



Alcohol consumption alters anti-*Strongyloides stercoralis* antibodies production

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ABSTRACT

Individuals infected with *Strongyloides stercoralis* have been reported to produce different immunoglobulins isotypes, yet few studies have evaluated their use in strongyloidiasis diagnosis. The aim of this work was to evaluate the immunoreactivity of different classes and subclasses of anti-*S. stercoralis* circulating antibodies in alcoholic patients by ELISA and to perform immunoblotting in samples with discordant results between parasitological and immunological methods. 345 male patients with a clinical diagnosis of alcoholism hospitalized at a reference center for alcoholics in Salvador, Bahia, Brazil, were included in this study. The fecal samples were examined by three different parasitological methods (spontaneous sedimentation, Baermann-Moraes and Agar Plate Culture methods). The ELISA was performed for the detection of IgG, IgG1, IgG4, IgE and IgA1 anti-*S. stercoralis*. Immunoblotting, for the detection of specific IgA1, was used to elucidate discordant results between parasitological and immunological methods. *S. stercoralis* infection frequency in alcoholic patients by parasitological methods was 21.4% (74/345). Although IgE-ELISA demonstrated a high sensitivity and specificity in non-alcoholic patients, about 30% (22/74) of alcoholics with larvae in feces were negative. IgG1-ELISA detected the lowest frequency of antibodies in alcoholic patients with larvae in feces, only 57% (42/74). IgG4-ELISA was the best assay for *S. stercoralis* infection immunodiagnosis. Immunoreactivity in the immunoblotting for IgA1 at 90, 75, 26 and/or 17 kDa bands was observed in 92% (33/36) of alcoholics with larvae excretion and negative ELISA for one or more antibody isotypes. In conclusion, IgG4-ELISA showed the highest sensitivity and specificity, thus demonstrating its superiority for strongyloidiasis immunodiagnosis in alcoholic and non-alcoholic individuals. Both, IgE and IgG1-ELISA presented high sensitivities and specificities for *S. stercoralis* infection diagnosis in non-alcoholics, however there was low reactivity in alcoholic individuals. This can be associated with an increased susceptibility to severe strongyloidiasis in these patients. IgA1-immunoblotting can be used to confirm *S. stercoralis* infection when there are discordant results between parasitological methods and ELISA.

1. Introduction

Strongyloidiasis is a parasitic disease mainly caused by the helminth *Strongyloides stercoralis* (Teixeira et al., 2016; Nutman, 2017). It is generally asymptomatic, however when it occurs in parallel with an impaired host immune response, severe clinical conditions such as hyperinfection syndrome and disseminated disease can develop (Viney, 2017; Andreetta Corral et al., 2019a). The main co-morbidities associated with severe strongyloidiasis are co-infection with HTLV-1,

chronic use of corticosteroids, and alcoholism (Teixeira et al., 2016; De Souza et al., 2014; de Souza et al., 2015a; De Souza et al., 2018; de Souza et al., 2015b). In Brazil, *S. stercoralis* infection prevalence in alcoholic individuals is around 20%, almost 5 times higher than in the general population (Teixeira et al., 2016; Zago-Gomes et al., 2002; Silva et al., 2016). Chronic alcohol use alters the hypothalamic-pituitary-adrenal (HPA) axis function, raising the levels of endogenous corticosteroids and their metabolites, which can lead to increased susceptibility to severe strongyloidiasis (Teixeira et al., 2016). Also, there is a

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hypothesis where corticosteroids metabolites mimic an ecdysteroid hormone that regulates the fertility of *S. stercoralis* parthenogenetic females, inducing the transformation of rhabditiform to infective filariform larvae, which increases the autoinfection rate; however, more studies are necessary to prove this theory (Siddiqui et al., 2000; Genta, 1992). A recent study by our group demonstrated that increased levels of endogenous cortisol is directly related to an increased parasite load (Silva et al., 2016). Moreover, chronic alcohol consumption also has a toxic effect on the contractile proteins of the small intestine muscle and on vagal function, reducing gastrointestinal flow and favoring rhabditoid larvae permanence in the host intestinal lumen, which also enhances the autoinfection cycle (Addolorato et al., 1996; Leclercq et al., 2014).

Currently, *S. stercoralis* diagnosis depends on larvae detection in fecal samples. However, larval excretion occurs intermittently, raising the need for multiple samples analyses (Inês E de et al., 2011; Silva et al., 2019). Serological methods aid in diagnosis of strongyloidiasis, although their use is limited by false-positive results due to immunological memory and cross-reactions with other parasites and by the difficulties in producing homologous and heterologous antigens (Bisoffi et al., 2014; Buonfrate et al., 2015; Mendes et al., 2017). To circumvent the need for infected hosts for the native antigen preparation, recombinant antigens can be produced. They already have been used in *S. stercoralis* diagnosis, however more studies are necessary to establish their sensitivities and specificities in different groups of individuals (Krolewiecki et al., 2010; Rascoe et al., 2015).

