare *congenital* or *malignant diseases*
Intestinal ascariasis[a]	
Acute abdominal pain	*Infection* and/or *inflammation*[b], including appendicitis, cholelithiasis/cystitis, pancreatitis, diverticulitis, peritonitis, pyelonephritis *Vascular causes*, including intestinal ischaemia, abdominal aortic aneurysm, sickle cell disease crisis *Other*, including acute adrenal insufficiency, ectopic pregnancy, ovarian torsion, endometriosis, physiological
Ileus	*Bowel obstruction* due to American trypanosomiasis (Chagas disease), constipation, adhesions, hernia, volvulus, intussusception, tumours, IBD, congenital disorders *Intestinal paralysis* due to post-surgical paralytic ileus, drugs, acute pancreatitis or systemic disease
Acute pancreatitis	*Acute pancreatitis*, other causes of acute abdominal pain (see above)
Acute cholecystitis	*Acute cholecystitis*,other causes of acute abdominal pain (see above)
Liver abscess, cholangitis	*Infections*, including other parasites, enteropathogenic bacteria and opportunistic infections associated with AIDS; *cholelithiasis*; *tumours*

EBV Epstein-Barr virus, *CMV* cytomegalovirus, *HIV* human immunodeficiency virus, *COPD* chronic obstructive pulmonary disease, *IBD* inflammatory bowel disease, *AIDS* acquired immune deficiency syndrome

[a] In individuals with a high exposure to infection, elements from both stages may co-exist

[b] Apart from appendicitis, conditions most commonly affect adults

in contrast to other studies where helminth egg recovery decreases with preservation time [31]. The sensitivity of Kato-Katz and McMaster decreases following anti-helminthic treatment, whereas FLOTAC® remains high [21].

Diagnosis Using Mobile Phone Technology. Mobile devices have been adapted for examination of Kato-Katz slides and can accurately diagnose helminth eggs in moderate- to high-intensity infections, with a sensitivity of 81 % for *A. lumbricoides*, but lower for other STHs [45]. It is probable that mobile, lens-free devices, in combination with digital image analysis, may improve stool-based point-of-care diagnosis, particularly with further technological and software development [46•, 47, 48].

Serological Diagnosis

Detection of antibodies or antigens could provide a simpler, more rapid diagnosis of *Ascaris* infection than conventional stool microscopy. Point-of-care tests are available for other NTDs such as lymphatic filariasis (LF) [49••] and schistosomiasis [50]; however, currently, no such tests exist for STHs.

Humoural Immune Response. *A. lumbricoides* generates an antibody production that varies with exposure and intensity of infection, particularly in high-endemic areas [51, 52]. Importantly, factors such as age, genetic predisposition, atopy, nutritional status and co-infections may affect the humoral response to *Ascaris* [17, 53, 54]. Total immunoglobulin (Ig) titres are associated with worm burden in individuals living in endemic areas [51]. In some studies, certain Ig isotypes, such as IgG4, have been found to be sensitive and specific markers of chronic *A. lumbricoides* infection, and to positively correlate with intensity of infection [55, 56]. These findings are consistent with other parasite infections [57], although others have found more variable results for *Ascaris* [52].

Cross-reactivity of anti-*Ascaris* antibodies with epitopes of other helminths is common [51], and standardisation of *Ascaris* antigens for research and diagnostic purposes is warranted, including recombinant antigens, *Ascaris*-associated allergens and antigens of other ascarid species [56, 58, 59].

Antibodies as Markers of Active Infection. Few studies have evaluated the use of serological diagnosis of *Ascaris* at the community level. In one study of individuals with high-intensity infections treated repeatedly over several months, anti-*Ascaris* IgG4 fell to levels equal to negative controls [60]. In another study, however, antibody titres did not correlate with worm - load expulsion after treatment [56].

