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Review Article

Serodiagnosis and early detection of *Strongyloides stercoralis* infection

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Abstract Strongyloidiasis is a major neglected tropical disease with the potential of causing lifelong infection and mortality. One of the ways for effective control of this disease is developing improved diagnostics, particularly using serological approaches. A serological test can achieve high diagnostic sensitivity and specificity, has the potential for point-of-care translation, and can be used as a screening tool for early detection. More research is needed to find clinically important antibody biomarkers for early disease detection, mapping, and epidemiological surveillance. This article summarizes human strongyloidiasis and the available diagnostic tools for the disease, focusing on describing the current antibody assays for strongyloidiasis. Finally, prospects of developing a more effective serodiagnostic tool for strongyloidiasis are discussed.

Introduction

Strongyloidiasis is a human intestinal parasitosis caused by *Strongyloides stercoralis* and to a lesser extent by *Strongyloides fuelleborni, and S. f. kellyi*, with the first being the most pathogenic species.[1] While half of *S. stercoralis* infections progress asymptomatically, symptoms of gastroenteritis, urticaria and larva currens are commonly reported. Importantly, infected individuals may undergo persistent replication of the parasite or "autoinfection", which may result in long-lasting infection and death, particularly among patients with immunosuppression, comorbidities or malnutrition.[2,3] Persistent infection is reported to be a significant risk factor for stunting in

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children.[4] Strongyloidiasis is also significantly associated with solid organ transplantation (SOT), particularly renal transplantation.[5]

Epidemiological information on strongyloidiasis is relatively scarce and the disease is severely underestimated due to variability in the disease distribution across countries, limited number of studies and suboptimal diagnostic methods.[6] Approximately 370 million people are estimated to be infected worldwide, most commonly those residing in tropical and subtropical regions.[7] Brazil and Thailand have by far the most extensive studies on the prevalence of strongyloidiasis, with reported prevalence of 13% and 23.7%, respectively.[6] Strongyloidiasis is reportedly endemic in Okinawa, Japan with a prevalence rate of 5.2% amongst older persons.[8] Meanwhile in China, endemic infections were mostly found among farming communities with a reported prevalence of 11.7%.[9] In Australia, strongyloidiasis is hyperendemic among aboriginal communities with reported prevalence ranging from 35 to 60%,[10] however, a significant decrease in seropositivity from 21 to 5% was recently reported in an aboriginal community post-mass administration with ivermectin.[11] Data from North America and Europe are based on immigrants, refugees and travelers. For example, Canada reported a prevalence rate of 9—77% among immigrants and refugees, mostly from Southeast Asia, while Spain reported strongyloidiasis as the most common parasitic infection, detected in 17.2% of African migrants.[12,13]

Despite the global prevalence, disease burden and the high impact of strongyloidiasis alone or as a risk factor for other diseases of public health concern, major deficiencies exist in the current control programs, especially in terms of available diagnostic tools. While several diagnostic methods exist, none of them are ideal and tend to underdiagnose and/or misdiagnose the disease in endemic areas. Recent increase in global prevalence of *S. stercoralis* is attributed to poor personal hygiene and inadequate safe water sources in endemic settings,[14] increase in international travel, and rise in transplant procedures in tropical countries. Thus, there is a pressing need to improve diagnosis of strongyloidiasis, in particular, for use in areas with little infrastructure and skilled human resources. Furthermore, a tool that can be used for screening and early diagnosis is very important in detecting acute cases, especially when 50% of patients with strongyloidiasis are asymptomatic. The acute cases may require different case management strategies compared to individuals with a chronic or established infection. Diagnostic tests with robust sensitivity are needed for screening immunosuppressed patients prior to initiating any immunosuppressive therapy, including donors and recipients prior to SOT, as such measures could avoid patient mortality.[15] In this respect, serological testing is arguably the most suitable approach for diagnosing strongyloidiasis, and has the most potential for commercial translation, up-scale and making an impact on disease control.[16]

Patients with strongyloidiasis have been reported to secrete different isotypes of immunoglobulins that help the body to combat the parasite, i.e. immunoglobulin A (IgA), E (IgE), M (IgM), and G (IgG), and the subclasses of IgG antibody (i.e IgG1, IgG2, IgG3 and IgG4). However, current serological tests to diagnose strongyloidiasis primarily detect anti-*Strongyloides* IgG and to a lesser extent, anti-*Strongyloides* IgG4 from patient serum. These assays may differ by the antigens used for detection (crude lysate versus purified or recombinant protein), methodology such as enzyme-linked immunosorbent assay (ELISA), indirect fluorescent antibody test (IFAT), and luciferase immunoprecipitation system (LIPS), and whether the assay is commercially available or laboratory-based.[17] A critical limitation of current serological assays is their tendency to overestimate the prevalence of the disease due to cross-reactivity with other nematode infections such as filarial parasites, schistosomes, and *Ascaris lumbricoides*.[18] Additionally, the assays may not be detecting acute cases, as IgG and IgG4 antibodies mostly detect chronic and severe stages of the infection.[19]

Presently, there are few previous studies which highlighted the importance of identifying potential serological biomarkers for detection of acute cases.[20] More research should be performed into developing more accurate serological assays, especially for early case detection, disease mapping, and epidemiological surveillance. Hence, this article aims to describe the current antibody assays for strongyloidiasis and their potential towards developing a more effective and early detection of human strongyloidiasis.

