

# *Strongyloides stercoralis*: current perspectives

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**Abstract:** *Strongyloides stercoralis* is an intestinal nematode parasite with a global distribution. Most infected individuals have few or no symptoms. Strongyloidiasis is of primary medical importance, and fatal disease can occur in infected people who become immunosuppressed/immunocompromised through the administration of steroids or because of coinfection with human T-lymphotropic virus I. Often, misdiagnoses of strongyloidiasis in patients leads to expensive, nonspecific, invasive diagnostic techniques, including endoscopy, barium swallow, cancer biopsies, chest X-rays, and computerized tomography (CT) scans. Delayed treatment for strongyloidiasis brings in medical complications, such as vomiting, diarrhea, anemia, weight loss, pulmonary abnormalities, and septicemia. Chronic infection is difficult to diagnose by standard stool examination; hence, a reliable recombinant antigen-based serodiagnosis is important. Though albendazole and thiabendazole reduce the burden of the disease, they are not effective in an immunocompromised host. Ivermectin has the advantage of eradicating the disease, even in an immunocompromised host, with fewer side effects compared to albendazole. However, drug treatment is a temporary solution since reinfection can often occur. Thus, developing effective vaccine candidate antigens is imperative to stop the disease.

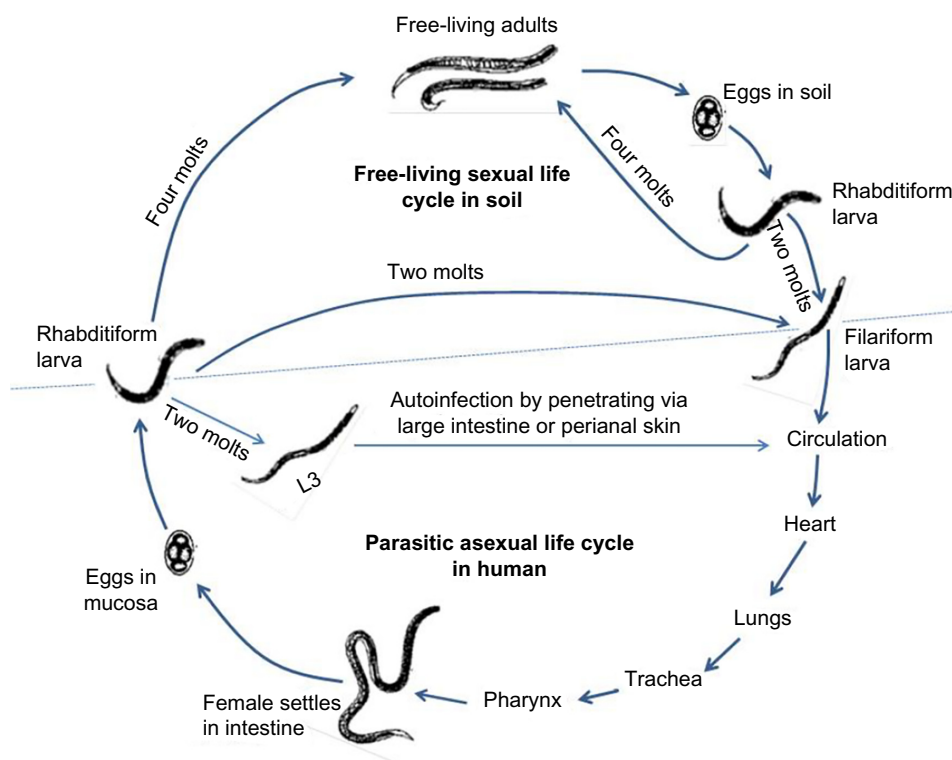
**Keywords:** strongyloidiasis, hyperautoinfection, albendazole, thiabendazole septicemia, immunocompromised host, HTLV-1, ivermectin, gastrointestinal

## Introduction

Strongyloidiasis, a human intestinal parasitic infection, is caused by *Strongyloides stercoralis* and *S. fuelleborni*. *S. stercoralis* accounts for >90% of infections and is endemic in Africa, South America, and Southeast Asia. *S. fuelleborni* is primarily found in nonhuman primates, but human infections have also been reported from Central Africa<sup>1</sup> and Papua New Guinea.<sup>2</sup> Strongyloidiasis infection is endemic in areas with poor hygiene, unorganized and ineffective health care facilities, and unequal socio-economic conditions. Other major causes for the spread of infection are high humidity, open defecation, walking barefoot, or playing in such an environment. However, the occurrences of *S. stercoralis* were reported even from temperate and subtropical regions, such as southern part of USA, Europe,<sup>1,3</sup> and Okinawa in Japan.<sup>1</sup> Several case reports from developed countries suggest that strongyloidiasis is an emerging disease in even nonendemic regions due to *S. stercoralis*-infected people emigrating from endemic to developed countries and travelers and veterans returning after serving in the endemic countries.

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**Figure 1** The *Strongyloides* life cycle is complex with its alternation between free-living and parasitic cycles.

