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Diagnostic Potential of an IgE-ELISA in Detecting Strongyloidiasis

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Abstract. *Strongyloides stercoralis* infection is prevalent worldwide and can cause lifelong infection in immunocompetent individuals, and potentially death in immunosuppressed patients. The diagnosis is hindered by the low sensitivity of microscopic examination, thus making serology an important complementary test to improve the detection rate. However, there were reports that some *Strongyloides*-infected individuals were negative with specific IgG and IgG4 assays, and other helminth infections were positive with commercial *Strongyloides* IgG-ELISAs. Thus, there is a need to develop better serodiagnostic methods for strongyloidiasis. We investigated the diagnostic potential of IgE-ELISAs using *Strongyloides* larval lysate. Sera from two groups infected with *Strongyloides* served as the positive reference, that is, 1) positive by commercial IgG-ELISAs and IgG4 rapid test, and stool samples positive by microscopy and/or PCR (group IA; $n = 20$); and 2) negative by IgG-ELISAs and IgG4 rapid test, but stool samples were PCR positive (group IB sera; $n = 11$). Sera from another two groups served as negative reference (controls), that is, 1) infected with other parasites (group II; $n = 73$) and 2) healthy donors (group III; $n = 22$). Results showed a 100% diagnostic sensitivity in detecting sera from groups IA and IB. The latter group of individuals probably had early infection because their IgG and IgG4 assays were negative. The optical density values of group IB sera were also significantly lower than those of group IA ($P < 0.003$). The IgE-ELISA was 100% specific when tested against sera from groups II and III. This study highlights the diagnostic potential of IgE-ELISA using larval lysate to detect strongyloidiasis, especially those with probable early infection.

INTRODUCTION

Strongyloidiasis is a soil-transmitted helminthiasis that is mainly caused by *Strongyloides stercoralis* and is hyperendemic in tropical and subtropical areas of the world.[1,2] Although the disease is less endemic in European countries, the worldwide prevalence has been rising because of the ongoing movements of migrants and travelers, in addition to the increasing number of patients with immunosuppression.

Strongyloidiasis is challenging to diagnose, and the development of sensitive diagnostic methods for *S. stercoralis* infection is crucial for detecting the disease as well as for epidemiological studies. Several direct and indirect detection methods have been developed but showed variable diagnostic sensitivities and specificities. Although direct stool microscopy is the routine method in clinical settings, the results are insensitive.[3,4] Agar culture plate followed by microscopy increases the diagnostic sensitivity but is inconvenient and time consuming. Current advances in direct detection methods have introduced several nucleic acid amplification tests (NTTs), for example, multiplex PCR, which is capable of simultaneously detecting parasite coinfections.[5] However, the intermittent release of larvae affects the sensitivity of NTTs,[6] and the need for expensive equipment and reagents makes NTTs unsuitable for low-resource settings.

Meanwhile, indirect assays that detect anti-*Strongyloides* antibodies have been developed using various formats, such as ELISA, Western blot, indirect fluorescent antibody test, and a luciferase immunoprecipitation system using somatic and/or recombinant antigens.[7,8] Serologically, patients may secrete significant levels of IgA, IgM, IgE, and IgG antibodies, including its subtypes (IgG1–IgG4), in response to *Strongyloides* infection.[9] Of these, IgG and the IgG4 subclass are the two most common types of antibodies found associated with an infection with *S. stercoralis.* The presence of both antibodies typically indicates a more established and chronic infection.[10] By contrast, it is reported that IgM, IgE, and IgG1 are secreted early in the infection, suggesting their role in detecting acute cases.[11–13] However, there are several significant drawbacks to the current strongyloidiasis serodiagnostics, such as cross-reactivity problems with other nematode infections, particularly with filariasis, schistosomiasis, and ascariasis.[8,14]

Detection of specific IgG is the most common serologic method for strongyloidiasis. However, several reports have shown that a specific IgG response was not detected in patients with proven infection; thus, it is not surprising that most serological assays have diagnostic sensitivities of less than 95%. In one study of 413 outpatients in London, 21% of the cohort had confirmed infection based on positive microscopy or culture, and of these, 9.3% had negative IgG serology.[15] In another study, the sensitivities of five serological tests ranged from 75.4% to 93.9% when tested with 114 sera from patients with microscopy-positive stool samples.[8] A CDC *Strongyloides* enzyme immunoassay was 94.6% sensitive when tested with 74 serum samples from individuals with *Strongyloides* larvae in stool samples.[16] Hence, the development of an improved serodiagnostic for strongyloidiasis with consistent high sensitivity is desirable.

