Introduction

Strongyloides stercoralis is an intestinal nematode transmitted by skin penetration of the infective larvae (L3) present in contaminated soil. It infects around 370 millions people worldwide, especially in tropical countries, where poor socio-economic and sanitary conditions combined with a suitable environment contribute to the maintenance of its transmission [1]. However, in spite of being a neglected tropical disease (NTD), human strongyloidiasis is an increasing problem in developed countries, where travelers and immigrants from tropical areas are the most commonly affected individuals [2]. Usually, this parasite develops a chronic and asymptomatic infection and the host can remain infected and undiagnosed for decades, despite the life span of Strongyloides stercoralis parthenogenic female be around five years. This occurs because this parasite has an unusual characteristic: the ability of produce an autoinfecctive cycle [3].

In this way, the parasite can reproduce within the host and produce new generations, leading to a lifelong infection. However, when an alteration in the immune system occurs, the parasite-host balance can be broken and it results in life-threatening conditions such as hyperinfection syndrome and disseminated strongyloidiasis. Factors predisposing to severe strongyloidiasis include corticosteroid therapy, Human T-cell lymphotropic virus type 1 (HTLV-1) infection and chronic use of alcohol [4-6]. Therefore, an effective diagnosis combined with an adequate treatment are essential for the severe strongyloidiasis prevention. Currently, S. stercoralis infection diagnosis is still performed through parasitological methods, by the finding of larvae in feces. The most used technique is Baermann-Moraes, which is based in the positive larval thermo-hydrotropism. However, studies have shown that Agar Plate Culture (APC) is more sensitive, yet it is underused due to the more complex methodology and higher cost [7]. Nonetheless, independent on the technique used, all parasitological methods are limited by the parasite load and larvae output intermittence, which requires the analysis of, at least, three fecal samples collected on alternate days. Besides, other factors also influence in the parasitological diagnosis efficacy, such as the amount, preservation and processing of stool samples and the experience of the microscopist in the identification of larva.

Immunological methods represent an alternative for S. stercoralis diagnosis. Few studies have evaluated coproantigen diagnostic tests, which use has been limited by the difficulty in producing monoclonal or polyclonal antibodies to S. stercoralis antigens and the low specificity of developed tests [8,9].

Most works have evaluated immunoassays for antibodies detection. Although different biological materials, such as urine [10], saliva [11] and breast milk [12], can be suitable for this purpose, the most common are serum samples. The first articles, to our knowledge, on this matter were published in 1981 by
groups from United States of America (USA) and Australia using *S. stercoralis* and *S. ratti* antigen to detect IgG anti-*S. stercoralis* by enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescent assay (IFA) [13,14]. Since then, several improvements have been made trying to increase the sensitivity and specificity of diagnostic methods based on antibody detection. The sensitivity and specificity of various reported serological tests ranged from 56 to 100% and 29–100%, respectively, depending on the type of assay, antigen, antibody-isotype, cut-off, study population and reference method used [15]. Several techniques, such as the luciferase immunoprecipitation system (LIPS) [16], immunoblotting [17] and fluorescent bead (Luminex) [18] have been studied, but the use is usually restricted to research centers or reference laboratories.

The most commonly used method is still ELISA. As matter of fact, there are three commercial assays available, the Bordier-ELISA (Bordier Affinity Products SA, Switzerland) and IV-D-ELISA (Scimedx Corporation, USA) using *Strongyloides ratti* and *S. stercoralis* crude antigens, respectively, and one with a recombinant antigen (NIE-ELISA, Novalisa; NovaTec Immunodiagnostica, Dietzenbach, Germany). However, two major limitations hamper the use of antibodies detection as a routine technique: false-positive results and the antigen production. False-positive results are common in an indirect diagnosis and can be caused by remaining antibodies after the parasitological cure (immunological memory) or by cross-reactivity with other parasites [19]. These problems can be reduced by the use of different antibodies classes and subclasses. Patients with strongyloidiasis have been reported to produce different isotypes of immunoglobulins against 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 detect mainly specific IgG and, to a lesser extent, IgG4 [15]. Some authors have suggested that IgE is a better marker for recent infections [20,21] and that IgG4 provides the highest sensitivity and specificity for *S. stercoralis* diagnosis [18]. Indeed, an increased specificity, of around 13%, was observed when IgG was changed by IgG4 as the secondary antibody in a *Strongyloides*-ELISA test (22). Concerning to the antigen production, the limitation comes from the difficulties in produce either homologous or heterologous antigens, once both depend on infected hosts. Recombinant antigens are a good alternative to substitute crude antigens and overcome those difficulties. Three recombinant antigens to *S. stercoralis* diagnosis are described in the literature: NIE, who is already commercially available as an ELISA test, and has been tested in other techniques, such as Luciferae Immunoprecipitation Systems Assay (NIE-LIPS) [16] and Luminex [18]; Ss-IR, who was used as a diagnostic tool and tested as a vaccine candidate in mice [23-25] and a recent discovered S. stercoralis recombinant protein named as rSs1a, used in an IgG4-ELISA [26]. A study comparing the sensitivity and specificity of Bordier-ELISA and NIE-ELISA found that despite the NIE-ELISA be more specific than the Bordier-ELISA, its low sensitivity limits its use in *S. stercoralis* screening [27]. Furthermore, more studies are needed to evaluate these antigens for *S. stercoralis* diagnosis in different endemic areas, where cross-reactions with other parasites may occur.