S. stercoralis infection induces rapid production of IgE, IgG1, IgG2 and IgG3 antibodies, followed by an increase in IgG4 levels weeks after infection (Atkins et al., 1997, 1999). Despite this, few studies have evaluated the immunoreactivity of different classes and/or subclasses of antibodies in *S. stercoralis* infection diagnosis (Rossi et al., 1993; Rodrigues et al., 2007; Bosqui et al., 2015). Among the available immunodiagnostic methods, the most used is IgG-ELISA (Corstjens et al., 2014; Fradejas et al., 2018). However, immunoblotting can be an extremely useful tool because it allows the recognition of specific molecules, which makes the diagnosis more sensitive and specific (Corral et al., 2017; Andreetta Corral et al., 2019b). The objective of this work was to evaluate the immunoreactivity of different classes and subclasses of anti-*S. stercoralis* circulating antibodies in alcoholic patients by ELISA and to perform immunoblotting in samples with discordant results between parasitological and immunological methods.

2. Materials and methods

2.1. Serum sample sets

This study enrolled 345 male patients, aged 24–78 years old, with a clinical diagnosis of alcoholism, hospitalized for alcoholic detoxification at the Centro de Acolhimento e Tratamento de Alcoolistas (CATA), Obras Sociais Irmã Dulce, Salvador, Brazil. The patient selection occurred from August 2015 to June 2017. Parasitological examination was performed on three fecal samples for each patient by spontaneous sedimentation (Hoffman et al., 1934), Baermann-Moraes (MORAES, 1948), and Agar Plate Culture (Koga et al., 1991) methods. The patient sample was defined as *S. stercoralis* positive if larvae were detected by any of those methods. The larvae number was quantified in one gram of feces using the Baermann-Moraes method.

As a comparison group, 100 sera were selected from non-alcoholic individuals seen at the Laboratório de Análises Clínicas e Toxicológicas da Faculdade de Farmácia (LACTFAR), Universidade Federal da Bahia, Salvador, Brazil. Among the non-alcoholic individuals, 50 were infected with *S. stercoralis*, 20 were infected with other intestinal helminths (*Schistosoma mansoni*, hookworm, *Trichuris trichiura* and *Enterobius vermicularis*) and 30 were apparently healthy individuals whose parasitological examination produced a negative result. Individuals using glucocorticoids and coinfecting with HTLV-1 were excluded from this

study.

The project was approved by the Research Ethics Committee of the Escola de Enfermagem, Universidade Federal da Bahia, Salvador, Brazil, under the reference number 367.464. All patients were informed about the study and those who agreed to participate signed an Informed Consent Form.

2.2. Antigen production

S. stercoralis larvae were obtained from the feces of a hyperinfected patient and *S. venezuelensis* larvae were recovered from Wistar rat feces (*Rattus norvegicus*) (provided by the Departamento de Parasitologia, Universidade de Campinas, São Paulo, Brazil). The larvae were cultured in animal charcoal at 28 °C for five and three days, respectively, to obtain filariform larvae. Filariform larvae were collected and concentrated by Rugai's method (Rugai et al., 1954), then washed 5 times in 0.15 mol/L phosphate buffered saline (PBS, pH 7.2) and centrifuged for 7 min at 1,800 × g. The parasites were suspended for 5 min in 0.25% sodium hypochlorite and washed 5 times in PBS as described above. The larvae were suspended in PBS with protease inhibitors (5 mmol/L EDTA, 1 mmol/L phenyl-methyl sulfonylfluoride, 0.05 mmol/L TPCK/TLCK, 1 µg/mL leupeptin) and sonicated (Branson Sonifier Cell Disruptor, Branson Instruments, Danbury, CT, USA) in an ice bath until there was total larvae disruption. The larvae homogenate was then centrifuged at 17,500 × g for 30 min at 4 °C. The supernatant (soluble antigen) was stored at −70 °C and the resulting *S. venezuelensis* larvae pellet was re-suspended in 1% sodium deoxycholate and incubated overnight in a cold room (at 4 °C), under agitation. After incubation, the material was again centrifuged at 17,500 × g for 30 min at 4 °C and the supernatant (membrane antigen) stored at −70 °C. The protein content of both antigens was measured by the Bradford method (Bradford, 1976).

2.3. ELISA for specific antibodies detection

A pilot study was conducted to establish the ELISA conditions. Briefly, microtiter plates (Corning Costar polystyrene EIA/RIA plates, Corning) were coated with 3, 5, 5, 10 and 20 µg/mL *S. stercoralis* antigen in 0.06 mol/L carbonate-bicarbonate buffer (pH 9.6) for IgA1, total IgG, IgG1, IgE and IgG4-ELISA, respectively, incubated overnight at 4 °C, and washed 3 times with PBS containing 0.05% Tween-20 (PBS-T). The plates were blocked with 100 µL/well of PBS-T containing 8% w/v of skim milk (blocking buffer) for 1 h. After blocking, the wells were washed as described previously.