**Notes:** Free-living cycle: the rhabditiform larvae passed in the stool can become either infective filariform larvae (direct development) or free-living adult males and females that mate and produce eggs from which rhabditiform larvae hatch and eventually become infective filariform larvae. The filariform larvae penetrate the human host skin to initiate the parasitic cycle. Parasitic cycle: filariform larvae in contaminated soil penetrate the human skin and by various, often random, routes migrate into the small intestine. Historically, it was believed that the L3 larvae migrate via the bloodstream to the lungs, trachea, and pharynx where they are eventually coughed up and swallowed. However, there is also evidence that L3 larvae can migrate directly to the intestine via connective tissues. In the small intestine, they molt twice and become adult female worms. The females live threaded in the epithelium of the small intestine and parthenogenetically produce eggs, which yield rhabditiform larvae. The rhabditiform larvae can either be passed in the stool for free-living cycle or develop to filariform larvae within the large intestine and can cause autoinfection.

**Abbreviation:** L3, third larval stage.

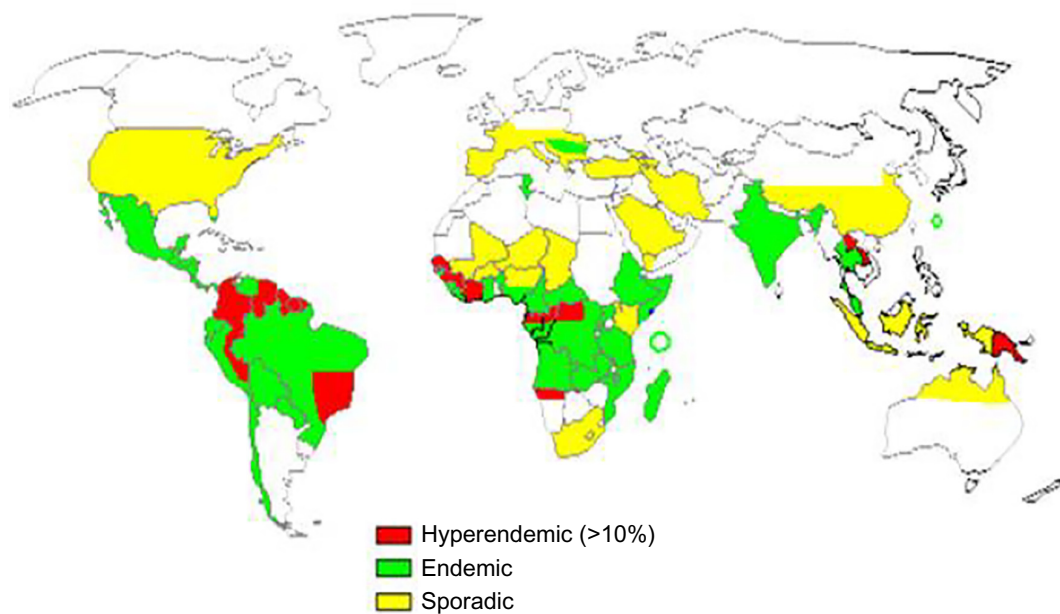
## Life cycle

Figure 1 shows two life cycles of *S. stercoralis* depending on the environment: a free-living, nonparasitic sexual life cycle and parasitic asexual life cycle. The free-living male and female worms mate and the female releases the rhabditiform (first-stage larva [L1]) larvae in the moist soil. These rhabditiform larvae molt four times to become young male and female worms for the next cycle of free-living sexual reproduction, whereas some rhabditiform larvae molt twice to become infective third-stage larvae (L3) (filariform), which can enter the human host to lead a parasitic asexual life cycle. The infected filariform larvae pass through the blood, transmigrate into the alveoli of lungs, trachea, pharynx, esophagus, and stomach, and finally reach into the submucosal layer of duodenum where they molt twice to become adult female worms.<sup>4-6</sup> Female adult worms that burrow into the submucosa of the small bowel asexually produce eggs. The eggs hatch into rhabditiform larvae (L1), which are released into the lumen of the intestine and are excreted in stool. However, in some cases, delayed defecation or constipation can induce

the L1 larvae to molt to L2 and then to infective filariform larvae (L3). The infective filariform larvae gain access to the bloodstream by penetrating the colonic or rectal mucosa or perianal region. This particular process of infection is called autoinfection and is responsible for the perpetuation of the parasite even after a long period after original infection. However, in immunosuppressed individuals, this process of autoinfection is enhanced multiple times that leads to larval dissemination of severe strongyloidiasis. Therefore, early diagnosis helps treatment with antihelminthic drugs for this potentially fatal but eminently curable disease.