Pathogenesis and disease manifestations

Transmission of strongyloidiasis is primarily through exposure to soil. Thus, due to the nature of work and behaviors that favor the dissemination of this soil-transmitted helminth, farmers, coal miners and children are prone to this disease.[14] The unique life cycle of *Strongyloides stercoralis* has been reviewed extensively elsewhere.[21] Briefly, *Strongyloides* infection begins when the parasite, present in stool, water or contaminated food or soil, penetrates human skin and enters the bloodstream. The parasite undergoes heart-lung migration until it finally reaches the intestine and matures into an adult female worm capable of producing eggs by parthenogenesis or is excreted in the feces. From there, the larvae continue the free-living life cycle in the environment or alternatively, the eggs that hatch in the intestine enters the parasitic life cycle, reinvading the same host causing sequential rounds of infection known as autoinfection. Auto infective generations may occur at low, well-regulated levels,[22] leading to persistent and lifelong infection in immunocompetent individuals. The most worrisome condition, however, could occur when the person becomes immunosuppressed, as this may lead to exacerbation of the autoinfection, followed by development of hyper infection and the dissemination of strongyloidiasis, with mortality rate as high as 87%.[5]

Infection by *S. stercoralis* can manifest as five clinical syndromes 1) acute infection combined with Loeffler's syndrome, 2) chronic infection of the intestine, 3) autoinfection without any symptom, 4) symptomatic autoinfection, and 5) hyper infection syndrome (HS) with dissemination (DS).[5] When the larvae penetrate human skin, acute local reaction occurs almost immediately at the site of larval entry lasting up to several weeks. Subsequent larval migration through the lungs raises pulmonary

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symptoms mimicking bronchitis such as cough and tracheal irritation. The gastrointestinal symptoms begin about two weeks post-infection manifesting as diarrhea, constipation, anorexia, and abdominal pain, and the larvae are detectable in the stool after 3 to 4 weeks. Filariform larvae may also penetrate the colonic mucosa or perianal skin (autoinfection), leading to chronic infection, in which almost 50% of individuals are infected asymptomatically, while others may have symptoms such as epigastric pain and tenderness, nausea, vomiting diarrhea, constipation, and weight loss. Pruritus and dermatologic symptoms such as urticaria and larva currens may be observed. The autoinfection in an immunosuppressed individual may result in HS, with more severe forms of the aforementioned symptoms and occasionally gastrointestinal bleeding. The pulmonary indicators of HS include asthma-like symptoms such as cough and wheezing, with diffuse bilateral infiltrates on the chest X-ray. Other symptoms such as pneumonia and pulmonary hemorrhage may also be observed. When the invasive filariform larvae spread to the sites outside their normal migration route, the disease reached the disseminated stage. DS affects other organs including liver, gallbladder, pancreas, kidneys, ovaries, diaphragm, skeletal muscle, mesenteric lymph nodes, heart and brain.[23]

Immunosuppressed patients including those with human T-cell lymphotrophic virus type I (HTLV-I) infection, hematologic malignancies, recipients of systemic corticosteroids (usually as part of organ transplant procedures) or allogeneic hematopoietic stem cell transplantation (HSCT) are among the populations most at-risk of developing the life-threatening HS and DS.[5,15] HTLV-1 is a human retrovirus associated with three major types of disease: i) neoplastic diseases (adult T-cell leukaemia/lymphoma, ATLL), ii) inflammatory syndromes (tropical spastic paraparesis/HTLV-1 associated myelopathy), and iii) opportunistic infections (including *S. stercoralis* hyperinfection).[24] It is hypothesized that the increased proportion of regulatory T cells (Treg), i.e. CD4+CD25 + FoxP3+ leads to the dissemination of *S. stercoralis* amongst this group of patients.[25] Of note, leukemia and lymphoma account for up to 90% of the cases of malignancy associated with severe strongyloidiasis, while more than one-half of cancer patients who had strongyloidiasis also had an underlying solid-organ malignancy.[26,27] Most *Strongyloides* infections in organ transplant recipients experience exacerbation of donor-derived infection or reactivation of chronic infection after the initiation of immunosuppressive therapy. However, the guidelines for screening prior to solid organ transplantation by the American Society for Transplantation only recommends screening recipients for *Strongyloides*.[28,29] Importantly, most strongyloidiasis diagnosis is only made post-transplantation, after complications or death occurs, and mild/early infections are often missed using existing screening tools. This highlights a clear gap in pre-transplantation screening of both recipient and donor.[30]

Diagnosis and associated issues

Standard diagnosis of strongyloidiasis relies on the demonstration of the parasite in feces, body fluids, or tissue samples. The diagnostic methods that have been developed include direct parasite detection methods such as fecal smear microscopy, concentration methods (Baermann's technique, formalin-ether concentration technique), culture methods (Harada-Mori filter paper, agar plate culture), polymerase chain reaction (PCR) and gastrointestinal aspirate or biopsy (especially in cases of HS). Serological assays, as indirect methods, are usually in the formats of ELISA and IFAT.[25] However, there is variability in detection rates across the different methods and problems with diagnostic sensitivity, specificity or availability.[11]