Sera samples were diluted in blocking buffer according to each antibody isotypes (1:100 for IgG and IgA1, 1:4 for IgG1 and IgG4 and 1:2 for IgE) and incubated at a volume of 100 µL/well and incubated for 1 h. After washing as before, 100 µL of conjugated anti-human IgG, IgG1, IgG4 and IgA1 linked to horseradish peroxidase (Sigma-Aldrich, St. Louis, MO, USA) diluted in blocking buffer at 1:2000, 1:500, 1:500 and 1:1000, respectively, was added to the plate and incubated under the same conditions as previously described. A biotinylated conjugate (Sigma-Aldrich, St. Louis, MO, USA) diluted 1:1000 was used for IgE-ELISA and, in this case, the reaction was amplified with 100 µL/well of streptavidin (Sigma -Aldrich, St. Louis, MO, USA) diluted at 1:500 in blocking buffer with 30 min incubation. All reactions were visualized by the addition of 100 µL of substrate (0.051 mol/L citrate-phosphate buffer [pH 5.0] containing 0.0037 mol/L p-phenylenediamine and 0.04% hydrogen peroxide) and incubation for 20 min in the absence of light, followed by the addition of 20 µL of 8 N sulfuric acid to stop the reaction. The absorbance (Abs) was measured at 450–630 nm with a microplate reader (Awareness Technology, USA).

2.4. Polyacrylamide gel electrophoresis (SDS-PAGE) and Immunoblotting (IB)

After a previous study of our group using several antibodies isotypes and subtypes in immunoblotting, it was demonstrated that IgA1 presented the better results, with reactivity in bands at 90, 75, 26 and 17 kDa in most *S. stercoralis* infected patients (data not published). Therefore, this assay was chosen for the confirmatory diagnostic test. In this way, IgA1-IB was used to confirm the result in 36 samples with larvae excretion in the feces and negative ELISA for one or more antibody classes and/or subclasses. The preparation of IB strips and the IB development has been described previously (Tsang et al., 1983). Briefly, *Strongyloides venezuelensis* membrane antigen at a concentration of 50 ng/ml was subjected to SDS-PAGE using 8–16% pre-fabricated gel (Criterion™ TGX™ Precast Gel, Bio-Rad Laboratories, USA). Samples were re-suspended in a sample buffer [10% SDS, 6% glycerol, and tracking dye (50 mg bromophenol blue, 8 ml glycerol, 1 ml 0.5 M Tris HCL (pH 8.0), 1 ml distilled water)] and heated at 65 °C for 15 min and electrophoresed at 200 V for 30 min at room temperature. Immediately after electrophoresis, the proteins on the gel were transferred onto nitrocellulose membranes with a semi-dry trans-blot apparatus (Bio-Rad). The membranes were incubated overnight with sera diluted with 5% skim milk at 1:100. After three washes with PBS Tween (0.05%), the membranes were again incubated with a secondary biotinylated-conjugated anti-human IgA1 antibody diluted at 1:1000 for 1 h at room temperature. After washing, peroxidase-streptavidin diluted at 1:2000 was added, followed by further incubation for 1 h. Bands were visualized after the strips were incubated with 0.05% diaminobenzidine (DAB; Sigma-Aldrich), 0.015% H₂O₂, in PBS, pH 7.4. Samples with reactivity to bands at 90, 75, 26 and/or 17 kDa were considered positive.

2.5. Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Science (version 19.0 for Windows, SPSS) and Graph Pad Prism 5.0 (Graph Pad Software, Inc., San Diego, California, USA) softwares. To test the data normality assumption, the coefficients of skewness and kurtosis were calculated, and a Gaussian distribution was considered when the coefficient of asymmetry presented values between -1 and +1. The quantitative variables were presented through the descriptive measures (mean and standard deviation), while the semi-quantitative variables were presented in terms of frequency. Differences between means were evaluated using the unpaired *t*-test to compare two groups, while the differences in frequencies were measured using the chi-square test. All tests were bi-causal and the statistical significance was considered when *p* presented a value lower than 0.05.

The cut-off point, sensitivity and specificity for ELISA were established using the Receiver operating characteristic (ROC) curve with sera from non-alcoholic individuals infected with *S. stercoralis* or other intestinal helminths and negative for any intestinal parasites.

3. Results

S. stercoralis infection frequency by parasitological methods was 21.4% (74/345), with the parasite load ranging from 1 to 958 larvae/gram of feces. The mean was 47.6 larvae/g of feces, with a standard deviation of 150.

The sensitivity and specificity for each ELISA using sera from non-alcoholic patients are shown in Fig. 1. The possible cross-reactions occurred only with sera from patients infected with *Schistosoma mansoni* and hookworm. The IgG-ELISA had the highest rate of cross-reactivity, with 40% (8/20) of sera from individuals with other helminth infections testing positive (Fig. 1A). Although the IgE-ELISA demonstrated a high sensitivity and specificity in non-alcoholic patients, 30% (22/74) of alcoholics with larvae in the feces were negative for specific IgE

antibodies. The mean parasite load in alcoholics testing negative by IgE-ELISA was significantly higher than in those testing positive by IgE-ELISA, 62.2 and 41.4 larvae/g of feces, respectively ($p < 0.01$) (Fig. 2). IgG1-ELISA detected the lowest frequency of antibodies in alcoholic patients with confirmed *S. stercoralis* infection, only 57% (42/74) (Table 1). Moreover, the mean optical density in IgG1-ELISA for *S. stercoralis*-infected alcoholic patients was statistically lower compared to non-alcoholic infected patients ($p < 0.05$) (Table 1). In addition, there was a statistically significant negative correlation between parasite load and IgG1 levels in alcoholic patients ($r = -0.273$, $p < 0.05$) (Table 1). Also, similarly to IgE, the mean parasite load in negative IgG1-ELISA alcoholic patients was higher than in positive IgG1-ELISA (Fig. 2).