Anti-*Ascaris* antibody titres have been associated with larval stage ascariasis in particular and may remain elevated for several months, even following treatment, especially in areas where re-infection is frequent [7, 60]. Anti-*Ascaris* antibodies are therefore generally not seen as suitable to detect active *Ascaris* infection and could overestimate the number of individuals in need of treatment in mass control programmes. A number of commercial diagnostic tests are available for detection of anti-*Ascaris lumbricoides* IgG and IgM; however, to our knowledge, most are based on somatic *A. lumbricoides* worm antigens and frequently cross-react with other helminths. Interestingly, saliva-based detection of IgG performed well in high-intensity *T. trichiura* infection, but not in *Ascaris* infection [61].

Antigen Detection. Whilst antibody detection could represent past infection or exposure, as well as current infections, antigen detection only represents current infections. We did not find any studies of detection of antigens in blood or other specimens for *A. lumbricoides* infection. Detection of schistosome antigen in urine is highly sensitive and is available as a commercialised point-of-care test [50, 62]. Similar tools for STHs may be limited by the location of the adult worms in the intestines, rather than in the blood vessels as is the case for schistosomes, and it is possible that coproantigen tests would be more sensitive than urine or blood tests for STHs.

Serological Diagnosis in Children. As control programmes potentially move towards elimination of STHs, antibodies may provide a good marker of infection in young children, especially in areas where children are frequently exposed to intestinal pathogens [63, 64].

Biomedical Markers. Few studies have identified biomedical target markers for *A. lumbricoides* infection. Fatty acid products of *A. lumbricoides* infection may be detected in urine by gas-liquid chromatography, and the levels correlate well with worm burden [65]; however, to our knowledge, no such tests are currently available as commercial products.

Molecular Diagnosis

Molecular diagnostic tools are highly sensitive and specific, and rapid advances are being made, resulting in reduced costs and improved techniques such as real-time quantitative PCR (qPCR) and multiplex assays.

The DNA extraction and amplification of the nuclear first internal transcribed spacer region (ITS1) from single *Ascaris* eggs have primarily been optimised for population genetic analyses [66]. These techniques, used on stool samples, could enable highly sensitive detection of *Ascaris*, particularly by amplification of DNA from single eggs. Methods to detect small amounts of ancient DNA, such as molecular paleoparasitological hybridization approach [67], may improve sensitivity for very low infections.

Multiplex PCR enables the detection of multiple parasite species in a single reaction and can simplify diagnostics by replacing several individual tests with one molecular test. High-throughput PCR assays have been developed, and a multiplex PCR showed promising results for *A. lumbricoides*, *T. trichiura* and *N. americanus* [68].

Unlike conventional PCR, which can only indicate presence of infection, qPCR enables quantification of amplicon (and associated infection intensity). High intensity reactions result in rapid amplification and early fluorescence. qPCR is more sensitive than Kato-Katz and the flotation technique (FS7) for detection of *A. lumbricoides* infections and co-infections [69, 70••, 71]. Multiplex qPCR assays have successfully detected *A. lumbricoides* infection alongside multiple intestinal parasites [69, 70••, 72], with over 90 % of children under 10 years of age harbouring two or more parasites [72]. These findings highlight the importance of multi-species diagnostic tests, even in young children, including common intestinal infections that are often neglected by control programmes. All primers had high sensitivity and specificity, and the quantified DNA correlated strongly with EPG [70••], indicating its potential for measuring parasite reduction following anti-helminthic treatment [70••].

Alternatively, amplicons for several STHs and protozoa can be hybridised to beads for probe-based detection on a Luminex platform providing a high-throughput diagnostic tool with less equipment required than for qPCR [73]. Further, reverse transcriptase PCR can identify specific stages of schistosomes [74] and could be useful for distinguishing new and treatment-resistant *Ascaris* infections.

The significantly higher sensitivity of qPCR over stool microscopy typical for a number species is not always observed for *Ascaris* due to high egg output and technical challenges related to isolating parasite DNA from the resistant, four-layered *Ascaris* egg shell [75]. This may limit the usefulness of future PCR methods such as USB DNA-chip technology [76] for field diagnosis of ascariasis. Also, specific multiplex assays are limited to the species targeted in the respective tests, and DNA from high-intensity infections will compete for dNTPs, thereby deterring detection of species of lower infection intensities. Finally, the price of molecular diagnostic techniques limits its use in endemic areas [69, 70••], and until equipment costs decrease, other diagnostics may remain more cost-effective.

