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Update on immunologic and molecular diagnosis of human strongyloidiasis

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ABSTRACT

Human strongyloidiasis is an intestinal parasitosis that may affect 100 million individuals. However, the prevalence rates of this infection may represent smaller values than the actual data, mainly due to difficulties in its diagnosis. The aim of this study was to update the immunological and molecular methods applied to the diagnosis of human strongyloidiasis. There is a great diversity of techniques used in the diagnosis of this parasitosis, such as immunofluorescence antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA), immunoblotting, luciferase immunoprecipitation system (LIPS), dispstick and polymerase chain reaction (PCR), all with advantages and disadvantages, and with unique features for specific purposes. Considering the magnitude of strongyloidiasis and the importance of early diagnosis, due to the possibility of chronicity and hyperinfection, this study analyzes the different methods currently employed, and demonstrates the necessity of developing innovative methodologies, which also maintain diagnostic accuracy, particularly for regions with limited technological resources.

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1. Introduction

Human strongyloidiasis is a neglected condition of major global distribution, particularly common in tropical and subtropical regions which present bad sanitary conditions, favorable to their development. Considering only acute infections, strongyloidiasis is a major intestinal infection in humans; however, the number of people potentially exposed or with subclinical infections represents a much higher value. Despite the fact that infection with *Strongyloides stercoralis* is usually self-limited and with low morbidity in immunocompetent individuals, it may become severe in cases of immunosuppression (Montes et al., 2009; Olsen et al., 2009; Paula and Costa-Cruz, 2011; Schär et al., 2013).

The interaction between *S. stercoralis* and the human host is complex due to its intrinsic ability to development, so that in infected individuals there may be three modes of progression of

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the disease: eradication of infection, chronicity and possibility of dissemination (Paula and Costa-Cruz, 2011). The presence of eggs, larvae and adult parasites in the intestinal mucosa resulting in an inflammatory reaction may cause malabsorption syndrome, chronic diarrhea with protein loss, hemorrhage and development of hypoalbuminemia, anemia and eosinophilia (Grove, 1996; Siddiqui and Berk, 2001). In pathological changes, where the parasitic load is increased, the enteritis is ulcerative, resulting in inflammation with severe ulceration with bacterial invasion, and subsequently replacement of the intestinal epithelium by fibrotic tissue (Juchems et al., 2008).

Infected individuals are asymptomatic in most cases, but some may experience abdominal discomfort. Pulmonary symptoms have varying intensity, featuring a cough with or without expectoration and dyspnea (Kunst et al., 2011). The migration of larvae may induce rupture of the alveolar capillaries causing hemorrhage and inflammatory infiltrate. More severe cases may lead to the development of Loeffler's syndrome with pulmonary edema and respiratory insufficiency (Marcos et al., 2008; Mirdha, 2009). However, symptoms resulting from these processes are similar to other parasitic diseases, which make complicate the clinical diagnosis (Siddiqui and Berk, 2001; Agrawal et al., 2009). The symptoms of strongyloidiasis are not only related to the parasitic load, but also to the immunosuppression conditions. In patients with hyperinfection, diagnosis of the parasitic form is possible by examination of the bronchial lavage secretion and alveolar fluid by fluorescence microscopy, peripheral blood and cerebrospinal fluid, in addition to biopsy of the digestive system (Normura et al., 1996; Siddiqui and Berk, 2001).

Patients with strongyloidiasis develop specific antibodies of the IgG, IgA, IgM and IgE classes. IgG titers are noticed two weeks post-infection (pi), with a peak around the sixth week that persists for up to 20 weeks pi. In human strongyloidiasis, most diagnostic methods involve the detection of IgG, since patients affected by this parasite exhibit high levels of this immunoglobulin in their serum (Genta, 1989; Grove, 1996). The cycle of the parasite suggests that it can also stimulate a local response mediated by systemic IgA antibodies. This IgA response, which provides protection against mucosal parasites, is the second most prevalent serum immunoglobulin and represents the most prominent class in the mucosal surface seromucous and secretions such as saliva, tears, cerebrospinal fluid and colostrum (van Egmond et al., 2001; Costa et al., 2003; Yoo and Morrison, 2005; Mestecky and Russell, 2009; Ribeiro et al., 2010).

The *Strongyloides*-specific IgM, which represents recent infection by *S. stercoralis*, shows a peak output one week post-infection and maintains high levels for more than two or three weeks. Elevated levels of IgE are found in immunocompetent patients with strongyloidiasis; however, in cases of hyperinfection in immunosuppressed individuals, levels of total and specific IgE may be within the normal range (Porto et al., 2001; Rodrigues et al., 2004, 2007; Marcos et al., 2008). This hypersensitivity mediated by antigen-specific IgE is the faster immune response against parasites (Galioto et al., 2006).