## Epidemiology and clinical symptoms

Figure 2 shows the worldwide distribution of strongyloidiasis. It is commonly distributed in tropical and subtropical countries, highly endemic in Southeast Asia, Africa, South America, and has a frequent occurrence even in Europe and USA.<sup>7</sup> Based on fecal tests, clinical presentations, and duodenal lavage biopsy tests, it is estimated that globally 30–100 million people are infected. However, the estimated



**Figure 2** Global distribution of *Strongyloides stercoralis*.

**Notes:** Sporadic reports of prevalence are shown in yellow. Endemic countries are shown in green. Hyperendemic regions are shown in red. Reused with permission from the World Gastroenterology Organisation from WGO Practice Guideline: Management of Strongyloidiasis, October 2004.

global burden of strongyloidiasis is arbitrary due to the lack of accurate diagnostic methods. Insensitive and laborious fecal tests underestimate the disease prevalence, whereas a sensitive enzyme-linked immunosorbent assay (ELISA) method using crude *S. stercoralis* somatic antigen overestimates the disease prevalence due to cross-reactivity with other nematode parasite infections in *S. stercoralis*-endemic areas. Accurate statistics of global distribution of the parasite remains uncertain until a gold standard diagnostic method is available.

In the immunocompetent human host, mild persistent internal autoinfections can occur for many years or life-long without eliciting obvious clinical symptoms. More than 50% of patients infected with the parasite report no symptoms.<sup>8,9</sup> Minor clinical manifestation due to acute infection is rarely diagnosed. Acute infection may result in chronic disease with various clinical symptoms mostly associated with gastrointestinal tract and in some cases include pulmonary manifestation. Immune suppression due to steroid therapies in tissue transplantation cases or cancer therapies leads to chronic hyperautoinfection and disseminated strongyloidiasis. Disseminated autoinfection transports intestinal fauna into the bloodstream along with the penetrating parasites across the intestinal mucosal layer that could cause septic shock.

*S. stercoralis* infection in nonendemic area goes unnoticed or often misdiagnosed since this infection is unexpected by

the physicians. Without knowing the underlying strongyloidiasis infection, patients often undergo various complicated clinical procedures and nonspecific chemotherapies.<sup>8,10</sup>

## Strongyloidiasis in children

Children growing in poor hygienic and unsanitary conditions are at high risk for *S. stercoralis* infection. However, >50% of infected children report no symptoms. Severe cases of *S. stercoralis* infection in children present clinical ailments, such as malnourishment due to malabsorption, intermittent diarrhea, vomiting, poor immunity, fragile body structure, fatigue, growth failure and weight loss, abdominal distention and tenderness, abdominal bloating and discomfort, upper abdominal pain, eosinophilia, steatorrhea, and protein loss.<sup>11</sup> Paralytic ileus may cause frequent constipation due to acute strongyloidiasis in children. A more recent study in Darwin, Australia, found that children with *S. stercoralis* were more likely to be hypokalemic and wasted than children with diarrhea caused by other pathogens.<sup>12</sup> Gastrointestinal symptoms were present in 72% of these cases and half of the children were younger than 5 years.<sup>13</sup> Refugees' children (n=163 patients) were admitted between 2008 and 2009 to Pediatric Internal Health Clinic, State University of New York Hospital, Stony Brook, USA. The median patient age of children was 8 years with a range of 8 months to 18 years who arrived from Southeast Asia (59%) or Africa (27%) and Middle East (14%). Overall, 24% of these patients were seropositive for *Strongyloides*, 8%

were positive for schistosomes, and 19% had absolute eosinophilic count  $>400/\mu\text{L}$ .<sup>14</sup> *S. stercoralis* infections were prevalent in 27% of children in Doomadgee and Gununa, Australia, after thiabendazole treatment fell  $<7\%$ .<sup>15</sup>

## Pulmonary strongyloidiasis

*S. stercoralis* infections are often mild and associated with few or no symptoms. However, during the lung migratory phase, filariform larvae may induce inflammatory reactions, such as pneumonitis, bronchopneumonia, and pulmonary hemorrhage, including fever,<sup>16</sup> productive cough, wheezes, dyspnea hemoptysis, and bronchospasm.<sup>17–19</sup> Pulmonary symptoms, including dyspnea, cough, and sputum discharge, were recorded in 15.7% of *S. stercoralis*-infected cases.<sup>20</sup> People with chronic bronchitis, emphysema, pulmonary fibrosis, asthma,<sup>21,22</sup> and immunosuppression have a higher risk of pulmonary strongyloidiasis (PS).<sup>23–25</sup> Chronic pulmonary abnormalities such as mucous plugging of bronchi, bullae, pulmonary fibrosis, and any preexisting pulmonary-associated infection may delay the transmigration of filariform larvae through alveoli and trachea, which allows infective larvae to mature well in the lung itself.<sup>18</sup> A matured adult female lays embryonated eggs that hatch into rhabditiform larvae. The repetition of the entire life cycle within the lungs can then lead to severe PS. Widespread dissemination of *S. stercoralis* throughout the thorax may occur, resulting in pulmonary hemorrhage with hemoptysis, pleuritis with hemorrhagic pleural effusion, severe bronchopneumonia,<sup>21,26</sup> hoarseness,<sup>27</sup> chest pain,<sup>28</sup> dyspnea,<sup>29</sup> and respiratory failure.<sup>25</sup> Clinical diagnosis of PS is often delayed because of nonspecificity of signs and symptoms. Treatment courses are often misdirected toward asthma, allergy, and chest X-ray and in some cases diagnosed as lung cancer. Inappropriate therapy with a corticosteroid may then lead to worsening of the patient's condition. It is necessary to educate physicians to investigate strongyloidiasis as a probable cause based on the endemic region or travel history of patients with pulmonary and gastrointestinal symptoms and with peripheral blood eosinophilia. If strongyloidiasis is suspected, examination of sputum, stool, and duodenal aspirates or parasite-specific ELISA<sup>30</sup> is warranted for diagnosing strongyloidiasis in patients with steroid-dependent unexplained lung disease or lung shadows of miliary nodulation or diffused interstitial reticulation in chest X-rays or computerized tomography (CT) scans.<sup>18,24,26,31</sup>