Parallels to Diagnostic Tools for *Ascaris suum*

Recent studies suggest that *A. suum* may be a relatively common cause of infection in humans, also in areas non-endemic for *A. lumbricoides*, and tools for species-specific diagnosis are required on a larger scale than previously anticipated [6, 77–82]. The zoonotic potential of *Ascaris* spp. may be reinforced by drug resistance to anti-helminthic treatment in domestic pigs [83] and could change public health strategies [77]. Although adult *A. lumbricoides* and *A. suum* worms differ in structure [84], the absence of differences in egg morphology makes stool-based species diagnosis difficult.

Serological Diagnosis An *A. suum* antigen-based immunoblot assay was developed that successfully diagnosed human visceral larva migrans (VLM) syndrome assumed to be caused by *A. suum* [85]. An enzyme-linked immunosorbent assay (ELISA) using the *A. suum* haemoglobin antigen correlates well with EPG and worm load, is more sensitive than microscopy and has low cross-reactivity with *Trichuris suis* in experimentally infected pigs [86]. Although developed as a veterinary tool, it could be useful for rapid, multi-species diagnosis in human *Ascaris* infection [87•].

Molecular Diagnosis Although PCR may detect a single *Ascaris* egg, it does not appear to discriminate *A. lumbricoides* from *A. suum* [88]. Additional studies from sympatric populations using multi-locus genotype data are required to determine if cross-transmission is a global issue, and to determine what diagnostics are required. Detailed comparisons [89] of the published mitochondrial genome of *A. lumbricoides* [90] and *A. suum* [91] and the complete *A. suum* [92] and *A. lumbricoides* genome (Wellcome Trust Sanger Institute for the 50 Helminth Genomes Initiative) may reveal genes suitable for differentiating infections. However, the mitochondria vary by only 1.9 % [89] and differentiation may not be possible if they are in fact not two distinct species [93, 94].

Discussion

Despite global efforts to control STH-related morbidity, only approximately 30 % of children worldwide in need of treatment are currently receiving preventive chemotherapy [4]. Key factors for optimal planning, M&E and surveillance of control programmes include accurate diagnostic tools and optimal survey protocols with appropriate sample sizes, number of repeated measurements and timing. The choice of diagnostic technique and protocol will vary depending on the research question being addressed. To date, the development of diagnostic tests for ascariasis has been limited by largely insufficient investments and is further complicated by the fact that no true gold standard exists for comparison of tests. There is a need for tests which compare adult worm expulsion (for up to a week), repeated Kato-Katz and/or other tests that estimate EPG, and PCR, to standardise analyses of current and future diagnostic methods. Ideal relationships should be linear, with low variance. We have reviewed the published literature to identify currently available diagnostic tests that may support endemic countries to achieve global targets, and below we provide our recommendations for each of the components of such control programmes (summarised in Table 2).

Geographical Mapping of Disease Distribution

Currently Available Tests Mapping of disease for defining appropriate frequency of MDA is currently done through stool microscopy, most commonly Kato-Katz, with *A. lumbricoides* EPG categories of light (1–4999 EPG), moderate (5000–49,999 EPG) and heavy (>50,000 EPG) infections [3]. These thresholds need to be refined, and more research is required to determine the correlation between EPG calculated by FLOTAC, McMaster and Kato-Katz. Unlike some common NTDs, questionnaires are not a sensitive tool for identification of communities targeted for STH treatments [95].