Although the IgA1-ELISA sensitivity and specificity were slightly lower than IgE-ELISA, in alcoholic patients, IgA1-ELISA was able to detect a higher number of individuals with fecal larvae excretion (80%; 59/74) (Table 1). Also, the mean parasite load in positive IgA1-ELISA alcoholic patients was lower than in negative IgA1-ELISA (Fig. 2). Among the tested antibodies, IgG4-ELISA was the best assay for *S. stercoralis* infection diagnosis for both alcoholic and non-alcoholic patients (Fig. 1E, Table 1).

A total of 36 individuals with positive parasitological examination were negative for one or more antibody classes in the ELISA, 11 for IgG4, 15 for IgA1, 17 for total IgG, 22 for IgE and 32 for IgG1 (Table 1). Among these, immunoblotting was positive in 92% (33/36) of the cases.

4. Discussion

S. stercoralis infection can progress to hyperinfection and/or dissemination in immunocompromised patients. In a previous study conducted in our laboratory, the frequency of this infection in alcoholic patients was 23.5% (Silva et al., 2016), which is in agreement with the result found in this most recent study. Other authors have also found a high strongyloidiasis prevalence in alcoholic patients, ranging from 20.5–40.2% (Zago-Gomes et al., 2002; Gaburri et al., 1996; Marques et al., 2010). However, this prevalence is probably underestimated, since the intermittent larval excretion and the parasite load fluctuation can influence the parasitological diagnostic methods' sensitivity (Inês E de et al., 2011). Serological tests are an alternative that does not depend on larval elimination in the feces, but more studies are necessary to evaluate such tests, since the current methods still have problems with sensitivity, specificity and cross-reactions with other helminths.

In this work, the ELISAs for detection of different classes and subclasses of circulating antibodies demonstrated sensitivity and specificity varying from 80 to 96% and 70 to 94%, respectively. This finding is in agreement with the results obtained by other authors (de Souza et al., 2015a; Silva et al., 2016; Buonfrate et al., 2015; Inês et al., 2013; Bosqui et al., 2017; Ruantip et al., 2019). The IgG-ELISA is the most used method for *S. stercoralis* immunodiagnosis, probably because IgG is the most abundant circulating immunoglobulin (Marcos et al., 2011). However, its use has been limited by the elevated frequency of cross-reactions with other helminths and the persistence of high levels of antibodies after cure, making it difficult to distinguish between active and past infections (van Doorn et al., 2007). In this study, IgG-ELISA presented a higher number of cross-reactions with sera from individuals with other infections, resulting in the lowest specificity between the tested antibodies, only 70%.

IgG antibodies fall into four subclasses: IgG1, IgG2, IgG3 and IgG4 (Schur, 1988). Although they have more than 90% similarity at the amino acid level, each subclass has a unique profile in the antigen binding, immune complex formation, complement activation, effector cell stimulus and half-life (Vidarsson et al., 2014). Despite the high sensitivity and specificity of IgG1-ELISA in non-alcoholic patients, immunoreactivity in alcoholic patients with confirmed *S. stercoralis* infection was only 57% (42/74). Additionally, the mean optical density in

