Strategies for production and evaluation of recombinant antigens for *Strongyloides stercoralis* infection diagnosis

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Currently, *Strongyloides stercoralis* infection diagnosis is, mainly, carried out through parasitological methods. However, its sensitivity varies according to several factors, which makes the result unreliable. Serological methods can be an alternative. But crude antigens are difficult to produce and presents cross-reactions. Thus, the objective of this study is to produce and evaluate the sensitivity and specificity of recombinant antigens for *S. stercoralis* infection diagnosis. Initially, *Strongyloides venezuelensis* soluble (SvS) and *Strongyloides venezuelensis* membrane (SvM) antigens were separated into different fractions according to molecular weight through FPLC (Fast Protein Liquid Chromatography). Each fraction was tested by immunoblotting, finding six immunoreactive fractions for each antigen. These fractions were analyzed by Tandem Mass Spectrometry (MS/MS), through which 15 proteins were identified as a potential antigen. The gene sequence of each protein of interest was sent to the GenScript® laboratory and inserted into the pGs21a vector for preparation of recombinant plasmids. *Escherichia coli* BL21(DE3)/pLysS strains were transformed to express these proteins and its sensitivity and specificity will be tested by ELISA and immunoblotting. Meanwhile, two recombinant antigens already described in the literature, rSs-NIE and rSs-IR, were evaluated through IgG-Immunoblotting in comparison to three crude antigens: *Strongyloides stercoralis* soluble (SsS), *Strongyloides venezuelensis* soluble (SvS) and *Strongyloides venezuelensis* membrane (SvM) antigens using sera from 50 alcoholic patients in which 20 were infected with *Strongyloides stercoralis*, 15 were infected with other intestinal helminths (8 with *Schistosoma mansoni* and 7 with hookworms) and 15 with negative parasitological diagnosis in three stool samples, analyzed on alternate days, using three different methods (Spontaneous sedimentation, Baermann-Moraes and Agar Plate Culture (APC)). It was observed that SvM presented the best result, with a sensitivity and specificity of 100 (20/20) and 90% (27/30), respectively, with four bands chosen as diagnostic criteria, 90, 75, 26, and 22 kDa. rSs-NIE, rSs-IR, SvS and SsS presented a sensitivity of 95 (19/20), 85 (17/20), 75 (15/20) and 75% (15/20), respectively. The specificity presented by the same antigens were 67 (20/30), 33 (10/30), 87 (26/30) and 87 (26/30), respectively. Both recombinant antigens, rSs-NIE and rSs-IR, presented a high rate of cross-reactions, 60 (9/15) and 87% (13/15), respectively. Thus, the best antigen for diagnosing strongyloidiasis through immunoblotting was the crude membrane antigen of *S. venezuelensis*, which points to the need to produce new recombinant antigens with lower rates of cross-reactions.

Keywords: *Strongyloides stercoralis*, Diagnosis, Recombinant Antigen.