According to the United States Centers for Disease Prevention and Control (US CDC), serial microscopic examination of *S. stercoralis* larvae in faecal samples is the gold standard for strongyloidiasis diagnosis. However, up to seven stool examinations are required to reach a sensitivity of 100% due to low parasite load and intermittent larvae excretion.[3] Other challenges include the difficulty in visible detection of eggs in the feces, and simultaneous presence of both rhabditiform and filariform larvae (presumably signifying autoinfection or disseminated strongyloidiasis), which further impede the detection of acute infection. Arguably, these limitations make fecal microscopy a rather controversial gold standard.[24,31] The Baermann, Harada Mori filter and agar plate culture methods are much more sensitive than single stool-smears, but they are rarely standard procedures in clinical parasitological laboratories.[32,33] Although the agar plate method is laborious and time-consuming (requiring 2−3 days), the sensitivity of this method has been found to be 96%, that is, 4.4 times greater than the direct smear method.[33] Modifications to this method have significantly increased the sensitivity of the test, making it the most accurate diagnostic test for strongyloidiasis, and has been recommended as a second-tier laboratory test. Meanwhile, positive Gram and/or routine acid-fast staining of sputum samples were found to be diagnostic for pulmonary strongyloidiasis.[30] In patients with gastrointestinal symptoms, string test is used for sampling the duodenal aspirates, while endoscopic-pathology assessment is the main key to diagnosing strongyloidiasis following appearances of a broad range of endoscopic features such as edema, subepithelial haemorrhages, megaduodenum, thickened folds and mucosal erosions of the stomach.[34,35] Eosinophilia is an important clue to the presence of the strongyloidiasis among refugees and immigrants from regions where the parasites are endemic, but it contributes little to the diagnostic accuracy in returning travelers and patients who are immunocompromised or with severe strongyloidiasis, thus making it less suitable for use as first line diagnostic testing for strongyloidiasis.[36]

A more recent and popular development is diagnosis by detecting *S. stercoralis* nucleic acid through molecular methods, whether from stool or urine samples.[37,38] Molecular detection of *S. stercoralis* has demonstrated better sensitivity as compared to direct detection methods. However, in asymptomatic patients with low levels of larval output, it is very unlikely to achieve high sensitivity. The techniques include conventional polymerase chain reaction (PCR) and its other variants (i.e nested-PCR, real time PCR, RFLP-PCR, FRET-PCR, loop-mediated isothermal amplification (LAMP), multiplex-PCR). However, the high cost, and laboratory equipment/infrastructure as well as skilled

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personnel required reduces the application of molecular detection in low resource settings where strongyloidiasis is endemic. Nevertheless, all these diagnostic tools have difficulty in diagnosing acute cases, as direct detection-based methods require significant parasite burden to be sufficiently sensitive.

Serological diagnosis

Detection of the host antibodies produced against *Strongyloides* antigen forms the backbone of serodiagnosis of strongylodiasis. As they are also the most amenable to point-of-care translation, commonly in a lateral flow assay (LFA), serological detection is arguably the best screening method for detecting acute and early cases of strongylodiasis.[39] Serological detection requires the use of antigens with high sensitivity and specificity. For this purpose, various antigenic preparations have been produced from various components of *S. stercoralis*. Other heterologous species such as *Strongyloides ratti*, *Strongyloides venezuelensis* and *Strongyloides cebus*, have also been used as antigens but these result in assays with highly variable diagnostic sensitivity and specificity.[40,41] The sensitivity and specificity of various reported serological tests ranged from 56 to 100% and 29—100%, respectively, depending on the method, antigen, antibody-isotype, cut-off, study population, and reference method used.

Assays using crude and recombinant antigens for serodiagnosis of strongylodiasis have been reported in many studies. Commonly used commercial ELISA kits that employ somatic antigens are Bordier-ELISA (Bordier Affinity Products SA, Switzerland) and *Strongyloide*s-ELISA (Scimedx Corporation, USA). The diagnostic performance of these commercial assays have been reviewed elsewhere, with sensitivity and specificity ranging between 67.5-98.3% and 87.3—100.00%, respectively.[42] Meanwhile *Strongyloides* IgM/IgG ELISA (NovaTec Immunodiagnostica, Germany) uses a chimeric recombinant antigen, however there is still no publication on this kit. Sensitive and specific serologic tests using recombinant antigens have been reported by research laboratories. Two main *S. stercoralis* recombinant antigens that have been used in ELISA and LIPS platforms are 32 kD NIE and SsIR. In addition a newly reported *S. stercoralis* recombinant protein named as rSs1a has been used in an IgG4-ELISA.[43,44] Other methods under investigation include a newer iteration of the conventional ELISA based on fluorescent bead assay such as the Luminex.[45] These tests, however, require the use of readers for result measurements, which may limit their translation into point-of-care tools in resource-limited settings. At present, there is no commercialized rapid LFA for strongyloidiasis.

Antigen preparation constitutes only one of the key components in developing a reliable serological assay. Variations in the specificity and sensitivity of serological tests are also dependent on the antibodies and conjugates used in the assays. Currently, the majority of serological assays developed for strongyloidiasis are based on detection of anti-*Strongyloides* IgG antibody in ELISA and its other variants (i.e. LIPS and Luminex) including dipstick assay, with a smaller focus on detection of anti-*Strongyloides* IgG4 antibody.[43,44,46] Thus far, none of the tests developed specifically detects other antibody isotypes or has been specifically evaluated for their potential for detection of acute/early infection. In this regard, the limited understanding of the seroimmunological profile in strongyloidiasis, as well as limited access and availability of samples from acute infection are some of the problems faced. Variability of individual responses to parasitic helminths and the possibility of cross-reactions between helminthic antigens are factors that must also be addressed. A serological diagnostic test with a combination of two (or more) clinically important biomarkers and/or the use of different antibody-isotypes may be a practical approach worth investigating for improved serodiagnosis of acute and chronic strongylodiasis.

Immunoglobulin responses and their potential role for early serodiagnosis

Although serological assays have the best potential for point-of-care translation, development of a reliable and sensitive serologic tool poses many challenges, chiefly from cross-reactivity with nematodes such as filariasis[47,48] difficulty in detecting early/mild infections, and discriminating acute from chronic infections. Previously developed ELISA-based tests focused on detection of anti-*Strongyloides* IgG and IgG4 antibodies, which typically indicate more established and chronic infections. IgG is the most abundant circulating antibody produced against *S. stercoralis,* seen with 95% of patients with the disease.[49] It has been detected after the sixth week of infection and remains elevated during chronic infection.[50] The IgG level was found to be higher in people with asymptomatic or mild symptomatic infections, but lower in patients with severe strongyloidiasis and co-infected patients with HTLV-1.[51,52] Therefore, for many years, detection of circulating IgG antibody has been central to serological diagnosis of strongyloidiasis.