During helminth infection, polarized T helper type 2-type responses promote B cell class switching to IgE and IgG4 antibodies, a reaction mediated by interleukin-4 receptor signaling and cognate T–B cell interactions.[17] IgE potently activates mast cells and basophils, and the former is crucial for protection against *Strongyloides venezuelensis* or *Trichinella spiralis*.[18] It is also important to note that the IgE levels peak during acute or early phase of infection. This finding was supported by a report that the level of IgE increased in 90% of patients during the course of acute *Strongyloides* infection and declined in cases of chronic infection and coinfection with human T-cell lymphotropic virus type 1.[10,13,19] Although IgE is

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commonly associated with allergic reactions, this isotype is also significantly present in asymptomatic individuals harboring parasitic infection and is detectable in immunocompetent patients with strongyloidiasis but not in disseminated and immunosuppressed patients.[20] In addition, the level of *Strongyloides*-specific IgE is also significant in copronegative individuals, implicating the role of IgE in infection intensity and larval output.[12]

Therefore, a specific IgE response may be an important marker for strongyloidiasis, especially in cases of symptomatic individuals who are copronegative and for epidemiological studies. In the present study, we used a larval lysate of *S. stercoralis* and monoclonal antihuman IgE as a probe to develop an IgE-ELISA for strongyloidiasis.

MATERIALS AND METHODS

Serum samples. Anonymized samples were obtained from serum bank at the Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia. In determining the diagnostic value of the IgE-ELISA, the serum samples were divided into four groups. Two of the groups (groups IA and IB) were samples from *Strongyloides*-infected individuals as positive reference, and two others were from control groups as negative reference (groups II and III). Some samples from group IA and group II and all samples from Group IB were from previous field studies involving asymptomatic aborigines in Peninsular Malaysia.

All serum samples were previously tested with a commercial *Strongyloides* IgG-ELISA Kit (SciMedx Corporation, Denville, NJ). In addition, group IA and IB samples were also tested with an in-house IgG4 rapid test, following our published report.[21] The rapid test is a lateral flow assay using a mixture of NIE and SS1a recombinant proteins with reported diagnostic sensitivity and specificity of 91.3% and 100%, respectively. Thus, the *Strongyloides* IgG and/or IgG4 status of all samples were known which helped in the categorization of the serum samples.

Group IA were serum samples from individuals who were positive by both the *Strongyloides* IgG-ELISA and IgG4 rapid test, and their stool samples were positive by microscopy and/or PCR ($n = 20$). Group IB comprised serum samples that were negative by both the serology tests, but were positive by stool PCR ($n = 11$). The stool PCR was performed using Rotor Gene 6000 real-time thermocycler (Rotorgene-Q, Hilden, Germany). Each PCR run included a tube containing plasmid harboring the target sequence as positive control and a tube without template as the negative control. Cycle threshold (Ct) value > 38 was considered as negative based on previously determined detection limit of the assay.[22] Real-time PCR assays were repeated on several samples with a Ct value > 35 to confirm the results.

To determine the diagnostic specificity, 73 serum samples from patients with other infections (group II) and negative by the *Strongyloides* IgG-ELISA were used, that is, amebiasis ($n = 4$), ascariasis ($n = 4$), filariasis ($n = 12$), giardiasis ($n = 1$), hookworm ($n = 6$), schistosomiasis ($n = 11$), taeniasis ($n = 2$), toxocariasis ($n = 20$), toxoplasmosis ($n = 5$), trichuriasis ($n = 4$), and echinococcosis ($n = 4$). All infections were diagnosed based on microscopy, except for toxoplasmosis and echinococcosis. Toxoplasmosis was diagnosed based on clinical presentations and the presence of specific high IgM and IgG antibodies using a commercial ELISA. Echinococcosis was diagnosed based on clinical presentation, ultrasound, and ELISA results. In addition, diagnostic specificity was also tested using group III comprising serum samples from healthy individuals collected from volunteers at INFORMM who were negative by the *Strongyloides* IgG-ELISA ($n = 22$).

IgE is generally known to be more labile than other antibodies. However, this was not a problem in the present study because the samples were stored in small volume aliquots at –20°C, and thawed on ice before each experiment. The ethical approval for use of the serum samples was obtained from Universiti Sains Malaysia Human Research Ethics Committee (ref: USM/JEPeM/19070400).

Preparation of crude lysate antigen. Infective third-stage larvae (L3i) of *S. stercoralis* were isolated from charcoal coprocultures prepared from feces of immunosuppressed laboratory dogs experimentally infected with the University of Pennsylvania, Dog (canine) strain of *S. stercoralis*.[23,24] *Strongyloides stercoralis* was maintained under biosafety level 2 conditions in purpose bred mix breed dogs, treated with prednisolone, under protocol 804883 approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Pennsylvania, Philadelphia, PA. This IACUC protocol and all routine husbandry care of the animals were conducted in strict accordance with the *Guide for the Care and Use of Laboratory Animals* of the U.S. NIH. The coprocultures were prepared as described[25] and incubated at 25°C for seven days. Infective third-stage larvae were isolated from the coprocultures by the Baermann funnel technique as described,[25] and cleaned through low-gel agar.[26] The recovered larvae were washed once in distilled water, centrifuged, and the larval pellets (containing approximately 250,000 L3i/mL) were stored at –80°C until shipment on dry ice from the University of Pennsylvania (Philadelphia, PA) to the Universiti Sains Malaysia (Penang, Malaysia).