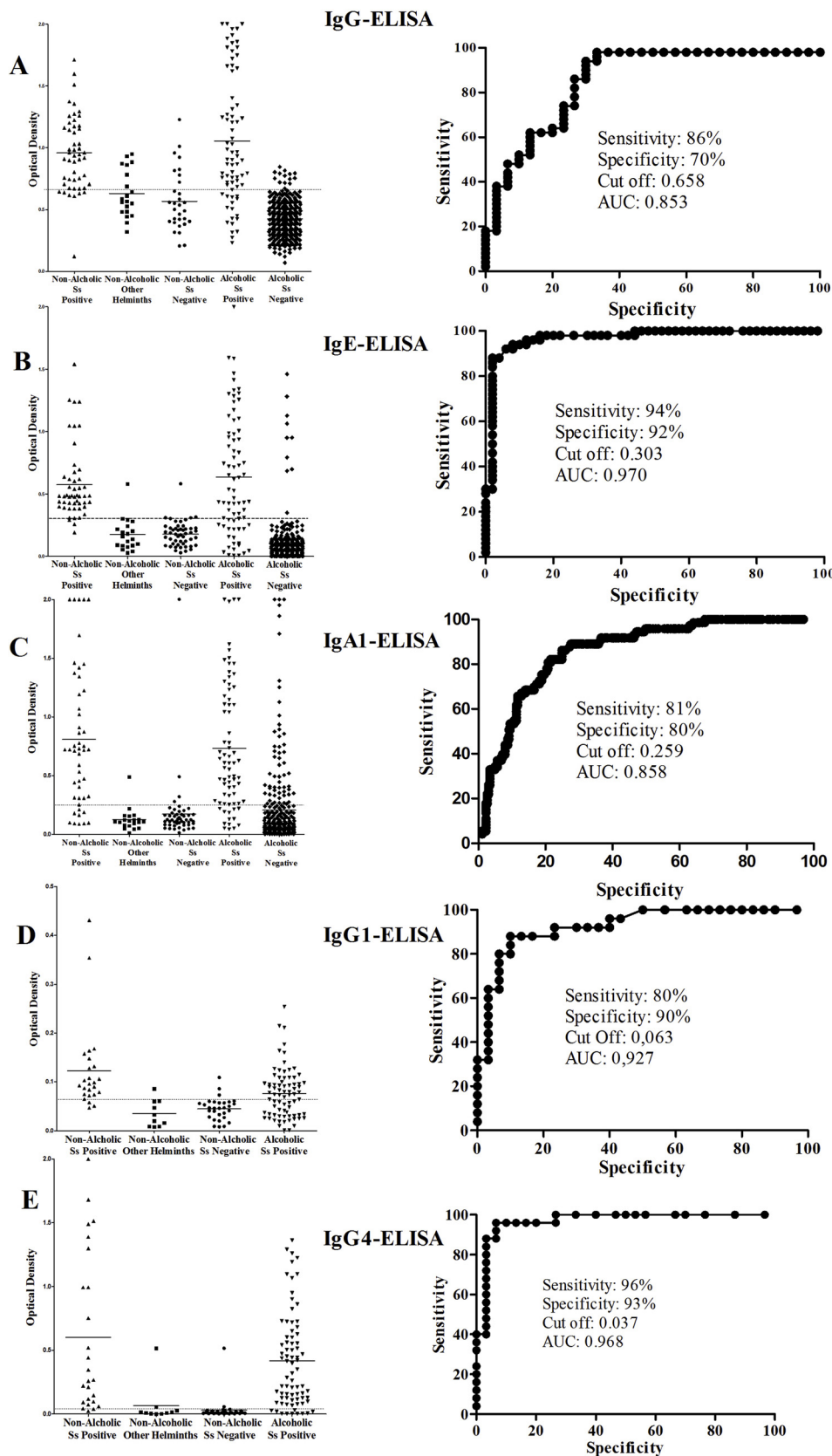


Fig. 1. IgG (A), IgE (B) and IgA1(C), IgG1 (D) and IgG4 (E) anti- *S. stercoralis* ELISA in non-alcoholic and alcoholic patients attended at CATA and LACTFAR, UFBA. 100 non-alcoholic patients: 50 infected with *S. stercoralis* (▲); 20 infected with other helminths (■) and 30 without any parasites in feces (•). 345 alcoholic patients: 74 infected with *S. stercoralis* (▼) and 271 non-infected with *S. stercoralis* (◆) and ROC Curve indicating sensitivity, specificity, cut off and Area Under the Curve (AUC). For IgG1 and IgG4, 60 non-alcoholic patients (25 infected with *S. stercoralis*; 10 infected with other helminths and 25 without any parasites in feces) and 74 alcoholic patients with larvae excretion were selected.

infected alcoholic individuals was significantly lower than in non-alcoholic infected individuals, demonstrating a low IgG1-specific reactivity in alcoholics. This fact may be related to the increased susceptibility to severe strongyloidiasis in alcoholics, since specific IgG1

was the only antibody that showed a negative correlation with the parasite load ($p < 0.05$). Also, we observed that the parasite load was significantly higher in IgG1-ELISA negative alcoholics than in IgG1-ELISA positive alcoholics infected with *S. stercoralis* ($p < 0.05$). On the

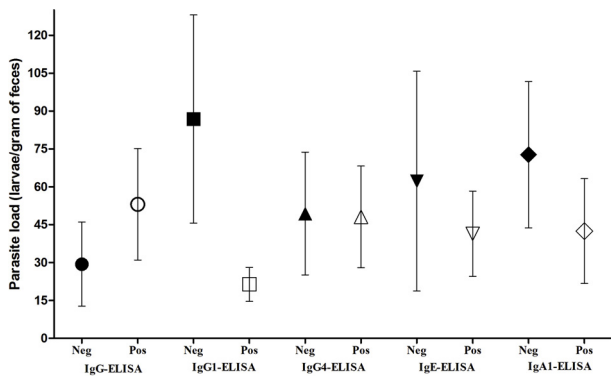


Fig. 2. Mean parasite load and standard deviation in alcoholic patients infected with *S. stercoralis* (n = 74) positive or negative in the ELISA for different antibodies isotypes and subtypes.

other hand, IgG4-ELISA detected the highest number of alcoholic and non-alcoholic individuals with larvae excretion in feces, confirming its potential to be used in the strongyloidiasis diagnosis and demonstrating a preserved IgG4 specific response in alcoholic patients.