Amongst the subclasses of IgG, IgG1 is reportedly upregulated early in infection, and prominently observed in younger rather than older individuals.[53] Meanwhile, specific IgG2 level was found to be significantly elevated, with higher levels in immunocompetent individuals than in immunosuppressed patients.[50] Detection of IgG3 is less informative as it has a shorter life span, with a half-life of only seven to nine days, thus it is unlikely to be suitable for use in clinical applications.[54] IgG4 represents the least abundant of the four IgG subclasses in healthy human serum, accounting for 3—6% of the total IgG.[55] IgG4 in particular has been extensively researched because it is highly specific for the detection of helminth infections, including strongyloidiasis. An increased specificity of 13.3% was observed when IgG was substituted with IgG4 as the secondary antibody in a *Strongyloides* ELISA test.[47] While it is noted to play a prominent role in chronically infected individuals, several reports have indicated that levels of IgG4 was higher in non-cured treated strongyloidiasis patients, prompting the hypothesis that the increased elevation of *S. stercoralis*-specific IgG4 antibody is associated with resistance to treatment.[56] Therefore, treatment efficacy in patients with higher *S. stercoralis*-specific IgG4 antibody titers may be a challenge. When the IgG4 level is

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high, conversely the elevation of IgE level was found to be suppressed, suggesting that the IgG4 acts as a modulator in the IgE-mediated immune response by blocking the IgE-basophil system. Such a situation was also seen in a group of patients following treatment of allergic rhinitis with specific hyposensitization, implicating the role of specific IgG4 as blocking antibodies in allergic reactions.[57]

While there are limited studies on the use of serological follow-up for monitoring seroreversion in patients after treatment, however, in non-endemic areas, the commercial IgG-ELISA and NIE-ELISA were found to demonstrate the most significant decline in monitoring the seroreversion.[58] It is not yet clear as to which immunoglobulin isotypes works best to evaluate the treatment efficacy, although some studies have shown clear tendency of decline in antibody titer based on IgG-assays.[58] It has been suggested that for monitoring of cure in patients with low serological responses, such as those who are immunosuppressed or receiving rituximab, serology should be mandatory and be used in combination with a faecal test. A cure can be determined by negativitization of faecal test plus negativization or consistent reduction in the titer of a serology test.[20]

Notably, very few researchers had focused on detecting other biomarkers (i.e IgM, IgA, and IgE antibody isotypes) that may indicate different stages of infection. Secretion of IgM usually indicates acute infection, as it has been recovered at one-week post-immunization in mice[59] and has been shown to act in concert with eosinophils in killing of filariform larvae in the BALB/cByJ mice model.[60] However, the results on IgM in the serum samples of *S. stercoralis* infected patients have been less consistent, thus there are limited studies on this isotype.[49] The IgA antibody isotype controls larva excretion, and it can be used for the recognition of immunodominant antigenic components in the absence of detectable *S. stercoralis* larvae in stool.[53] While studies have shown limited detection of antigen-specific IgA in serum of patients with strongyloidiasis, recent studies suggest that it is more readily detectable in saliva.[61] However, salivary samples are typically less accessible for further investigation as they are more challenging to obtain and store compared to blood samples.

In helminthic infections, antigen-specific IgE antibodies constitute approximately 10% of the total IgE in serum.[62] While IgE is commonly associated with allergic reactions, this isotype is also found to be significantly present in asymptomatic individuals harboring parasitic infection and has been shown to be detectable in immunocompetent patients with strongyloidiasis rather than in disseminated and immunosuppressed patients.[63] Similar to IgA, the elevation of IgE has also been found to be significant in copronegative individuals, implicating the role of IgE in infection intensity and larval output.[53] It has reported that the level of IgE had increased in 90% of patients during the course of acute infection and declined in cases of chronic infection and coinfection with HTLV-1.[49,64] This was also seen during acute infection of toxoplasmosis, implying a potential role for IgE in improving early detection of helminthic infections.[65] Additionally, the IgE levels are found to be down-regulated with increasing duration of the infection, as it is seen in only 10% of the ex-Far East prisoners of war with strongyloidiasis.[66]

The IgE antibody is also found to be a significant constituent of host-protective immune response against the helminthic parasites that are endemic in most of the world population. There are two types of IgE responses to helminthic infection; one is host defensive response to produce IgE that are specific to parasitic antigen and the second response is the host production of Th2/IL-4 dependent polyclonal formation of IgE that increases the total serum IgE level.[67] The relationship between IgE antibody and helminthic infection are rooted along two hypotheses; first, IgE antibody can mediate the cytotoxic activity of eosinophils along with the local reaction in the gut for intestinal parasite, lending credence to the idea that the primary function of the allergic response is part of the protective mechanisms against the parasite. Second, IgE secreting B cells are abundant in skin, gut and lungs, which are the main sites of parasitic invasion.[68] The anti-helminthic response not only stimulates the production of IgE antibodies, it can also non-specifically induce polyclonal IgE production, which increases the total serum IgE levels. Such polyclonal stimulation can reduce specific IgE antibody responses.[67] The polyclonal IgE may be the helminths defense mechanisms against anti-parasitic IgE.[69] However, although helminthic parasites may be the most effective inducers of IgE, the capacity of common environmental allergen to stimulate IgE responses and to produce allergic symptoms may overshadow any existing anti-helminthic IgE responses.[63]

In summary (Table 1), while IgG and IgG4 are found to be up-regulated in chronic and long-standing infection, IgE and

Table 1 The level of secretion of each immunoglobulin in strongyloidiasis.