A 50× protease inhibitor cocktail tablet (Roche Diagnostics, Basel, Switzerland) was dissolved in 2 mL of deionized water to produce a 25× protease inhibitor solution. A volume of 60 µL of the solution was added to 1.5 mL of *S. stercoralis* infective larvae and incubated on ice for 30 minutes. This mixture was then lysed by sonication on ice using a microtip probe (QSonica LLC., Newtown, CT)) for 3 minutes (30 seconds ON, 30 seconds OFF, 4.5 Hz). The lysed mixture was transferred to a cryovial and dipped into liquid nitrogen for 30 seconds (snap freeze) and then immediately thawed in a water bath for 20 minutes. The freeze-thawed mixture was centrifuged at 14,000 rpm for 5 minutes, and the supernatant (soluble larval lysate) was collected. The protein concentration of the lysate was determined by the RC DC protein assay (Bio-Rad, Hercules, CA) and stored at –20°C until further use.

IgE-ELISA optimization. The conditions of the IgE-ELISAs were optimized by varying the protein concentration of the larval lysate, dilutions of primary and secondary antibodies, and the incubation period of the peroxidase-conjugated antibody. For optimizations and each ELISA run, duplicate wells using a pooled of three serum samples from groups IA and IB were used as positive controls, whereas duplicate wells using pooled of three serum samples from groups II and III were used as negative controls. The antigen in carbonate buffer (pH 9.6) was coated in microtiter plate wells (MaxiSorp; Nalge Nunc International, Rochester, NY) at 4°C overnight. The next day, the plate was incubated for 1 hour at 37°C, washed five times with phosphate-buffered saline containing 0.1% Tween-20, and then blocked for 1 hour with 3% bovine serum albumin (Amresco, Solon, OH). Subsequently, samples (2 wells/serum) were incubated at 37°C for 2 hours, washed, and then mouse antihuman IgE-HRP (Southern Biotech, Birminghan, AL) was

incubated for 2 hours at 37°C. Finally, the plate was washed again and ABTS substrate (Roche Diagnostics GmbH, Mannheim, Germany) added at 100 µL/well and incubated for 30 minutes. All the incubation steps were performed on a microplate shaker at 300 rpm, whereas washing steps were performed at 700 rpm. The optical density (OD) readings were read at 405 nm/490 nm using the Thermo Multiskan Spectrum Reader (Multiskan Spectrum, Thermo Scientific, Rockford, IL).

Statistical analysis. Diagnostic sensitivity and specificity of the IgE-ELISA were determined by standard methods. Graph Pad Prism version 8.0.2 (GraphPad Software, San Diego, CA) was used to perform one-way analysis of variance (ANOVA) with Bonferroni multiple comparisons test for all groups, and *t*-test was used to compare between groups. A statistical probability (*P*) of less than 0.05 was considered statistically significant. Receiver operator characteristic (ROC) curve analysis of the IgE-ELISA was performed using MedCalc statistical software 19.0.7 (MedCalc Software Ltd., Ostend, Belgium).

RESULTS

The optimized parameters of the IgE-ELISA were as follows: 100 µL larval lysate antigen at 5 µg/mL (0.5 µg/well), serum dilution at 1:100, and secondary antibody at 1:500 with 2 hours incubation period. Between-day precision values of the control wells were as follows: group IA 0.722 ± 0.012, group IB 0.553 ± 0.017, group II 0.110 ± 0.013, and group III 0.089 ± 0.002. Meanwhile, within-day precision values of the control wells were as follows: group IA 0.720 ± 0.0071, group IB 0.557 ± 0082, group II 0.106 ± 0063, and group III 0.084 ± 0015. The *Strongyloides*-specific IgE responses by ELISA showed a cutoff OD value of 0.19 and area under the ROC curve of 1.0 (95% CI: 1.000–1.000, $P < 0.01$). A diagnostic sensitivity of 100% was achieved with positive reference sera of 20 samples from group IA (microscopy and/or PCR positive; *Strongyloides* IgG and IgG4 positive) and 11 serum samples from group IB (PCR positive; *Strongyloides* IgG and IgG4 negative). A diagnostic specificity of 100% was obtained using 73 samples with negative reference (control) sera from group II (other infections; *Strongyloides* IgG negative) and 22 samples from group III (healthy individuals; *Strongyloides* IgG negative).