## Strongyloidiasis in HIV/AIDS

Patients with HIV infection or AIDS are susceptible to strongyloidiasis coinfection since humoral and CD4-

mediated immunities are severely compromised. Patients with advanced HIV-positive and *S. stercoralis* coinfection failed to respond to the standard course of thiabendazole or ivermectin treatment and died due to disseminated infection.<sup>32,33</sup> HIV and *S. stercoralis* coinfection were strongly associated with other bacterial infections, especially those transferred from intestines, and caused *Escherichia coli* meningitis. However, the incidence of strongyloidiasis is low in the AIDS pandemic/strongyloidiasis-endemic areas, and it was shown that the frequency of strongyloidiasis is not significant between HIV-infected and normal population. In Lusaka, Zambia, only 6% of patients with HIV infection ( $n=63$ ) were *S. stercoralis* positive by stool examination.<sup>34</sup> Dually infected patients show almost similar clinical complications to those of *S. stercoralis*-infected immunocompetent patients. Recent evidence suggested that even with severe depletion of CD4 cells, patients with AIDS control dissemination of *S. stercoralis*. Since HIV infection predominantly impairs Th1 response and not Th2, the unaffected Th2 response effectively controls the parasite by eliciting parasite-specific IgE antibody production and eosinophil response.<sup>35</sup>

## Strongyloidiasis and human T-lymphotropic virus

Human T-lymphotropic virus I (HTLV-1) is endemic in Africa, Japan, the Caribbean, and South America. HTLV-1 is a retrovirus that primarily affects CD4 T-lymphocytes, alters gene expression, and increases T-cell proliferation. Most HTLV infections have long latent period and are asymptomatic. HTLV-1 is primarily associated with two diseases, adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis. The prevalence rate of *Strongyloides* infection was significantly higher in HTLV-1 carriers (31.6%) than in those without HTLV-1 (6.3%) infection in Okinawa, Japan.<sup>36,37</sup> The high prevalence of strongyloidiasis infection in HTLV-1-infected individuals is attributed to immunosuppression. Approximately 39% of patients with *S. stercoralis* infection stimulated the oligoclonal expansion of HTLV-1-infected lymphocytes in HTLV-1 asymptomatic carriers, suggesting the shortened period of latency in ATL patients with strongyloidiasis.<sup>38</sup> *S. stercoralis*-induced HTLV-specific oligoclonal expansion is partly mediated by the IL-2/IL-2R upregulation caused by the viral transactivator Tax protein. Thus, strongyloidiasis is frequently associated with lymphoid malignancies, including ATL.<sup>39</sup> Significantly elevated provirus load positively correlated with the population of CD4<sup>+</sup>25<sup>+</sup> T-cells in dually infected carriers.<sup>38</sup> In addition, *S. stercoralis* antigen significantly induced IL-2 production by peripheral



blood mononuclear cells (PBMC) and activated IL-2 promoter that helps Tax-immortalized T-cells in vitro.<sup>39</sup>

High mortality rate of ~36% was observed in hyperauto-infective strongyloidiasis in HTLV-1 carriers.<sup>40</sup> Furthermore, there is a decrease in the efficacy of treatment in patients coinfecting with HTLV-1 and *S. stercoralis*.<sup>5</sup> The cure rate of *S. stercoralis* was lower in HTLV-I carriers<sup>41,42</sup> due to decreased IgE ( $P<0.05$ ) and increased IgG4 ( $P<0.05$ ) observed in the noncured group, especially in HTLV-I carriers.<sup>43</sup> It has been reported that despite several courses of thiabendazole therapy, *S. stercoralis* reappeared in stool samples, resulting in the recommendation of a double dose of ivermectin (200 µg/kg) in patients positive for anti-HTLV-1 antibody.<sup>44</sup> Hyperinfective strongyloidiasis may also be a clinical marker of HTLV-1 infection in areas where both entities are endemic or in immigrants from such areas (Figure 2). *Strongyloides* hyperinfection syndrome in HTLV-1 carriers was due to selective impairment of parasite-specific IgG as well as IgE antibody production.<sup>36,45</sup> HTLV-1 coinfection with *S. stercoralis* decreased the production of IL-4, IL-5, IL-13, and IgE antibodies. These cytokines are very important for humoral as well as cellular immunities against helminths. Eosinophils and IgE antibody levels were low even after treatment with ivermectin in patients with *S. stercoralis* and HTLV coinfections,<sup>37</sup> suggesting that HTLV-1 dysregulated parasite-specific immunity. It was reported that HTLV-1 and *S. stercoralis* coinfection increased regulatory T-cells (CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup>) that downregulate parasite-specific IL-5 and eosinophil production, as a selective impairment of immunity against parasites.<sup>46,47</sup>