Thus, *S. stercoralis* diagnosis still remains a challenge since there is not a gold-standard method. Larvae absence in feces cannot be used as definitive non-infection evidence, pointing out serological methods as an alternative. However, they also have limitations for *S. stercoralis* diagnosis. Despite this, its use is extremely important, especially in patients in risk to develop severe strongyloidiasis. In these cases, a high specific antibodies level can justify the treatment for strongyloidiasis to prevent a life-threatening infection [28]. While there are limited studies on the use of serological follow-up for monitoring seroreversion in patients after treatment, negativization or even the decrease in antibody reactivity are strong indicators of a successful treatment [29]. In our research group, in two different patients with strongyloidiasis, one with Hansen’s diseases and under corticosteroid chronic use [5] and one with HTLV-1 [4], it was possible to observe a negativization to IgG-ELISA after a successful treatment. In this way, studies that pursue the improvement of immunoassays to *S. stercoralis* infection diagnosis are extremely needed. Meanwhile, physicians should be aware of the antibody detection usefulness as a diagnostic tool in patients at risk of developing severe strongyloidiasis.

**References**

1. Bisoffi Z, Buonfrate D, Montesor A, Requena Méndez A, Muñoz J, et al. (2013) Strongyloides stercoralis: a plea for action. PLoS Negl Trop Dis 7(5): e2214.

2. Buonfrate D, Angheben A, Gobbi F, Muñoz J, Requena Mendez A, et al. (2012) Imported Strongyloidiasis: Epidemiology, Presentations, and Treatment. Curr Infect Dis Rep 14(3): 256-262.

3. Viney M (2017) Strongyloides. Parasitol Today marco de 144(3): 259-262.

4. De Souza JN, Soares BNRR, Goes LL, Lima CS, Barreto NMVP, et al. (2018) Case Report: Strongyloides stercoralis Hyperinfection in a Patient with HTLV-1: An Infection with Filariform and Rhabditiform Larvae, Eggs, and Free-Living Adult Females Output. Am J Trop Med Hyg 99(6): 1583-1586.

5. De Souza JN, Machado PR, Teixeira MC, Soares NM (2014) Recurrence of Strongyloides stercoralis infection in a patient with Hansen's disease: a case report. Leprosy review 85(1): 58-62.

6. Teixeira MCA, Pacheco PTF, Souza JN, Silva MLS, Inês EJ, et al. (2016) Strongyloides stercoralis Infection in Alcoholict Patients. BioMed Research International.

7. Inês E, De J, Souza JN, Santos RC, Souza ES, Santos FL, et al. (2011) Efficacy of parasitological methods for the diagnosis of Strongyloides stercoralis and hookworm in faecal specimens. Acta Tropica 120(3): 206-210.

8. Sykes AM, McCarthy JS (2011) A Copromatigen Diagnostic Test for Strongyloides Infection. PLoS Neglected Tropical Disease 5(2): e955.

9. El Badry AA (2009) ELISA-based coproantigen in human strongyloidiasis: a diagnostic method correlating with worm burden. J Egypt Soc Parasitol 39(3): 757-768.

10. Ruantip S, Eamudomkarn C, Tepchat S, Wangboon C, Sthithaworn J, et al. (2019) Accuracy of Urine and Serum Assays for the Diagnosis of Strongyloidiasis by Three Enzyme-Linked Immunosorbent Assay Protocols. Am J Trop Med Hyg 100(1): 127-129.
11. Bosqui LR, Gonçalves ALR, Gonçalves Pires MRF, Pavanelli WR, Conchon Costa I, et al. (2018) Immune complex detection in saliva samples: an innovative proposal for the diagnosis of human strongyloidiasis. Parasitol Today 14(8): 1090-1094.

12. Mota Ferreira DM, Gonçalves Pires Mdo R, Júnior AE, Sopelete MC, Abdallah VO, et al. (2009) Specific IgA and IgG antibodies in paired serum and breast milk samples in human strongyloidiasis. Acta Trop 109(2): 103-107.