As shown in Figure 1, one-way ANOVA showed statistically significant differences among the four different sera groups, with five comparisons showing *P*-values < 0.01 and one comparison with $P < 0.026$. The OD values of group IA were

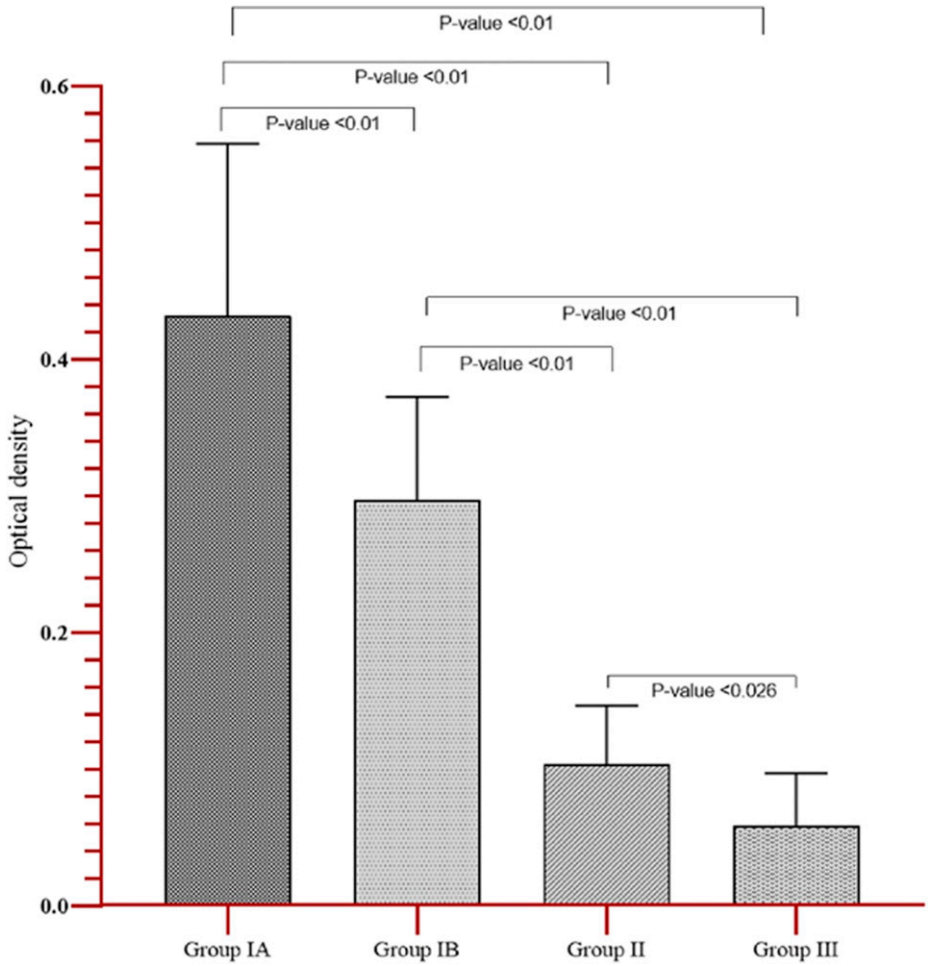

FIGURE 1. One-way ANOVA comparison of the optical density (OD) values (mean + 1 SD) of the different serum groups. Group 1A: positive by *Strongyloides* IgG-ELISA and IgG4 rapid test, and stool samples were positive by microscopy and/or PCR ($n = 20$); group 1B: negative by *Strongyloides* IgG-ELISA and IgG4 rapid test, but stool samples were PCR positive ($n = 11$); group II: infected with other parasites and negative by *Strongyloides* IgG-ELISA ($n = 73$); group III: healthy donors who were negative by *Strongyloides* IgG-ELISA ($n = 22$). This figure appears in color at www.ajtmh.org.

significantly higher than those of group IB at $P < 0.003$. The figure also shows that the OD values of group IB were significantly higher than those of group II at $P < 0.001$, and group III at $P < 0.01$. The distribution of OD values of the positive and negative serum samples by IgE-ELISA is shown in Figure 2.

DISCUSSION

Antibody responses are induced upon infection with pathogens, and the types of antibodies produced depend on the specific infectious agent. In response to *S. stercoralis* infection in humans, the immune system secretes different classes of antibodies at various phases of the disease. For example, specific IgM, IgA, and IgE are secreted in the early stages of infection, indicating their importance as diagnostic markers of acute strongyloidiasis. However, the IgM response declines quickly, and thus detection of IgM in patients infected with *S. stercoralis* has not shown to be diagnostically significant.[11,27] Meanwhile, the IgA level declines 4–5 weeks after exposure, showed limited detection in serum, and was more readily detected in saliva.[28]

The levels of IgG and IgG4 levels are high in patients with chronic *Strongyloides* infection, demonstrating their utility in the diagnosis of the chronic phase of the disease.[9] Although IgG is abundant in 95% of the patients infected with *S. stercoralis*,[27] its main drawback is potential cross-reactivity, especially with antibodies from patients with filariasis, ascariasis, and schistosomiasis.[29] Among the subclasses of IgG, the IgG1 response is significantly depressed in older populations and is also more often associated with long-standing chronic infections.[10,30] Meanwhile, IgG4 antibody constitutes a more specific response to *S. stercoralis* infection and is consistently found in chronically infected patients.[10,30,31]

Strongyloides-specific IgE antibodies can remain in patient serum for years, indicating the presence of long-lived memory B cells or plasma cells.[32] The level of IgE antibodies seemed to depend on whether the infection is active. Albendazole treatment in children every 2 months for over a year is associated with a significant reduction in total IgE levels.[33] Specific IgE antibody decreases to a low level or disappears within 6–12 months after effective treatment of strongyloidiasis with ivermectin.[34] An elevated IgE level is also one of the most consistent findings in human helminthic infection. Significant associations between elevated IgE level and the risk of schistosomiasis,[35,36] hookworm disease,[37,38] trichuriasis,[39] and ascariasis[40,41] pertain in both mice and humans. Susceptibility to infection by various parasitic helminths, including *Schistosoma mansoni*,[42] *T. spiralis*,[43,44]and *Brugia malayi*,[45] increases in IgE-deficient mice.