Immunoglobulin	Level of secretion	
	High	Low
IgG	Asymptomatic and mild symptomatic patient[46] Remain elevated in chronic patient[45]	Severe strongyloidiasis and co-infected patients with HTLV-1[46,47]
IgG1	Early infection in younger patients[48]	Long standing infection[48]
IgG2	Immunocompetent individuals[45]	Immunosuppressed patients[45]
IgG4	Non-cured strongyloidiasis patients[52] Remain elevated in chronic patient[48,52]	
IgA	Copronegative individuals[48]	
IgE	Asymptomatic and immunocompetent individuals[58] Copronegative individuals[48] Acute infection[59]	Chronic disseminated, immunosuppressed and co-infected patients[48,58,59] Patients with long standing infection[49]

IgG1, IgG2 and IgG3 are excluded due to lack of information.

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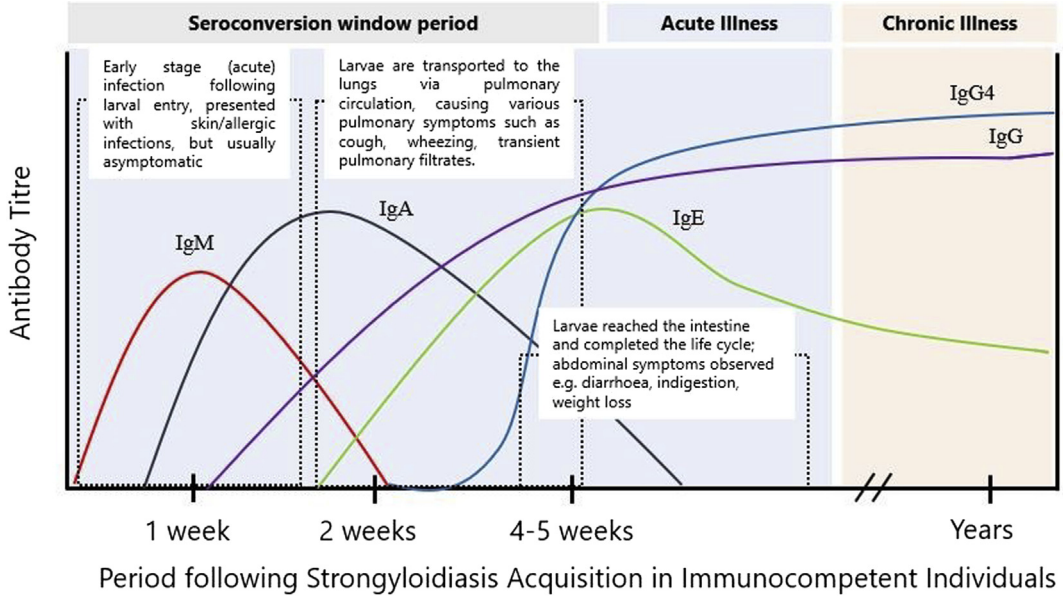

Figure 1. Hypothetical figure on the titers of different immunoglobulin isotypes during the course of infection with strongyloidiasis in immunocompetent individuals[49,50,56,59]

IgG1 are more prominent in early infection, implying that these antibodies have potential to be used as biomarkers for early diagnosis. A hypothetical figure on the titers of different immunoglobulin isotypes during course of infection are further illustrated in Fig. 1, based on findings from the literature.[49,50,56,59]

Conclusion

Recent advances in diagnosis have led to the development of new diagnostic tools for strongyloidiasis. While numerous intervention tools have been introduced, none have met the ideal criteria of early detection, point-of-care test, reliability and cost-effectiveness. The paucity of information/discoveries on acute biomarkers for strongyloidiasis is of concern in view of the need for early case management, especially in patients who are immunocompromised or immunosuppressed (due to co-infections or transplantation procedures). Currently available serologic tests also rely on imperfect gold standards that only detect chronic and long-standing infection, making it unfeasible for early case detection in most-at-risk patients of whom the diagnosis and treatment is of utmost priority to avoid fatal consequences of strongyloidiasis.

In terms of detecting acute cases, there are a limited number of clinically relevant biomarkers identified, and none have reached the stage of commercialization. Based on a summary of available literature, we propose further investigation into the potential of antigen-specific IgE antibodies as indicators for detection of acute infection especially in cases with asymptomatic/mild symptomatic infection, skin and allergic reactions. The combination of IgE with IgG4, may improve diagnostic sensitivity and specificity of the assays, and separately yet simultaneously detect acute and chronic cases. Different antigens should likely be coupled with each antibody isotype, since different antigens probably induce divergent antibodies profiles during infection. Ideally, multiplexing of acute and chronic biomarkers in LFAs may help to advance the existing diagnostic tools for improved serodiagnosis of human strongyloidiasis at the point-of-care.

Author contributions

NA and KMH wrote the manuscript with additional input from HA and RN. RN is the principal investigator of this project. All authors contributed to the final version of the manuscript.

Conflicts of interest

RN and NA are named as inventors in a patent titled "*Strongyloides stercoralis* protein and/or corresponding DNA and RNA sequences for application in diagnosis" filed in Malaysia (PI 2015002836) and at PCT [PCT/MY 2016/050053].

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References

1. Ashford RW, Barnish G, Viney ME. *Strongyloides fuelleborni kellyi*: infection and disease in Papua New Guinea. *Parasitol Today* 1992;**8**(9):314—8.