## Drug treatment

The goal of drug treatment is to eliminate both the adult worms and larvae in order to eradicate the infection, reduce the morbidity, and prevent complications. It is recommended to treat all the patients who harbor *Strongyloides*, even if they are asymptomatic, because of the risk of hyperinfection. *Strongyloides* hyperinfection syndrome, usually precipitated by immune suppression, should be considered in patients who reside in the endemic regions or traveled from endemic regions to other nonendemic countries. Attempts at the detection and eradication of this infection are recommended to prevent this potentially fatal complication. However, for infected pregnant patients, clinicians may prefer to defer treatment for strongyloidiasis until after the first trimester. *Strongyloides* species are the hardest worms to eradicate and to ensure this, posttherapy stool examinations are also recommended to verify *Strongyloides* eradication and to

exclude other parasitic infections. Empirical corticosteroid administration for treating wheezing could be problematic, because it may cause life-threatening hyperinfection.

Benzimidazoles (thiabendazole, mebendazole, and albendazole) are anthelmintic agents that disrupt energy production in the parasites by inhibition of beta-tubulin polymerase, which causes disruption of cytoplasmic microtubule formation. Ivermectin inhibits neurotransmission in nematodes by stimulating the release of gamma-aminobutyric acid-dependent neurotransmission. Ivermectin and thiabendazole have shown to be superior to albendazole, and ivermectin is becoming the drug of choice in many countries due to its more favorable side effects compared to benzimidazoles. A standard dose of 200 µg/kg ivermectin for 2 days is recommended. However, patients with hyperinfection and disseminated disease should be administered ivermectin daily until symptoms have resolved and larvae have not been detected for at least 2 weeks. A newer drug, tribendimidine, remains under investigation in the People's Republic of China and shows some promise in the treatment of strongyloidiasis.<sup>48</sup>

The efficacy and safety of thiabendazole and ivermectin were evaluated, with thiabendazole at 25 mg/kg/12 h for 3 consecutive days and ivermectin at 200 µg/kg as single dose and another group with the same dose of ivermectin for 2 consecutive days. It was found that the criteria for cure were met with 78% treated with thiabendazole, 77% treated with ivermectin single dose, and 100% treated with 2 days ivermectin regimen. These studies further suggested that ivermectin has fewer complaints than thiabendazole.<sup>49,50</sup> In another study, comparing the efficacy of single-dose ivermectin (200 µg/kg) with 3 days of albendazole (400 mg/d) for the treatment of *S. stercoralis* in 301 children resulted in 83% and 45% efficacy, respectively.<sup>51</sup> Another advantage of ivermectin is its effect in eliminating *Ascaris lumbricoides*, another common intestinal nematode parasite in children.<sup>51</sup> In a case study, a 10-year-old boy receiving chemotherapy for T-cell lymphoblastic lymphoma became severely ill with disseminated strongyloidiasis. He responded to antibiotics and albendazole treatment but required ivermectin to eradicate the *Strongyloides* infection.<sup>52</sup> *S. stercoralis*-infected children received prednisolone therapy, and concomitant thiabendazole treatment was ineffective in eliminating the parasite; all these patients died due to disseminated strongyloidiasis.<sup>53</sup> Genetic predisposition plays an important role in treatment, as described by Satoh et al, who showed albendazole treatment failure due to a significantly elevated *S. stercoralis*-specific IgG4 in HLA-DRB1\*0901 carriers.<sup>54</sup>

Thiabendazole was successfully used in patients with HIV infection and most of them responded well. Before the start of chemotherapy for HIV, prescreening was needed for strongyloidiasis based on a report of dissemination of *S. stercoralis* as an immune restoration phenomenon in an HIV-1-infected man on antiretroviral therapy.<sup>55–57</sup> Because of the risk of hyperinfection and/or disseminated disease in patients with AIDS, multidose courses of ivermectin 200 µg/kg/d are warranted till the cure.<sup>57</sup>

## Diagnostic advances

Direct microscopic observation of the parasite in thick wet smeared feces or Saline/Lugol's iodine staining method shows ~30% sensitivity for *S. stercoralis* diagnosis.<sup>58</sup> This poor sensitivity is due to low larval output in the case of mild infection of parasite and sporadic release of the larvae. Many techniques were periodically used to improve the sensitivity to detect *Strongyloides* larvae in stool samples. Various other standard methods that increased the sensitivity of diagnosis are larval concentration and culture methods, including Baermann, formalin-ethyl acetate sedimentation techniques, Harada-Mori, charcoal filter paper, and nutrient agar culture plate methods. The Baermann technique is laborious and unpleasant but increased the diagnostic sensitivity only by 7.4% compared to microscopic examination of direct thin smear.<sup>59</sup> Although the agar plate method increased the sensitivity by an additional 6.4% over the Baermann method, this method needs >48 hours. Many investigators relied on the agar plate method since it is simpler than the Baermann method.<sup>59</sup> However, Sato et al showed that 40% of samples consisted of false-negative (did not detect) larvae by agar culture plate techniques, concluding that sensitivity of this method is only 60%.<sup>60</sup> With these disadvantages and due to sporadic release of larvae, many investigators adopted to repeated sampling for increasing the diagnostic sensitivity.<sup>59</sup>