Numerous serodiagnostic tools have been introduced to detect strongyloidiasis. An ideal test would be able to detect early infection and differentiate between acute and chronic diseases. The latter would be much more challenging to achieve because strongyloidiasis is usually detected at the chronic stage of the infection. The most widely used serodiagnostic test format for strongyloidiasis is the ELISA, most of which use a crude extract of *S. stercoralis* to detect specific serum IgG or IgG4 antibodies.[16,46–48] In the present study, ELISA plates coated with larval lysate of *S. stercoralis*

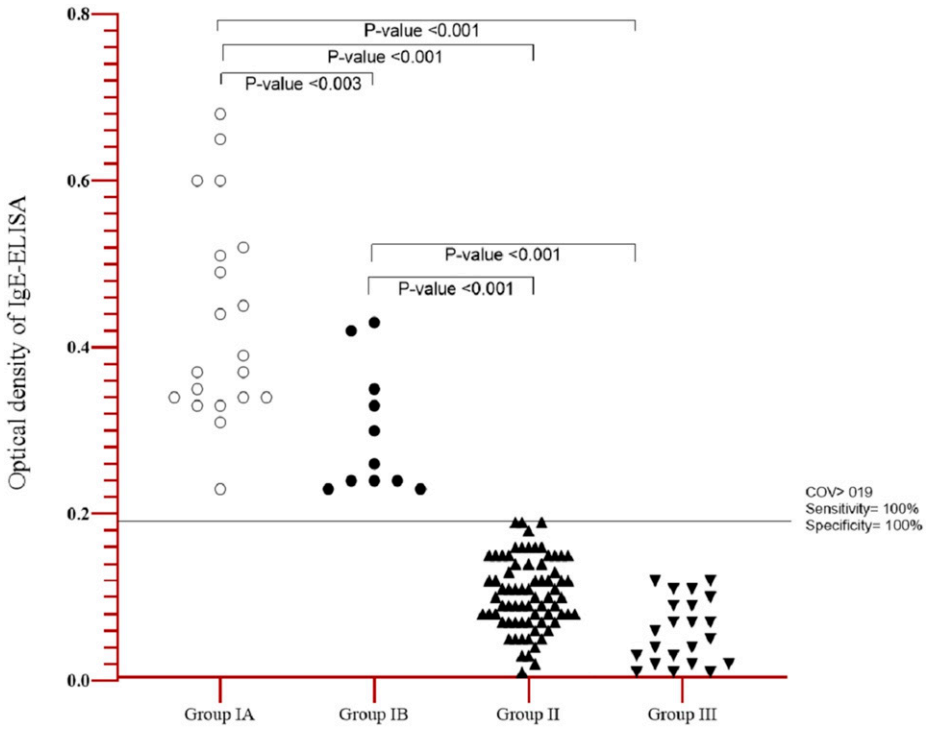

FIGURE 2. The distribution of optical density (OD) values of different serum groups by IgE-ELISA. The cutoff OD value to discriminate between positive and negative results was 0.19. Group 1A: positive by *Strongyloides* IgG-ELISA and IgG4 rapid test, and stool samples were positive by microscopy and/or PCR ($n = 20$); group 1B: negative by *Strongyloides* IgG-ELISA and IgG4 rapid test, but stool samples were PCR positive ($n = 11$); group II: infected with other parasites and negative by *Strongyloides* IgG-ELISA ($n = 73$); group III: healthy donors who were negative by *Strongyloides* IgG-ELISA ($n = 22$). This figure appears in color at www.ajtmh.org.

detected specific IgE antibodies from two groups of individuals with strongyloidiasis (positive reference), and two groups of controls (negative reference), with 100% diagnostic sensitivity and specificity. Positive test results among group IA individuals were not surprising because the samples were from individuals who were positive by direct methods (microscopy and/or PCR) and were seropositive. Notably, the IgE-ELISA was also positive with asymptomatic infected individuals who were negative for specific IgG and IgG4 antibodies (group IB). *Strongyloides stercoralis* infection was confirmed in these patients by PCR testing of their stool samples. We hypothesized that group IB individuals had early infection because their IgG serology was negative. This group of individuals is interesting because they could only have been detected by the IgE-ELISA in a field study among asymptomatic individuals. On the other hand, hospitalized chronic strongyloidiasis patients are commonly asymptomatic as well as positive for *Strongyloides* IgG. Our results are thus consistent with the earlier findings that IgE is an important antibody marker for strongyloidiasis, especially in cases of probable early/acute infection.[10,13]

Significantly higher OD values were observed in sera from group IA than in group IB sera. The results were consistent with the assumption that group IA individuals (likely to have more chronic infection) had the infection for a more extended period than group IB individuals (likely to have an early infection). Concerning the diagnostic specificity of the IgE-ELISA, it was found to be excellent, as shown by the significantly lower ODs of groups II and III (negative reference/control) as compared with groups IA and IB (positive reference).