Despite the IgE-ELISA high sensitivity, 30% (22/74) of alcoholic patients with larvae excretion were negative, indicating a low IgE-specific response. In addition, similar to the verified in IgG1-ELISA, we observed that the mean parasite load was significantly higher in IgE-ELISA negative than in IgE-ELISA positive alcoholic patients infected with *S. stercoralis* ($p < 0.05$), which may indicate an increase in the number of parasites in the absence of an IgE-specific response. IgE has been associated with acquired resistance to autoinfection, resulting in a decrease in the parasite load (Atkins et al., 1999). The immune response in alcoholic individuals is not yet fully understood, but several studies have demonstrated a profile of inflammatory response in alcoholism, responsible for several of the pathogenic manifestations of this disease (Teixeira et al., 2016). In contrast, high levels of total IgE have also been reported (Teixeira et al., 2016; González-Quintela et al., 2002; Lomholt et al., 2016). This is the first study, to our knowledge, which demonstrates a low specific IgG1 and IgE response in alcoholic individuals. Further studies are needed to clarify the role of alcohol in the specific production of these antibodies.

Although IgA1-ELISA did not present the highest sensitivity and specificity it was able to detect 80% (59/74) of alcoholic patients with larvae excretion. The presence of *S. stercoralis* in the intestinal submucosa probably stimulates local production of specific IgA, which modulates the infection and limits the number of larvae excreted in the feces (Atkins et al., 1997; Bosqui et al., 2015; Gonçalves et al., 2012). These findings explain the decrease in the mean parasite load in IgA1-ELISA positive patients. In addition, in this study, 20.3% (55/271) of the alcoholic patients without larvae fecal excretion were IgA1-ELISA

positive, whereas in non-alcoholic patients a IgA1-ELISA positive result was observed in only 10% (3/30) of the individuals. IgA, when present, can act in the destruction of the parasite, which can explain the non-elimination of larvae in the feces. However, cross-reactions and the IgA1-ELISA specificity can also be related to this finding.

Immunodominant proteins with molecular weights varying from 12 to 150 kDa were described in *S. venezuelensis* antigen in reaction to *S. stercoralis* infected individuals' sera (Andreetta Corral et al., 2019a; Corral et al., 2017). In this work, the IB using *S. venezuelensis* membrane antigen for detection of IgA1 anti-*S. stercoralis* was positive in 92% (33/36) of the patients with larvae excretion and ELISA negative for one or more classes of antibodies. This demonstrates the superiority of this method in comparison to ELISA, even when the antibody level is low (Silva et al., 2003).

In conclusion, both IgE and IgG1-ELISA presented high sensitivities and specificities for strongyloidiasis diagnosis; however, a low reactivity was found in alcoholics. Possibly, chronic alcohol intake interferes with the production of these antibodies and, therefore, IgE and IgG1-ELISA are not indicated for strongyloidiasis diagnosis in these patients. IgG4-ELISA showed the highest sensitivity and specificity, demonstrating its superiority for strongyloidiasis immunodiagnosis in alcoholic and non-alcoholic individuals. Immunoblotting with reactivity at 90, 75, 26 and/or 17 kDa bands for IgA1 can be used to confirm *S. stercoralis* infection in discordant cases between parasitological methods and ELISA.

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Declaration of Competing Interest

None.

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Joelma Nascimento De Souza: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Alana das Virgens Cruz:** Investigation. **Wéslei Almeida Costa Araújo:** Investigation. **Larissa Mota Sampaio:** Investigation. **Silmara Marques Allegretti:** Resources, Writing - review & editing. **Márcia Cristina Aquino Teixeira:** Methodology, Writing - review & editing, Supervision. **Sukwan Handali:** Investigation, Writing - review & editing, Supervision. **Bernardo Galvão-Castro:** Writing - review & editing, Supervision. **Neci Matos Soares:** Conceptualization, Methodology, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project

Table 1

IgG, IgE, IgA1, IgG1 and IgG4 specific antibodies evaluation by ELISA in alcoholic and non-alcoholic patients infected or non-infected with *S. stercoralis* (Ss) and correlation with optical density and parasite load.

ELISA detection	Optical density mean		Alcoholic patients antibodies frequency (%)		Correlation with parasite load ¹	
	Non-alcoholic Ss Positive ² (n = 50)	Alcoholic Ss Positive (n = 74)	Ss positive (n = 74)	Ss negative (n = 271)	R	p
IgG	0.958	1.053	77 (57/74)	5.2 (14/271)	-0.048	0.687
IgE	0.574	0.633	70 (52/74)	3.7 (10/271)	-0.151	0.197
IgA1	0.809	0.734	80 (59/74)	20.3 (55/271)	0.080	0.501
IgG1 ³	0.123	0.077*	57 (42/74)	**	-0.273	0.019*
IgG4 ³	0.603	0.417	85 (63/74)	**	-0.194	0.099

* $p < 0.05$, Unpaired t-test.

** IgG1 and IgG4-ELISA were performed only in alcoholic patients with *S. stercoralis* larvae in feces.

¹ Pearson test of parasite load in alcoholic patients, $p < 0.05$.

² Non-alcoholic individuals attended at LACTFAR, UFBA, Bahia, Brazil.

³ For IgG1 and IgG4 the total of non-alcoholic patients infected with *S. stercoralis* was 25.