Molecular diagnostic methods for the detection of *S. stercoralis*-specific DNA in stool samples were attempted by a few investigators using polymerase chain reaction (PCR) amplification techniques. The sensitivity of a real-time PCR-based diagnostic method of another tropical parasite, *Schistosoma mansoni*, was at least 100 eggs per gram of feces and failed to detect <100 eggs per gram of feces.<sup>61</sup> Similar approach was adopted to detect *S. stercoralis* in human feces using real-time PCR with species-specific target genes, such as cytochrome *c* oxidase, 18S rRNA, and 5.8S rRNA.<sup>62–66</sup> The real-time PCR was 100% specific but failed to enhance the sensitivity to detect strongyloidiasis compared to parasite

concentration methods.<sup>67</sup> The major disadvantage of DNA-based detection is that the amount of parasite-specific DNA in the feces is variable because of sporadic larval release, and in asymptomatic patients with very low levels of larval output, the test is unlikely to achieve a higher sensitivity unless repeated stool samples are tested. Moreover, this method is expensive and technically challenging for implementation in the Third-World countries.

To improve the sensitivity and specificity of *S. stercoralis* diagnosis, several investigators attempted to find a suitable protein candidate antigen(s) from the larval surface or the excretory secretory products<sup>68</sup> and somatic antigens. Some of the promising candidate antigens were identified from somatic extracts at molecular weights of 28, 31, and 41 kDa.<sup>69</sup> These antigens were recognized by antibodies in sera from *S. stercoralis*-infected individuals, but the specificity of these antigens is yet to be determined.<sup>70</sup> *S. stercoralis* crude somatic antigen detected 87%–94% of parasite-specific antibodies in strongyloidiasis as reported by various investigators.<sup>30,71–74</sup> However, the disadvantage of crude L3 somatic antigens is the presence of cross-reacting epitopes that are recognized by sera from other helminthic parasites: *Onchocerca volvulus* by 80%, *Wuchereria bancrofti* by 70%,<sup>29</sup> and other parasitic infections, such as Ascariasis and Schistosomiasis.<sup>74,75</sup>

Recombinant antigens 5a and 12a identified from *S. stercoralis* L3 larvae were capable of detecting IgG antibodies in sera from *S. stercoralis*-infected humans and did not cross-react with sera from filarial parasites.<sup>76</sup> A diagnostic ELISA developed to detect antibodies in *S. stercoralis*-infected humans using the recombinant NIE antigen showed 88% specificity and 95% sensitivity.<sup>30</sup> The recombinant NIE antigen assay that was used to study the seroprevalence of strongyloidiasis in Sudanese refugees in USA showed that 33% were positive to *S. stercoralis* infection.<sup>77</sup> This assay was also used to screen the refugees or immigrants to Canada from Asia, Africa, and South America and revealed a prevalence rate of >30% of *S. stercoralis* infection in this population (Greenway, unpublished data, 2007). NIE ELISA<sup>30</sup> and NIE luciferase immunoprecipitation system assays<sup>78</sup> showed no cross-reactivity with serum samples from patients with filarial infection.

Eosinophilia is another prognostic marker commonly seen in *Strongyloides*-infected patients with intact immune systems, but it may be absent in immunosuppressed patients.<sup>79,80</sup> Patients with hyperinfection who have peripheral eosinophilia appear to have a better prognosis than patients without peripheral eosinophilia.<sup>40</sup> However, eosinophils are not specific to *Strongyloides* since any helminthic infection can

elicit eosinophil response. A second episode of *S. stercoralis* infection is an important cause of severe pulmonary infection and obstruction and causes even death.<sup>81</sup> Microscopic observation of unstained or Lugol- or Gram-stained sputum or lung lavage is a diagnostic method for PS. Symptoms of PS are wheezing and shortness of breath, similar to asthma symptoms, with elevated peripheral eosinophil count and parasite-specific IgE antibodies known as tropical pulmonary eosinophilia.<sup>47</sup>

Invasive diagnostic methods such as duodenal aspirate, biopsies, and endoscopy are insensitive in the early stage of diagnosis. Examination of single specimens of duodenal fluid under the microscope revealed to be more sensitive than wet mount analysis of stool samples for the detection of larvae. However, it was observed that this method is 24% false negative.<sup>82</sup> Moreover, in some cases, histological observation of duodenal biopsy specimens may show the presence of *S. stercoralis* embedded in the mucosa.<sup>83,84</sup>

We conclude that the concentration methods of *Strongyloides* larvae, conventional crude somatic ELISA to detect parasite-specific antibodies, and PCR-based detections of parasite-specific ribosomal markers are plagued by poor specificity and sensitivity, higher cost, and labor-intensive methods. Commercial development and deployment of a simple, specific, and sensitive method using well-studied recombinant antigens is ideal for the detection of this parasitic infection and will be beneficial in eradicating this disease in combination with prompt drug treatment.