We previously reported an IgE-ELISA using *Strongyloides* larval lysate, which detected only 7.7% (n = 26) of sera from *Strongyloides*-infected individuals.[48] One possible reason for the different levels of diagnostic sensitivity between our previous and present studies could be attributed to the antihuman IgE-HRP secondary antibody, in terms of the source, dilution, and incubation time. The previous study used the antibody from Abcam (England, United Kingdom) at 1:2,000 for 30 minutes, whereas the present study used the antibody from Southern Biotech (Birmingham, AL) at 1:500 for 2 hours. However, both studies concurred that the diagnostic specificity of the IgE-ELISA was 100%.

An IgE assay using a recombinant protein would be more desirable in terms of ease of production and standardization. Thus, we previously attempted to optimize IgE-ELISAs using two recombinant antigens reported to be of diagnostic value, that is, NIE and recombinant *Strongyloides* Ss1a.[7,49] However, we were unable to obtain satisfactory diagnostic specificity despite many optimization attempts (data not shown); consequently, no further work along these lines was performed. Therefore, the discovery of new recombinant proteins for use in IgE assays would be beneficial for the improvement of strongyloidiasis serodiagnosis.

In conclusion, the IgE-ELISA developed in this study is highly sensitive and specific. However, clinicians who work in endemic areas of other parasitosis should carefully interpret because of the limited numbers of serum samples used for specificity evaluation. In the future, much larger number of serum samples from individuals with strongyloidiasis and other infections should be tested to confirm the findings of this study. The IgE-ELISA is promising for laboratory detection of strongyloidiasis, to diagnose suspected patients, especially those with early infection, and for epidemiological studies.

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REFERENCES

1. Marcos LA, Terashima A, DuPont HL, Gotuzzo E, 2008. *Strongyloides* hyperinfection syndrome: an emerging global infectious disease. *Trans R Soc Trop Med Hyg 102:* 314–318.
2. Schär F, Trostdorf U, Giardina F, Khieu V, Muth S, Marti H, Vounatsou P, Odermatt P, 2013. *Strongyloides stercoralis*: global distribution and risk factors. *PLoS Negl Trop Dis 7:* e2288.
3. Requena-Méndez A, Chiodini P, Bisoffi Z, Buonfrate D, Gotuzzo E, Muñoz J, Requena-me A, 2013. The laboratory diagnosis and follow up of strongyloidiasis: a systematic review. *PLoS Negl Trop Dis 7:* e2002.
4. Campo LP, Gutiérrez LA, Cardona JA, 2014. Diagnosis of *Strongyloides Stercoralis* infection: meta-analysis on evaluation of conventional parasitological methods (1980–2013). *Rev Esp Salud Publica 88:* 581–600.
5. Mejia R, Vicuña Y, Broncano N, Sandoval C, Vaca M, Chico M, Cooper PJ, Nutman TB, 2013. A novel, multi-parallel, real-time polymerase chain reaction approach for eight gastrointestinal parasites provides improved diagnostic capabilities to resource-limited at-risk populations. *Am J Trop Med Hyg 88:* 1041–1047.
6. Iatta R, Buonfrate D, Paradies P, Cavalera MA, Capogna A, Iarussi F, Šlapeta J, Giorli G, Trerotoli P, Bisoffi Z, 2019. Occurrence, diagnosis and follow-up of canine strongyloidiosis in naturally infected shelter dogs. *Parasitology 146:* 246–252.
7. Arifin N, Yunus MH, Nolan TJ, Lok JB, Noordin R, 2018. Identification and preliminary evaluation of a novel recombinant protein for serodiagnosis of strongyloidiasis. *Am J Trop Med Hyg 98:* 1165–1170.
8. Bisoffi Z et al., 2014. Diagnostic accuracy of five serologic tests for *Strongyloides stercoralis* infection. *PLoS Negl Trop Dis 8:* e2640.
9. Arifin N, Hanafiah KM, Ahmad H, Noordin R, 2019. Serodiagnosis and early detection of *Strongyloides stercoralis* infection. *J Microbiol Immunol Infect 52:* 371–378.
10. Rodrigues RM, de Oliveira MC, Sopelete MC, Silva DAO, Campos DMB, Taketomi EA, Costa-Cruz JM, 2007. IgG1, IgG4, and IgE antibody responses in human strongyloidiasis by ELISA using *Strongyloides ratti* saline extract as heterologous antigen. *Parasitol Res 101:* 1209–1214.
11. Bonne-Année S, Hess JA, Abraham D, 2011. Innate and adaptive immunity to the nematode *Strongyloides stercoralis* in a mouse model. *Immunol Res 51:* 205–214.
12. Atkins NS, Conway DJ, Lindo JF, Bailey JW, Bundy DA, 1999. L3 antigen-specific antibody isotype responses in human strongyloidiasis: correlations with larval output. *Parasite Immunol 21:* 517–526.
13. Hirata T, Uchima N, Kishimoto K, Zaha O, Kinjo N, Hokama A, Sakugawa H, Kinjo F, Fujita J, 2006. Impairment of host immune