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References

- Teixeira, M.C.A., Pacheco, F.T.F., Souza, J.N., Silva, M.L.S., Inês, E.J., et al., 2016. *Strongyloides stercoralis* infection in alcoholic patients. Biomed Res. Int.
- Nutman, T.B., 2017. Human infection with *Strongyloides stercoralis* and other related *Strongyloides* species. Parasitology 144 (3), 263–273.
- Viney, M., 2017. *Strongyloides*. Parasitology 144 (3), 259–262.
- Andreetta Corral, M., de Paula, F.M., Meisel, D.M.C.L., Abdala, E., Figueiredo Costa, S., Camera Pierrotti, L., et al., 2019a. IgG reactivity with 40–35 kDa soluble and membrane antigen of *Strongyloides venezuelensis* in immunocompromised patients. Acta Trop. 190, 357–360.
- De Souza, J.N., Machado, P.R., Teixeira, M.C., Soares, N.M., 2014. Recurrence of *Strongyloides stercoralis* infection in a patient with Hansen's disease: a case report. Lepr. Rev. 85 (1), 58–62.
- de Souza, J.N., Inês, E.D.J., Santiago, M., Teixeira, M.C.A., Soares, N.M., 2015a. *Strongyloides stercoralis* infection in patients with systemic lupus erythematosus: diagnosis and prevention of severe strongyloidiasis. Int. J. Rheum. Dis.
- De Souza, J.N., Ribeiro Soares, B.N.R., Goes, L.L., Souza, De, Lima, C., Vieira Barreto, N.M.P., Jacobina, B.S., 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.
- de Souza, J.N., Teixeira, M.C.A., Soares, N.M., 2015b. Associação entre hiperinfecção por *Strongyloides stercoralis* e HTLV-1: um relato de caso. Rev. Ciências Médicas E Biológicas 13 (3), 427–430.
- Zago-Gomes, M.P., Aikawa, K.F., Perazzio, S.F., Gonçalves, C.S., Pereira, F.E.L., 2002. Prevalence of intestinal nematodes in alcoholic patients. Rev. Soc. Bras. Med. Trop. 35 (6), 571–574.
- Silva, M.L.S., de J. Inês, E., da S. Souza, A.B., dos S. Dias, V.M., Guimarães, C.M., Menezes, E.R., et al., 2016. Association between *Strongyloides stercoralis* infection and cortisol secretion in alcoholic patients. Acta Trop. 154, 133–138.
- Siddiqui, A.A., Stanley, C.S., Skelly, P.J., Berk, S.L., 2000. A cDNA encoding a nuclear hormone receptor of the steroid/thyroid hormone-receptor superfamily from the human parasitic nematode *Strongyloides stercoralis*. Parasitol. Res. 86 (1), 24–29.
- Genta, R.M., 1992. Dysregulation of strongyloidiasis: a new hypothesis. Clin. Microbiol. Rev. 5 (4), 345–355.
- Addolorato, G., Montalto, M., Capristo, E., Certo, M., Fedeli, G., Gentiloni, N., Stefanini, G.F., Gasbarrini, G., 1996. Influence of alcohol on gastrointestinal motility: lactulose breath hydrogen testing in orocecal transit time in chronic alcoholics, social drinkers and teetotaler subjects. Hepatogastroenterology 44, 1076–1081.
- Leclercq, S., Matamoros, S., Cani, P.D., Neyrinck, A.M., Jamar, F., Stärkel, P., et al., 2014. Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. PNAS 111 (42), E4485–E4493.
- Inês E de, J., Souza, J.N., Santos, R.C., Souza, E.S., Santos, F.L., Silva, M.L.S., et al., 2011. Efficacy of parasitological methods for the diagnosis of *Strongyloides stercoralis* and hookworm in faecal specimens. Acta Trop. 120 (3), 206–210.
- Silva, M.L.S., Inês, E.J., Souza, J.N., Souza, A.B.S., Dias, V.M.S., Oliveira, L.N., et al., 2019. Influence of parasite load on the diagnosis and occurrence of eosinophilia in alcoholic patients infected with *Strongyloides stercoralis*. J. Helminthol. 93 (1), 21–25.
- Bisoffi, Z., Buonfrate, D., Sequi, M., Mejia, R., Cimino, R.O., Krolewiecki, A.J., et al., 2014. Diagnostic Accuracy of Five Serologic Tests for *Strongyloides stercoralis* Infection. PLoS Negl. Trop. Dis.
- Buonfrate, D., Formenti, F., Perandin, F., Bisoffi, Z., 2015. Novel approaches to the diagnosis of *Strongyloides stercoralis* infection. Clin. Microbiol. Infect. 21 (6), 543–552.
- Mendes, T., Minori, K., Ueta, M., Miguel, D.C., Allegritti, S.M., 2017. Strongyloidiasis current status with emphasis in diagnosis and drug research. J. Parasitol. Res. 2017, e5056314.
- Krolewiecki, A.J., Ramanathan, R., Fink, V., McAuliffe, I., Cajal, S.P., Won, K., et al., 2010. Improved diagnosis of *Strongyloides stercoralis* using recombinant antigen-based serologies in a community-wide study in Northern Argentina. Clin. Vaccine Immunol. 17 (10), 1624–1630.