Ideal Tools Although current stool-based tests may be sufficient to define mass treatment strategies, especially in moderate- to high-endemic areas, tests with higher sensitivity are needed as infection intensity is reduced [4]. Similar to rapid, point-of-care diagnostic tests developed for other infectious diseases [48, 96, 97], mapping for STH control programmes need more convenient, reliable and affordable

Table 2 Characteristics of the most common current and potential laboratory-based diagnostic techniques, and their use in national control programmes

	Strengths and limitations					Recommendations for use in control programmes				Integration with other NTDs	
	Spec	Sens	Field-based	Cost	Sample[a]	Mapping	M&E	Drug efficacy	Surveillance[b]	STHs	Common intestinal pathogens
Coprological											
Kato-Katz	✓✓✓	✓	✓✓	✓✓✓	F	✓✓	✓	✓		✓✓✓	
McMaster	✓✓✓	✓	✓✓✓	✓✓✓	F	✓✓	✓	✓		✓✓✓	
FLOTAC[c]	✓✓✓	✓✓	✓	✓✓	F	✓✓	✓✓	✓		✓✓✓	✓✓
Mini-FLOTAC[c]	✓✓✓	✓	✓✓	✓✓	F	✓				✓✓✓	✓✓
Serological											
Antibodies	✓✓	✓✓	✓	✓	B				✓✓✓	✓	✓✓
Antigens[d]	?	?	✓✓✓	✓✓	?[e]	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓
Molecular											
PCR	✓✓✓	✓✓✓	-	✓	F/B	✓	✓	✓	✓✓✓[f]	✓✓✓	✓✓✓
qPCR	✓✓✓	✓✓✓	-	✓	F/B		✓✓	✓✓✓	✓✓[f]	✓✓✓	✓✓✓

Spec specificity, *Sens* sensitivity, *M&E* monitoring and evaluation, *NTDs* neglected tropical diseases, *STHs* soil-transmitted helminths, *qPCR* quantitative PCR

[a] F=faeces, B=blood/serum

[b] Surveillance for elimination and/or recrudescence

[c] Choice of flotation solution affects diagnostic accuracy of different species, with FS2 recommended for hookworms, and FS7 for *A. lumbricoides* and *S. mansoni*. Duplicate FLOTAC® using two different flotation solutions is recommended in areas where multiple species co-exist

[d] No antigen tests are available; however, antigen detection has the potential for accurate, non-invasive and rapid diagnosis of active infection

[e] Future diagnostic tools based on non-invasive specimens such urine or oral fluid may be highly applicable for field use, as well as coproantigen tests

[f] Analysis of pooled samples in order to reduce costs

tools, including tests for detection of antigens, host immunological markers and/or parasite DNA, ideally in urine, blood or oral fluid [98–100]. However, due to the location of STHs in the intestines, it is possible that coproantigen tests will be more sensitive, although research to support this prediction is needed. Moreover, improved coordination of disease mapping, including specimens sampled for other NTD surveys, could strengthen cooperation between health and non-health sectors, as well as attract sustainable funding for control programmes [101, 102].

Monitoring and Evaluating Impact of Anti-helminthic Treatment

Currently Available Tests The impact of mass control programmes is currently evaluated through sentinel site surveys [3]. In some instances, evaluating impact through repeated mapping is conducted, although the value of comparing cross-sectional survey results, often with differing protocols and techniques, is debatable [101]. At present, stool-based microscopy, especially Kato-Katz, remains the main diagnostic test to evaluate impact of treatment, and outcomes include binary values of prevalence and cure rate (CR; recommended by WHO), and numeric values of EPG and egg reduction rates (ERRs). Although cost and ease of use have historically been more important than diagnostic sensitivity, especially for prevalence and CR, more sensitive tools may be needed as successful control programmes lead to reduced prevalence and intensity of infection.

Ideal Tools As infection intensity decreases, measuring disease transmission becomes increasingly important, and direct markers of infection, including antigens, will be required. As integrated control programmes develop, increased precision of diagnostic tests may improve the interpretation of the effect of complementing interventions, such as WASH [101]. As albendazole is used to treat both LF and STH infections, disease impact surveys may be coordinated [103], and collection of the same, conveniently sampled specimens would improve data validity and cost-effectiveness. Alternatively, techniques including a preservation stage, such as FLOTAC®, could be incorporated, and stools processed at a central location [36]. However, LF surveillance will probably scale down as the disease becomes eliminated ahead of STH programmes, and rapid on-site tools for STH diagnosis are highly required.