response against *Strongyloides stercoralis* by human T cell lymphotropic virus type 1 infection. *Am J Trop Med Hyg 74:* 246–249.
14. Koosha S, Fesharaki M, Rokni MB, 2004. Comparison of enzyme-linked immunosorbent assay and indirect immunofluorescence assay in the diagnosis of human strongyloidiasis. *Indian J Gastroenterol 23:* 214–216.
15. Ming DK, Armstrong M, Lowe P, Chiodini PL, Doherty JF, Whitty CJM, McGregor AC, 2019. Clinical and diagnostic features of 413 patients treated for imported strongyloidiasis at the Hospital for Tropical Diseases, London. *Am J Trop Med Hyg 101:* 428–431.
16. Loutfy MR, Wilson M, Keystone JS, Kain KC, 2002. Serology and eosinophil count in the diagnosis and management of strongyloidiasis in a non-endemic area. *Am J Trop Med Hyg 66:* 749–752.
17. Iriemenam NC, Sanyaolu AO, Oyibo WA, Fagbenro-Beyioku AF, 2010. *Strongyloides stercoralis* and the immune response. *Parasitol Int 59:* 9–14.
18. Harris N, Gause WC, 2011. To B or not to B: b cells and the Th2-type immune response to helminths. *Trends Immunol 32:* 80–88.
19. Hayashi J, Kishihara Y, Yoshimura E, Furusyo N, Yamaji K, Kawakami Y, Murakami H, Kashiwagi S, 1997. Correlation between human T cell lymphotropic virus type-1 and *Strongyloides stercoralis* infections and serum immunoglobulin E responses in residents of Okinawa, Japan. *Am J Trop Med Hyg 56:* 71–75.
20. Vercelli D, De Monte L, Monticelli S, Di Bartolo C, Agresti A, 1998. To E or not to E? Can an IL-4-induced B cell choose between IgE and IgG4? *Int Arch Allergy Immunol 116:* 1–4.
21. Yunus MH, Arifin N, Balachandra D, Anuar NS, Noordin R, 2019. Lateral flow dipstick test for serodiagnosis of strongyloidiasis. *Am J Trop Med Hyg 101:* 432–435.
22. Basuni M, Muhi J, Othman N, Verweij JJ, Ahmad M, Miswan N, Rahumatullah A, Aziz FA, Zainudin NS, Noordin R, 2011. A pentaplex real-time polymerase chain reaction assay for detection of four species of soil-transmitted helminths. *Am J Trop Med Hyg 84:* 338–343.
23. Schad GA, Hellman ME, Muncey DW, 1984. *Strongyloides stercoralis:* hyperinfection in immunosuppressed dogs. *Exp Parasitol 57:* 287–296.
24. Genta RM, Schad GA, Hellman ME, 1986. *Strongyloides stercoralis*: parasitological, immunological and pathological observations in immunosuppressed dogs. *Trans R Soc Trop Med Hyg 80:* 34–41.
25. Lok JB, 2007. *Strongyloides stercoralis*: a model for translational research on parasitic nematode biology. The *C. elegans* Research Community eds. *WormBook: The Online Review of* C. elegans *Biology*. Pasadena, CA: WormBook, 1–18.
26. De'Broski RH, Nolan TJ, Schad GA, Lustigman S, Abraham D, 2002. Immunoaffinity-isolated antigens induce protective immunity against larval *Strongyloides stercoralis* in mice. *Exp Parasitol 100:* 112–120.
27. Lindo JF, Lee MG, 2001. *Strongyloides stercoralis* and *S. fulleborni*. *Principles and Practice of Clinical Parasitology*. Chichester, United Kingdom: John Wiley & Sons Ltd, 479–500.
28. Bosqui LR et al., 2015. Detection of parasite-specific IgG and IgA in paired serum and saliva samples for diagnosis of human strongyloidiasis in northern Paraná state, Brazil. *Acta Trop 150:* 190–195.
29. Van Dam GJ, Stelma FF, Gryseels B, Falcão Ferreira STM, Talla I, Niang M, Rotmans JP, Deelder AM, 1996. Antibody response patterns against *Schistosoma mansoni* in a recently exposed community in Senegal. *J Infect Dis 173:* 1232–1241.
30. Atkins NS, Lindo JF, Lee MG, Conway DJ, Bailey JW, Robinson RD, Bundy DAP, 1997. Humoral responses in human strongyloidiasis: correlations with infection chronicity. *Trans R Soc Trop Med Hyg 91:* 609–613.
31. Satoh M, Toma H, Sato Y, Kikuchi M, Takara M, Shiroma Y, Kiyuna S, Hirayama K, 1999. 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 61:* 668–671.
32. Babu S, Nutman TB, 2019. Immune responses to helminth infection. Robert R, Thomas F, William S, Harry S, Anthony F, Cornelia W, eds. *Clinical Immunology*. Amsterdam, The Netherlands: Elsevier, 437–447.