13. Carroll SM, Karthigasu KT, Grove DI (1981) Serodiagnosis of human strongyloidiasis by an enzyme-linked immunosorbent assay. Trans R Soc Trop Med Hyg 75(5): 706-709.

14. Neva FA, Gam AA, Burke J (1981) Comparison of Larval Antigens in an Enzyme-Linked Immunosorbent Assay for Strongyloidiasis in Humans. J Infect Dis 144(5): 427-432.

15. Arifin N, Hanafiah KM, Ahmad H, Noordin R (2019) Serodiagnosis and early detection of strongyloides stercoralis infection. Journal of Microbiology Immunology and Infection 52(3): 371-378.

16. Anderson NW, Klein DM, Dornink SM, Jespersen DJ, Kabo Eck J, et al. (2014) Comparison of Three Immunoassays for Detection of Antibodies to Strongyloides stercoralis. Clin Vaccine Immunol 21(5): 732-736.

17. Carvalho EFG de, Sousa JEN de, Gonçalves ALR, da Cunha Junior JP, Costa Cruz JM (2015) Immunoblotting using Strongyloides venezuelensis larvae, parthenogenetic females or eggs extracts for the diagnosis of experimentally infected immunosuppressed rats. Experimental Parasitology 157: 117-123.

18. Rascoc LN, Price C, Shin SH, McAuliffe I, Priest JW, et al. (2015) Development of Ss-NIE-1 Recombinant Antigen Based Assays for Immunodiagnosis of Strongyloidiasis. PLOS Neglected Tropical Diseases 9(4): e0003694.

19. de Souza JN, Inês EDJ, Santiago M, Teixeira MC, Soares NM (2015) Specific antibody isotype responses in human strongyloidiasis: comparison with larval output. Parasite Immunol 21(10): 517-526.

20. 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(10): 517-526.

21. Costa Cruz JM, Madalena J, Silva DA, Sopelete MC, Campos DM, et al. (2003) Heterologous antigen extract in ELISA for the detection of human IgE anti-Strongyloides stercoralis. Revista do Instituto de Medicina Tropical de São Paulo 45(5): 265-268.

22. Norgyadh A, Riazi M, Sadjadi SM, Muhamad Hafiznur Y, Low HC, et al. (2013) Laboratory detection of strongyloidiasis: IgG, IgG4- and IgE-ELISAs and cross-reactivity with lymphatic filariasis. Parasite Immunol 35(5-6): 174-179.

23. Ramanathan R, Varma S, Ribeiro JM, Myers TG, Nolan TJ, et al. (2011) Microarray-Based Analysis of Differential Gene Expression between Infective and Noninfective Larvae of Strongyloides stercoralis. PLOS Neglected Tropical Diseases 5(5): e1039.

24. Ramanathan R, Burbelo PD, Groot S, Iadarola MJ, Neva FA, et al. (2008) A Luciferase Immunoprecipitation Systems Assay Enhances the Sensitivity and Specificity of Diagnosis of Strongyloides stercoralis Infection. J Infect Dis 198(3): 444-451.

25. Abraham D, Hess JA, Mejia R, Nolan TJ, Lok JB, et al. (2011) Immunization with the recombinant antigen Ss-IR induces protective immunity to infection with Strongyloides stercoralis in mice. Vaccine 29(45): 8134-8140.

26. Arifin N, Yunus MH, Nolan TJ, Lok JB, Noordin R (2018) Identification and Preliminary Evaluation of a Novel Recombinant Protein for Serodiagnosis of Strongyloidiasis. J Trop Med Hyg 98(4): 1165-1170.

27. Fradejas I, Herrero Martinez JM, Lizasoain M, Rodriguez de Las Parras E, Perez Ayala A (2018) Comparative study of two commercial tests for Strongyloides stercoralis serologic diagnosis. Trans R Soc Trop Med Hyg 112(12): 561-567.

28. Thamwiwat A, Mejia R, Nutman TB, Bates JT (2014) Strongyloidiasis as a Cause of Chronic Diarrhea, Identified Using Next-Generation Microarray-Based Analysis of Differential Gene Expression between Infective and Noninfective Larvae of Strongyloides stercoralis-Specific Immunoassays. Curr Trop Med Rep 1(3): 145-147.

29. Buonfrate D, Sequi M, Mejia R, Gimeno RO, Krolewicki AJ, et al. (2015) Accuracy of Five Serologic Tests for the Follow up of Strongyloides stercoralis Infection. PLOS Neglected Tropical Diseases 9(2): e0003491.