## Vaccine development

Strongyloidiasis is a long-neglected parasitic disease, and research studies to develop a suitable vaccine are also scanty. Only few authors have reported the attempts of identifying and developing novel and potential vaccine candidate antigens in a mice model using deoxycholate-soluble affinity-purified L3 antigens of various molecular sizes of 80, 75, 61, 57, 43, and 42 kDa. This antigen pool stimulated the proliferation and secretion of IL-5 by splenocytes that were recovered from mice immunized with live L3. Vaccination of mice with these immunoaffinity-isolated antigens significantly increased protective immunity by killing 83% of challenged larvae.<sup>85</sup> Recently, the same group reported yet another potential recombinant surface antigen known as SS-IR, a *S. stercoralis* surface-specific antigen, that decreased 80% of worm burden.<sup>86</sup> In another study, intradermal vaccination of mice with Na<sup>+</sup>-K<sup>+</sup>ATPase DNA significantly reduced larval survival.<sup>87</sup> Protective IgG from mice immunized with live L3 requires complement activation and neutrophils

for the killing of L3 through an antibody-dependent cellular cytotoxicity mechanism.<sup>88</sup> Abraham et al showed that immunization of BALB/c mice with 10,000 live L3 larvae eliminated 97% of the larvae either contained in diffusion chambers or free within the tissue of the mouse within 24 hours of postinfection. Sera from these immunized mice had elevated levels of IgG1, IgM, and IgA isotypes specific to the parasite; IgM was the only antibody isotype that recognizes surface antigens of L3.<sup>89</sup> In another closely related *Strongyloides ratti* (Sr) species study, mice immunized with a native form of SrHsp60 or with complete Freund's adjuvant developed predominantly dysregulated Th1 response that allowed a higher larval output and was not protective. However, the same SrHsp60 precipitated with alum induced Th2 cell response that conferred partial protection against the challenge infections, suggesting the importance of adjuvants for vaccination studies.<sup>90</sup> In another study, the same group reported that passive immunization of mice with monoclonal anti-srHSP60 IgM led to reduced numbers of migrating larvae in the lung and head, reduced parasitic adults in the small intestine, and reduced the larval output upon *S. ratti* challenge infection.<sup>91</sup> Vlaminck et al reported the effect of adjuvant in inducing protective immunity, with deoxycholic acid-soluble antigen prepared from *S. venezuelensis* and used with saponin extract from *Quillaja saponaria* and with immunomodulatory substances. *Phlebotomus pseudoaureum* hydroalcoholic extracts induced the highest level of protection in terms of fecal egg count reduction by 93%–99%.<sup>92</sup> Since, both *S. ratti* and *S. venezuelensis* are primarily rodent infections, vaccination studies using these parasites need further validation for human-specific *S. stercoralis*.

## Genomics of Strongyloides

The genome of *S. stercoralis* is ~43 Mbp, organized in six chromosomes in the female and five chromosomes in the male. The sex is determined by one pair of X (XX) in the female and a single X (XO) in the male.<sup>93</sup> Because of the extra X chromosome, females are capable of reproducing parthenogenetically to both female and male offspring. It is estimated that the genome of *S. stercoralis* codes for 13,114 genes, which have significantly fewer and smaller introns compared to *Caenorhabditis elegans* and parasitic nematodes representing Clades I, III, and V.<sup>94,95</sup>

With the advent of DNA sequencing technology, the initial focus of genomic analysis was sequencing of expressed sequence tags (ESTs), or short DNA sequences of gene-coding regions reverse-transcribed from mRNA, to identify diagnostic and vaccine candidate genes. Ramachandran et al initially

submitted *S. stercoralis* filariform and rhabditiform ESTs to the GenBank, most of which were immunoreactive with human sera.<sup>76</sup> The program for nematode genome sequencing initiative included *S. stercoralis* and *S. ratti* (commonly found in rat host) EST sequencing. As of now, ~10,908 ESTs grouped into 3,479 contigs were sequenced from *S. stercoralis* and 14,761 ESTs grouped into 4,152 clusters and 5,237 contigs were sequenced from *S. ratti*.<sup>96,97</sup> Thus, a total of 25,669 EST sequences are available from the L1 and L3i larvae of *S. stercoralis* and five stages of *S. ratti* (<http://nematode.net>). Mitreva et al compared ESTs of L1 and L3i larvae of *S. stercoralis* with L3i-like dauer stage larvae of *C. elegans*.<sup>98</sup> Comparison of *S. stercoralis* L1 and L3i-biased or -specific clusters with *C. elegans* nutrient-rich or dauer-specific genes showed significant matches between *S. stercoralis* L1 and *C. elegans* nutrient-rich-specific transcripts only; thus, the conservation of transcriptional profiles of L3i of *S. stercoralis* and dauer larvae of *C. elegans* was not supported by this analysis.<sup>98</sup> The cluster consensus sequences of *S. ratti* were used to assign each cluster to one of the three databases: 1) *C. elegans* and *C. briggsae* sequences, 2) other nematode sequences, and 3) nonnematode sequences. The authors found that ~25% of clusters have no significant alignment and may therefore represent novel genes.<sup>96</sup> This high level of nonalignment is a feature of nematode EST analyses, which probably reflects both the large evolutionary distance between taxa and the diversity of life histories.<sup>99</sup> There was a specificity of substantial free-living and parasitic stages in the detected ESTs.<sup>100</sup>