Human patients with hyperinfection by immunosuppression show a significant reduction in IgA and IgM levels, but there is no change in IgG. In the development of the immune response against *S. stercoralis*, mainly IgG1 and IgG4 are prevalent. It is believed that IgG1 has a protective role against infection and that IgG4 is involved in blocking the protective response promoted by IgE, reducing the expulsion of the parasite (Atkins et al., 1997; Rodrigues et al., 2007; Marcos et al., 2011).

1.1. Laboratory diagnosis

Several techniques have been developed with the goal of diagnosing strongyloidiasis, such as stool tests, immunological methods and molecular biology techniques. The parasitological examination allows the identification of parasitic forms of *S. stercoralis*. Among the examinations that enable this identification, there are: direct smear in saline, the spontaneous sedimentation method (Hoffmann et al., 1934), centrifugation (Ritchie, 1948), the method of Baermann–Moraes (Baermann, 1917; Moraes, 1948) and its variations, the agar plate culture (Arakaki et al., 1988) and the method of Harada–Mori (Harada and Mori, 1955). Although the stool-based methods are less sensitive than other immunological methods, some of these parasitological methods, such as the Baermann or the agar place culture, are clearly much more sensitive than others (Requena-Méndez et al., 2013). Inês et al. (2011) demonstrated that agar plate culture is the most sensitive parasitological method; however, the cost-benefit ratio of this method should be assessed in relation to the Baermann–Moraes technique.

The parasitological methods have low sensitivity because of the small and irregular release of larvae in the feces (Sato et al., 1995; Uparanukraw et al., 1999; Siddiqui and Berk, 2001). It has been demonstrated that the collection of a larger number of samples on alternate days may increase the sensitivity (Hirata et al., 2007; Knopp et al., 2008). However, as the examination of several samples is time consuming and quite inconvenient for the patient, many physicians are reluctant to use it (Hira et al., 2004).

Immunological methods have the advantage of showing a high sensitivity when compared with parasitological methods, in addition to being useful in the evaluation of the host immune response, as well as their use in seroepidemiological surveys (Rossi et al., 1993; Uparanukraw et al., 1999; Machado et al., 2008; Gonzaga et al., 2011b). Despite the difficulties in obtaining and purifying the antigen as well as standardization of techniques, various immunological methods have been described.

Among the methods that have higher sensitivity and specificity are: the immunofluorescence antibody test (IFAT), directed to research different classes of antibodies in sera; the enzyme-linked immunosorbent assay (ELISA), to detect antigen, antibody and immune complexes in serum samples, or coproantigen in feces; and Immunoblotting (IB), which is considered highly sensitive and specific in the recognition of protein fractions of infective larvae by antibodies in sera from patients with strongyloidiasis (Costa-Cruz et al., 1997; Uparanukraw et al., 1999; Van Doorn et al., 2007; Sykes and McCarthy, 2011; Gonçalves et al., 2012a).

The major limitation found in the standardization of more specific serological tests is the difficulty in obtaining infective larvae of *S. stercoralis* (Rossi et al., 1993; Costa-Cruz et al., 1998; Ribeiro et al., 2010). Due to this, standardization and the use of heterologous antigens from *Strongyloides cebus*, *Strongyloides ratti* and *Strongyloides venezuelensis* have been convenient (Campos et al., 1988; Costa-Cruz et al., 1998; Rodrigues et al., 2007; Gonzaga et al., 2011a,b). The antigenic characterization of eight strains of *S. venezuelensis* using serum samples from patients that were positive for *S. stercoralis* was conducted by Machado et al. (2008). A common fraction of 45 kDa was recognized in the eight strains by anti-*S. stercoralis* at an average rate of sensitivity and specificity of 93% and 100%, respectively, for IFAT and ELISA. A transcriptome analysis of *S. stercoralis* with other nematodes including *S. ratti* revealed a similarity in the transcription of molecules that have key roles in host-parasite interactions, as well as important molecules in the diagnosis, such as excretory-secretory proteins (Marcilla et al., 2012).

Because of the fact that most patients are asymptomatic, the difficulty in diagnosing this parasite in stool samples and the absence of a gold standard for the diagnosis of this parasitosis, many cases of strongyloidiasis are not diagnosed. For this reason, strongyloidiasis can be regarded as a neglected tropical disease of major public health importance. The objective of this study was to review the literature on what is most recent and innovative in order to analyze the results of immunological and molecular methods used to detect the parasite.