Although the limitations of currently available STH stool tests may be overcome by adjusted reporting metrics [38••], a convenient point-of-care test is needed for M&E of STH control programmes, including ascariasis. Ideally, the test would also detect other common tropical diseases, such as malaria, through a multi-array platform [104, 105]. Although novel NTD diagnostic tools are currently moving towards urine and blood specimens [50, 102], coproantigen tests, such as those available for other intestinal infections [106–108], may be the most sensitive diagnostic marker in *A. lumbricoides* infection.

In areas where elimination of STH may become a target for control programmes, antigen, antibody and/or multiplex qPCR assays may improve detection of disease. However, unlike microscopy, PCR results do not correlate with morbidity, unless infection intensity is accounted for [71], and PCR remains prohibitively expensive at this stage.

Measuring Drug Efficacy

Currently Available Tests Drug resistance is not routinely monitored by STH control programmes. Although rarely detected to date, resistance to benzimidazoles may arise from parasite selection pressure due to high frequency of mono-drug treatment [109]. Few studies have assessed the accuracy of available coprological methods for estimating drug efficacy, either for CR or even more rarely for ERR, and tools for measuring drug efficacy are commonly neglected [110]. ERR determined by Kato-Katz is currently recommended for measuring anti-helminthic drug efficacy; however, other stool techniques may be more sensitive, including pooled stool samples which may reduce volume-to-weight ratio confounders, as well as the costs [21, 23, 26, 33, 111•, 112••, 113].

Ideal Tools Despite high costs, the increased sensitivity and specificity of PCR and qPCR could help establish accurate baseline prevalence and determine measures of drug efficacy, although a better understanding of the correlation between EPG and worm burden with qPCR quantifications is required. We recommend that PCR methods [70••] are used in conjunction with Kato-Katz, and ideally an additional stool-based microscopy method, for accurate measurement of drug efficacy. Single-nucleotide polymorphisms, associated with drug resistance in veterinary nematodes, may be useful molecular markers to detect early resistance in human *A. lumbricoides* infections [109]; however, more studies are needed to clarify their phenotypic relevance. Finally, studies suggest that monitoring of drug resistance could be integrated with other NTD control programmes [102].

Surveillance of Disease Elimination and Recrudescence

Currently Available Tests In contrast to other NTDs, global targets for STH control programmes do not currently include elimination. Nevertheless, recent guidelines [49••] recommend the coordination of various NTD surveillance surveys, and reports have highlighted how the need for stool collection for STH diagnosis, as opposed to blood or urine, remains an important challenge [114]. In areas where elimination is relevant, antibody detection in young children may be an appropriate measure of active transmission, although identification of appropriate Ig isotypes is needed.

Ideal Tools Serology and PCR-based diagnostic tests are currently being developed for other NTDs with *Ascaris* primers or antibodies as add-ons. In order to ensure integrated diagnosis for all STH species, it is essential that research on antigen, antibody and molecular-based diagnostic tools for *Ascaris* is not left behind.

Conclusions

There is a paucity of data on novel, convenient diagnostics for ascariasis, even compared to other NTDs. Standardised protocols and validated diagnostics are required for assessing the epidemiological situation, burden of disease and drug efficacy. Based on an updated review of the literature, we have presented the currently available tools for clinical diagnosis and for field tests used in national control programmes. It is possible that *Ascaris* diagnostics will shift to more sensitive techniques, such as FLOTAC®, serological tests and qPCR, as areas of low-intensity infection become more common. As control programmes are scaled up, the shifting epidemiology of STH will need to be addressed, and quantitative rapid, point-of-care tests are required for successful control. Increased investments in research on *Ascaris* and other STHs is urgently needed for the development of simple and affordable diagnostic tools to support efforts to reduce human suffering caused by these infections.

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Compliance with Ethics Guidelines

Conflict of Interest Poppy H L Lamberton and Peter M Jourdan declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with animal subjects performed by any of the authors. Among cited articles where one of the authors of the current

report was an author, local Institutional Review Board approval was obtained and maintained in all procedures performed involving human participants.

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- •• Of major importance

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