2. Becker SL, Sieto B, Silue KD, Adjossan L, Kone S, Hatz C, et al. Diagnosis, clinical features, and self-reported morbidity of *Strongyloides stercoralis* and hookworm infection in a co-endemic setting. *PLoS Neglected Trop Dis* 2011;**5**(8):e1292.
3. Khieu V, Srey S, Schar F, Muth S, Marti H, Odermatt P. *Strongyloides stercoralis* is a cause of abdominal pain, diarrhea and urticaria in rural Cambodia. *BMC Res Notes* 2013;**6**:200.
4. Forrer A, Khieu V, Schar F, Hattendorf J, Marti H, Neumayr A, et al. *Strongyloides stercoralis* is associated with significant morbidity in rural Cambodia, including stunting in children. *PLoS Neglected Trop Dis* 2017;**11**(10), e0005685.
5. Marcos LA, Terashima A, Dupont HL, Gotuzzo E. *Strongyloides* hyperinfection syndrome: an emerging global infectious disease. *Trans R Soc Trop Med Hyg* 2008;**102**(4):314–8.
6. Schar F, Trostdorf U, Giardina F, Khieu V, Muth S, Marti H, et al. *Strongyloides stercoralis*: global distribution and risk factors. *PLoS Neglected Trop Dis* 2013;**7**(7):e2288.
7. Beknazarova M, Whiley H, Judd JA, Shield J, Page W, Miller A, et al. Argument for inclusion of strongyloidiasis in the Australian national notifiable disease list. *Trop Med Infect Dis* 2018;**3**(2):1–11.
8. Tanaka T, Hirata T, Parrott G, Higashiarakawa M, Kinjo T, Kinjo T, et al. Relationship among *Strongyloides stercoralis* infection, human T-Cell lymphotropic virus type 1 infection, and cancer: a 24-year cohort in patient study in Okinawa, Japan. *Am J Trop Med Hyg* 2016;**94**(2):365–70.
9. Steinmann P, Zhou XN, Du ZW, Jiang JY, Wang LB, Wang XZ, et al. Occurrence of *Strongyloides stercoralis* in Yunnan Province, China, and comparison of diagnostic methods. *PLoS Neglected Trop Dis* 2007;**1**(1):e75.
10. Johnston FH, Morris PS, Speare R, McCarthy J, Currie B, Ewald D, et al. Strongyloidiasis: a review of the evidence for Australian practitioners. *Aust J Rural Health* 2005;**13**(4):247–54.
11. Kearns TM, Currie BJ, Cheng AC, McCarthy J, Carapetis JR, Holt DC, et al. Strongyloides seroprevalence before and after an ivermectin mass drug administration in a remote Australian Aboriginal community. *PLoS Neglected Trop Dis* 2017;**11**(5), e0005607.
12. Thompson C, Boggild AK. Strongyloidiasis in immigrants and refugees in Canada. *CMAJ* 2015;**187**(18):1389.
13. Salas-Coronas J, Cabezas-Fernandez MT, Lozano-Serrano AB, Soriano-Perez MJ, Vazquez-Villegas J, Cuenca-Gomez JA. Newly arrived African migrants to Spain: epidemiology and burden of disease. *Am J Trop Med Hyg* 2018;**98**(1):319–25.
14. Puthiyakunnon S, Boddu S, Li Y, Zhou X, Wang C, Li J, et al. Strongyloidiasis–an insight into its global prevalence and management. *PLoS Neglected Trop Dis* 2014;**8**(8):e3018.
15. Marcos LA, Terashima A, Canales M, Gotuzzo E. Update on strongyloidiasis in the immunocompromised host. *Curr Infect Dis Rep* 2011;**13**(1):35–46.
16. Mohd Hanafiah K, Garcia M, Anderson D. Point-of-care testing and the control of infectious diseases. *Biomark Med* 2013;**7**(3):333–47.
17. Anderson NW, Klein DM, Dornink SM, Jespersen DJ, Kubofcik J, Nutman TB, et al. Comparison of three immunoassays for detection of antibodies to *Strongyloides stercoralis*. *Clin Vaccine Immunol* 2014;**21**(5):732–6.
18. Koosha S, Fesharaki M, Rokni MB. Comparison of enzyme-linked immunosorbent assay and indirect immunofluorescence assay in the diagnosis of human strongyloidiasis. *Indian J Gastroenterol* 2004;**23**(6):214–6.
19. Rodrigues RM, de Oliveira MC, Sopelete MC, Silva DA, Campos DM, Taketomi EA, et al. IgG1, IgG4, and IgE antibody responses in human strongyloidiasis by ELISA using *Strongyloides ratti* saline extract as heterologous antigen. *Parasitol Res* 2007;**101**(5):1209–14.