The complete mitochondrial genome sequence of *S. stercoralis* is 13,758 bp in size coding for 36 genes (coding for 12 proteins, 22 *tRNAs*, and two *rRNAs*) and an AT-rich (control) region but lacks an *atp8* gene, similar to most nematodes examined to date. All genes are inferred to be transcribed in the clockwise direction.<sup>101</sup>

The *S. ratti* ESTs have been used to construct microarrays, which have been used for expression analysis of the free-living and parasitic stages.<sup>100</sup> Transcriptome analysis of L3 stage larvae of *S. stercoralis* revealed that 42.25% (3,412 ESTs) of putative proteins had homology with *C. elegans*, 47.96% had homology with *S. ratti* transcriptome, and 3,759 putative proteins were similar to human.<sup>102</sup> Polyadenylated transcriptome analysis of *S. stercoralis* and comparison with dauer stage *C. elegans* revealed that in both *S. stercoralis* and *C. elegans*, the genes encoding Cyclic Guanosine Monophosphate (cGMP) pathway components were coordinately upregulated in L3i, suggesting that dauer pathway genes are present in *S. stercoralis* and may play a role in L3i development.<sup>103</sup> Microarray analysis of L3i and L1 of *S. stercoralis*

helped to identify 935 differentially expressed genes between these two stages,<sup>104</sup> and similar results were reported using *S. ratti* transcriptome.<sup>100</sup> Overall, using *Strongyloides* EST representation and microarray data, there was rather little evidence for the conservation of transcriptional profiles between *S. ratti* and *S. stercoralis* or *C. elegans*.<sup>100</sup>

The Wellcome Trust Sanger Institute is now in the process of sequencing the *S. ratti* genome (together with four other helminth species) as a reference genome, with subsequent whole genome shotgun sequencing of the *S. stercoralis* genome planned (<http://www.sanger.ac.uk/Projects/Helminths/>). This gene discovery will facilitate research on these species. However, in view of the substantial diversity among the nematodes and the relative lack of tools for investigating gene function in parasitic species, a very substantial amount of work remains to be done.

Proteomic analysis of excretory/secretory proteins in *S. ratti* revealed specific expressions of 196, 79, and 35 proteins in infective larvae, parasitic female, and free-living stages, respectively. The study identified homologs of NIE and metalloprotease astacin expressed in the infective stage.<sup>105</sup> A systematic study identified batteries of *S. stercoralis* L3 surface antigens and somatic antigens by proteomic analysis.<sup>106</sup> These stage-specific proteins are crucial for host-parasite interactions. However, much remains to be done as it is very challenging to obtain pure parasite proteins.

## Conclusion

*S. stercoralis* is the only nematode parasite that completes its entire life cycle within the host and survives for a long time or throughout the lifetime with/without mild gastrointestinal symptoms. In majority of people, it succeeds many decades of survival by evading the host immune system. These hosts serve as reservoirs to infect people in close contacts with toiletries or contaminating soil to infect other primates. The advantage for *S. stercoralis* is its simplest life cycles, involving two alternative life cycles according to their environment: 1) capable of free-living sexual life cycle and 2) parasitic asexual life cycle. Thus, this parasite exhibits a global distribution emerging even in developed countries where there is rare fecal contamination. A balance between parasite and host immune system, which is affected due to HTLV-1 viral infection or corticosteroid therapies for asthma, tissue transplantation, and cancer treatment, allows parasite multiplication that leads to disseminated strongyloidiasis. Majority of the fatal disease is attributable to the invasion of parasite to all organs in the body. It is important to screen *Strongyloides*



infections before tissue transplantation, cancer therapies, or corticosteroid therapies. Health care providers should be educated and should consider patients' origin and travel history to endemic countries to recognize the possibility of this neglected parasitic infection. Recently a diagnostic assay developed based on a recombinant NIE antigen has been shown to be specific and sensitive to *S. stercoralis* infection and can be used for prescreening and estimating the global prevalence of the parasite. Early diagnosis of strongyloidiasis and use of ivermectin for treatment (a drug of choice that avoids various clinical complications and other unnecessary invasive diagnostic procedures) are vital in containment of the disease, whereas genomic and proteomic advances will unravel the understanding of biology of the parasite and aid in the development of an efficacious vaccine.

## Disclosure

The authors report no conflicts of interest in this work.

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