33. Cooper PJ, Alexander N, Moncayo A-L, Benitez SM, Chico ME, Vaca MG, Griffin GE, 2008. Environmental determinants of total IgE among school children living in the rural tropics: importance of geohelminth infections and effect of anthelmintic treatment. *BMC Immunol 9:* 33.
34. Lindo JF, Atkins NS, Lee MG, Robinson RD, Bundy DAP, 1996. Long-term serum antibody isotype responses to *Strongyloides stercoralis* filariform antigens in eight patients treated with ivermectin. *Am J Trop Med Hyg 55:* 474–476.
35. Rihet P, Demeure CE, Bourgois A, Prata A, Dessein AJ, 1991. Evidence for an association between human resistance to *Schistosoma mansoni* and high anti-larval IgE levels. *Eur J Immunol 21:* 2679–2686.
36. De Moira AP, Fulford AJC, Kabatereine NB, Ouma JH, Booth M, Dunne DW, 2010. Analysis of complex patterns of human exposure and immunity to *Schistosomiasis mansoni*: the influence of age, sex, ethnicity and IgE. *PLoS Negl Trop Dis 4:* e820.
37. Bethony J, Loukas A, Smout M, Brooker S, Mendez S, Plieskatt J, Goud G, Bottazzi ME, Zhan B, Wang Y, 2005. Antibodies against a secreted protein from hookworm larvae reduce the intensity of *hookworm* infection in humans and vaccinated laboratory animals. *FASEB J 19:* 1743–1745.
38. Pritchard DI, Quinnell RJ, Walsh EA, 1995. Immunity in humans to *Necator americanus*: IgE, parasite weight and fecundity. *Parasite Immunol 17:* 71–75.
39. Faulkner H, Turner J, Kamgno J, Pion SD, Boussinesq M, Bradley JE, 2002. Age-and infection intensity-dependent cytokine and antibody production in human trichuriasis: the importance of IgE. *J Infect Dis 185:* 665–674.
40. McSharry C, Xia Y, Holland CV, Kennedy MW, 1999. Natural immunity to *Ascaris lumbricoides* associated with immunoglobulin E antibody to ABA-1 allergen and inflammation indicators in children. *Infect Immun 67:* 484–489.
41. Turner JD, Faulkner H, Kamgno J, Kennedy MW, Behnke J, Boussinesq M, Bradley JE, 2005. Allergen-specific IgE and IgG4 are markers of resistance and susceptibility in a human intestinal nematode infection. *Microbes Infect 7:* 990–996.
42. King CL, Xianli J, Malhotra I, Liu S, Mahmoud AA, Oettgen HC, 1997. Mice with a targeted deletion of the IgE gene have increased worm burdens and reduced granulomatous inflammation following primary infection with *Schistosoma mansoni*. *J Immunol 158:* 294–300.
43. Dessein AJ, Parker WL, James SL, David JR, 1981. IgE antibody and resistance to infection. I. selective suppression of the IgE antibody response in rats diminishes the resistance and the eosinophil response to *Trichinella spiralis* infection. *J Exp Med 153:* 423–436.
44. Gurish MF, Bryce PJ, Tao H, Kisselgof AB, Thornton EM, Miller HR, Friend DS, Oettgen HC, 2004. IgE enhances parasite clearance and regulates mast cell responses in mice infected with *Trichinella spiralis*. *J Immunol 172:* 1139–1145.
45. Spencer L, Shultz L, Rajan TV, 2001. Interleukin-4 receptor–Stat6 signaling in murine infections with a tissue-dwelling nematode parasite. *Infect Immun 69:* 7743–7752.
46. Olsen A, van Lieshout L, Marti H, Polderman T, Polman K, Steinmann P, Stothard R, Thybo S, Verweij JJ, Magnussen P, 2009. Strongyloidiasis—the most neglected of the neglected tropical diseases? *Trans R Soc Trop Med Hyg 103:* 957–972.
47. Schaffel R, Nucci M, Carvalho E, Braga M, Almeida L, Portugal R, Pulcheri W, 2001. 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 65:* 346–350.
48. Norsyahida A, Riazi M, Sadjjadi SM, Muhammad Hafiznur Y, Low HC, Zeehaida M, Noordin R, 2013. Laboratory detection of strongyloidiasis: IgG, IgG4 and IgE-ELISAs and cross-reactivity with lymphatic filariasis. *Parasite Immunol 35:* 174–179.
49. Rascoe LN, Price C, Shin SH, McAuliffe I, Priest JW, Handali S, 2015. Development of Ss-NIE-1 recombinant antigen based assays for immunodiagnosis of strongyloidiasis. *PLoS Negl Trop Dis 9:* e0003694.