20. Buonfrate D, Formenti F, Perandin F, Bisoffi Z. Novel approaches to the diagnosis of *Strongyloides stercoralis* infection. *Clin Microbiol Infect* 2015;**21**(6):543–52.
21. Page W, Judd JA, Bradbury RS. The unique life cycle of *Strongyloides stercoralis* and implications for public health action. *Trop Med Infect Dis* 2018;**3**(2):53.
22. Lok JB. *Strongyloides stercoralis* and relatives: recent advances in general and molecular biology. *Curr Trop Med Rep* 2014;**1**(4):194–206.
23. Keiser PB, Nutman TB. *Strongyloides stercoralis* in the immunocompromised population. *Clin Microbiol Rev* 2004;**17**(1):208–17.
24. Verdonck K, González E, Van Dooren S, Vandamme AM, Vanham G, Gotuzzo E. Human T-lymphotropic virus 1: recent knowledge about an ancient infection. *Lancet Infect Dis* 2007;**7**(4):266–81.
25. Montes M, Sawhney C, Barros N. *Strongyloides stercoralis*: there but not seen. *Curr Opin Infect Dis* 2010;**23**(5):500–4.
26. Schaffel R, Nucci M, Carvalho E, Braga M, Almeida L, Portugal R, et al. The value of an immunoenzymatic test (enzyme-linked immunosorbent assay) for the diagnosis of strongyloidiasis in patients immunosuppressed by hematologic malignancies. *Am J Trop Med Hyg* 2001;**65**(4):346–50.
27. Safdar A, Malathum K, Rodriguez SJ, Husni R, Rolston KVI. Strongyloidiasis in patients at a comprehensive cancer center in the United States. *Cancer* 2004;**100**(7):1531–6.
28. Anjum H, Le M, Pasko J, Ravin KA. Transmission of *Strongyloides stercoralis* through transplantation of solid organs. *MMWR Morb Mortal Wkly Rep* 2013;**62**(14). 264-262, https://www.cdc.gov/mmwr/preview/mmwrhtml/mm6214a2.htm.
29. Fischer SA, Lu K. Screening of donor and recipient in solid organ transplantation. *Am J Transplant* 2013;**13**:9–21.
30. Chokkalingam Mani B, Mathur M, Clauss H, Alvarez R, Hamad E, Toyoda Y, et al. *Strongyloides stercoralis* and organ transplantation. *Case Rep Transplant* 2013, 549038.
31. Paula FM, Malta FM, Corral MA, Marques PD, Gottardi M, Meisel DM, et al. Diagnosis of *Strongyloides stercoralis* infection in immunocompromised patients by serological and molecular methods. *Rev Inst Med Trop Sao Paulo* 2016;**58**:63.
32. Martin-Rabadan P, Munoz P, Palomo J, Bouza E. Strongyloidiasis: the Harada-Mori test revisited. Clinical microbiology and infection : the official publication of the. *Clin Microbiol Infect* 1999;**5**(6):374–6.
33. De Kaminsky RG. Evaluation of three methods for laboratory diagnosis of *Strongyloides stercoralis* infection. *J Parasitol* 1993;**79**(2):277–80.
34. Liu HC, Hsu JY, Chang KM. *Strongyloides stercoralis* hyperinfection presenting with symptoms mimicking acute exacerbation of chronic obstructive pulmonary disease. *J Chin Med Assoc* 2009;**72**(8):442–5.
35. Thompson BF, Fry LaC, Wells CD, Olmos MN, Lee DH, Lazenby AJ, et al. The spectrum of GI strongyloidiasis: an endoscopic-pathologic study. *Gastrointest Endosc* 2004;**59**(7):906–10.
36. Nair D. Screening for Strongyloides infection among the institutionalized mentally disabled. *J Am Board Fam Pract* 2001;**14**(1):51–3.
37. Dacal E, Saugar JM, Soler T, Azcarate JM, Jimenez MS, Merino FJ, et al. Parasitological versus molecular diagnosis of strongyloidiasis in serial stool samples: how many? *J Helminthol* 2018;**92**(1):12–6.
38. Lodh N, Caro R, Sofer S, Scott A, Krolewiecki A, Shiff C. Diagnosis of *Strongyloides stercoralis*: detection of parasite-derived DNA in urine. *Acta Trop* 2016;**163**:9–13.
39. Dong MD, Karsenti N, Lau R, Ralevski F, Cheema K, Burton L, et al. Strongyloidiasis in Ontario: performance of diagnostic

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tests over a 14-month period. *Travel Med Infect Dis* 2016; **14**(6):625—9.
40. Fernández-Soto P, Sánchez-Hernández A, Gandasegui J, Santos CB, López-Abán J, Saugar JM. Strong-LAMP: a LAMP Assay for Strongyloides spp. detection in stool and urine Samples. Towards the diagnosis of human strongyloidiasis starting from a rodent model. *PLoS Neglected Trop Dis* 2016;**10**(7), e0004836.
41. Corral MA, Paula FM, Gottardi M, Meisel DM, Castilho VL, Goncalves EM, et al. Immunodiagnosis of human strongyloidiasis: use of six different antigenic fractions from *Strongyloides venezuelensis* parasitic females. *Rev Inst Med Trop Sao Paulo* 2015;**57**(5):427—30.
42. Bisoffi Z, Buonfrate D, Sequi M, Mejia R, Cimino RO, Krolewiecki AJ, et al. Diagnostic accuracy of five serologic tests for *Strongyloides stercoralis* infection. *PLoS Neglected Trop Dis* 2014;**8**(1):e2640.
43. Ramanathan R, Burbelo PD, Groot S, Iadarola MJ, Neva FA, Nutman TB. A luciferase immunoprecipitation systems assay enhances the sensitivity and specificity of diagnosis of *Strongyloides stercoralis* infection. *J Infect Dis* 2008;**198**(3):444—51.
44. Arifin N, Yunus MH, Nolan TJ, Lok JB, Noordin R. Identification and preliminary evaluation of a novel recombinant protein for serodiagnosis of strongyloidiasis. *Am J Trop Med Hyg* 2018; **98**(4):1165—70.
45. Rascoe LN, Price C, Shin SH, McAuliffe I, Priest JW, Handali S. Development of Ss-NIE-1 recombinant antigen based assays for immunodiagnosis of strongyloidiasis. *PLoS Neglected Trop Dis* 2015;**9**(4), e0003694.
46. Van Doorn HR, Koelewijn R, Hofwegen H, Gilis H, Wetsteyn JC, Wismans PJ, et al. Use of enzyme-linked immunosorbent assay and dipstick assay for detection of Strongyloides stercoralis infection in humans. *J Clin Microbiol* 2007;**45**(2):438—42.
47. Norsyahida A, Riazi M, Sadjjadi S, Muhammad Hafiznur Y, Low H, Zeehaida M, et al. Laboratory detection of strongyloidiasis: IgG-, IgG4-and IgE-ELISAs and cross-reactivity with lymphatic filariasis. *Parasite Immunol* 2013;**35**(5—6):174—9.
48. Conway DJ, Atkins NS, Lillywhite JE, Bailey J, Robinson RD, Lindo JF, et al. Immunodiagnosis of *Strongyloides stercoralis* infection: a method for increasing the specificity of the indirect ELISA. *Trans R Soc Trop Med Hyg* 1993;**87**(2):173—6.
49. Lindo JF, Lee MG. *Strongyloides stercoralis* and *S. fulleborni*. In: Gillespie SH, Pearson RD, editors. *Principles and practice of clinical parasitology*. Chichester: John Wiley & Sons Ltd; 2001. p. 485—90.
50. Genta RM, Lillibridge JP. Prominence of IgG4 antibodies in the human responses to *Strongyloides stercoralis* infection. *J Infect Dis* 1989;**160**(4):692—9.
51. Iriemenam NC, Sanyaolu AO, Oyibo WA, Fagbenro-Beyioku AF. *Strongyloides stercoralis* and the immune response. *Parasitol Int* 2010;**59**(1):9—14.
52. Porto AF, Neva FA, Bittencourt H, Lisboa W, Thompson R, Alcantara L, et al. HTLV-1 decreases Th2 type of immune response in patients with strongyloidiasis. *Parasite Immunol* 2001;**23**(9):503—7.
53. Atkins NS, Conway D, Lindo J, Bailey J, Bundy D. L3 antigen-specific antibody isotype responses in human strongyloidiasis: correlations with larval output. *Parasite Immunol* 1999;**21**(10): 517.
54. Gibaldi M. Antibodies and derivatives. In: Ho RJY, editor. *Biotechnology and biopharmaceuticals*. New Jersey: Wiley-Blackwell; 2013. p. 139—210.
55. Ebbo M, Grados A, Bernit E, Vely F, Boucraut J, Harle JR, et al. Pathologies associated with serum IgG4 elevation. *Int J Rheumatol* 2012;**2012**, 602809.
56. Satoh M, Toma H, Sato Y, Kikuchi M, Takara M, Shiroma Y, et al. Production of a high level of specific IgG4 antibody associated with resistance to albendazole treatment in HLA-DRB1*0901-positive patients with strongyloidiasis. *Am J Trop Med Hyg* 1999;**61**(4):668—71.
57. Panzani R, Ariano R, Augeri G. Monitoring of specific IgG4 antibodies in respiratory allergy due to the pollen of *Parietaria judaica*. Evidence for a protective role. *Allergol Immunopathol* 1996;**24**(6):263—8.
58. Buonfrate D, Sequi M, Mejia R, Cimino RO, Krolewiecki AJ, Albonico M, et al. Accuracy of five serologic tests for the follow up of *Strongyloides stercoralis* infection. *PLoS Neglected Trop Dis* 2015;**9**(2), e0003491.
59. Bonne-Année S, Hess JA, Abraham D. Innate and adaptive immunity to the nematode *Strongyloides stercoralis* in a mouse model. *Immunol Res* 2011;**51**(2—3):205—14.
60. Abraham D, Rotman HL, Haberstroh HF, Yutanawiboonchai W, Brigandi RA, Leon O, et al. *Strongyloides stercoralis*: protective immunity to third-stage larvae inBALB/cByJ mice. *Exp Parasitol* 1995;**80**(2):297—307.
61. Bosqui LR, Goncalves AL, Goncalves-Pires Mdo R, Custodio LA, de Menezes MC, Murad VA, et al. Detection of parasite-specific IgG and IgA in paired serum and saliva samples for diagnosis of human strongyloidiasis in northern Parana state, Brazil. *Acta Trop* 2015;**150**:190—5.
62. Jarrett EE, Miller HR. Production and activities of IgE in helminth infection. *Prog Allergy* 1982;**31**:178—233.
63. Vercelli D, De Monte L, Monticelli S, Di Bartolo C, Agresti A. To E or not to E? Can an IL-4-induced B cell choose between IgE and IgG4? *Int Arch Allergy Immunol* 1998;**116**(1):1—4.
64. Hirata T, Uchima N, Kishimoto K, Zaha O, Kinjo N, Hokama A, et al. Impairment of host immune response against *Strongyloides stercoralis* by human T cell lymphotropic virus type 1 infection. *Am J Trop Med Hyg* 2006;**74**(2):246—9.
65. Matowicka-Karna J, Kemona H. IgE antibodies in toxoplasmosis. *Adv Hyg Exp Med* 2014;**68**:597—602.
66. Atkins NS, Lindo JF, Lee MG, Conway DJ, Bailey JW, Robinson RD, et al. Humoral responses in human strongyloidiasis: correlations with infection chronicity. *Trans R Soc Trop Med Hyg* 1997;**91**(5):609—13.
67. Lynch NR, Hagel IA, Palenque ME, Di Prisco MC, Escudero JE, Corao LA, et al. Relationship between helminthic infection and IgE response in atopic and nonatopic children in a tropical environment. *J Allergy Clin Immunol* 1998;**101**(2):217—21.
68. Sutton BJ, Gould HJ. The human IgE network. *Nature* 1993; **366**(6454):421—8.
69. Hagel I, Lynch NR, Perez M, Di Prisco MC, Lopez R, Rojas E. Modulation of the allergic reactivity of slum children by helminthic infection. *Parasite Immunol* 1993;**15**(6):311—5.

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