- Rascoe, L.N., Price, C., Shin, S.H., McAuliffe, I., Priest, J.W., Handali, S., 2015. Development of Ss-NIE-1 recombinant antigen based assays for immunodiagnosis of strongyloidiasis. PLoS Negl. Trop. Dis. 9 (4), e0003694.
- Atkins, N.S., Lindo, J.F., Lee, M.G., Conway, D.J., Bailey, J.W., Robinson, R.D., et al., 1997. Humoral responses in human strongyloidiasis: correlations with infection chronicity. Trans. R. Soc. Trop. Med. Hyg. 91 (5), 609–613.
- Atkins, Conway, Lindo, Bailey, 1999. Bundy. L3 antigen-specific antibody isotype responses in human strongyloidiasis: correlations with larval output. Parasite Immunol. 21 (10), 517–526.
- Rossi, C.L., Takahashi, E.E.H., Partel, C.D., Teodoro, L.G.V.L., da, Silva L.J., 1993. Total serum IgE and parasite: specific IgG and IgA antibodies in human strongyloidiasis. Rev. Do Inst. Med. Trop. São Paulo 35 (4), 361–365.
- Rodrigues, R.M., de, Oliveira M.C., Sopelete, M.C., Silva, D.A.O., Campos, D.M.B., Taketomi, E.A., et al., 2007. IgG1, IgG4, and IgE antibody responses in human strongyloidiasis by ELISA using *Strongyloides ratti* saline extract as heterologous antigen. Parasitol. Res. 101 (5), 1209–1214.
- Bosqui, L.R., Gonçalves, A.L.R., Gonçalves-Pires M do, R.F., Custodio, L.A., de Menezes, M.C.N.D., Murad, V.A., 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.
- Corstjens, P.La.M., De Dood, C.J., Kornelis, D., Tjon Kon Fat, E.M., Wilson, R.A., Kariuki, T.M., et al., 2014. Tools for diagnosis, monitoring and screening of *Schistosoma* infections utilizing lateral-flow based assays and upconverting phosphor labels. Parasitology 141 (Special Issue 14), 1841–1855.
- Fradejas, I., Herrero-Martínez, J.M., Lizasoain, M., Rodríguez de las Parras, E., Pérez-Ayala, A., 2018. Comparative study of two commercial tests for *Strongyloides stercoralis* serologic diagnosis. Trans. R. Soc. Trop. Med. Hyg. 112 (12), 561–567.
- Corral, M.A., Paula, F.M., Meisel, D.M.C.L., Castilho, V.L.P., Gonçalves, E.M.N., Levy, D., et al., 2017. Potential immunological markers for diagnosis of human strongyloidiasis using heterologous antigens. Parasitology 144 (2), 124–130.
- Andreetta Corral, M., de Paula, F.M., Meisel, D.M.C.L., Abdala, E., Figueiredo Costa, S., Camera Pierrotti, L., et al., 2019b. IgG reactivity with 40–35 kDa soluble and membrane antigen of *Strongyloides venezuelensis* in immunocompromised patients. Acta Trop. 190, 357–360.
- Hoffman, W.A., Pons, J.A., Janer, J.L., 1934. The sedimentation-concentration method in schistosomiasis mansoni. Puerto Rico J. Public Health Trop. Med. [Internet] 9 (3).
- MORAES, R.G., 1948. Contribuição para o estudo do *Strongyloides stercoralis* e da strongyloidíase no Brasil. Rev. Serv. Esp. Saúde Pública. 1, 507–624.
- Koga, K., Kasuya, S., Khamboonruang, C., K S, M I, N T, et al., 1991. A modified agar plate method for detection of *Strongyloides stercoralis*. Am. J. Trop. Med. Hyg. 45 (4), 518–521.
- Rugai, E., Mattos, T., Brisola, A.P., 1954. Nova técnica para isolar larvas de nematóides das fezes: modificação do método de Baermann. Rev. Inst. Adolfo Lutz 14, 5–8.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72 (1), 248–254.
- Tsang, V.C., Peralta, J.M., Simons, A.R., 1983. Enzyme-linked immunoelectrotransfer blot techniques (ETTB) for studying the specificities of antigens and antibodies separated by gel electrophoresis. Meth. Enzymol. 92, 377–391.
- Gaburri, D., Ak, G., Mh L, E.H., Am, R., Ga de, P., et al., 1996. [Intestinal parasitosis and hepatic cirrhosis]. Arq. Gastroenterol. 34 (1), 7–12.
- Marques, C.C., Zago-Gomes M da, P., Gonçalves, C.S., Pereira, F.E.L., 2010. Alcoholism and *Strongyloides stercoralis*: daily ethanol ingestion has a positive correlation with the frequency of *Strongyloides* larvae in the stools. PLoS Negl. Trop. Dis. 4 (6), e717.
- Inês, Ede J., Silva, M.L.S., Souza, J.N., Teixeira, M.C.A., Soares, N.M., 2013. The role of glycosylated epitopes in the serodiagnosis of *Strongyloides stercoralis* infection. Diagn. Microbiol. Infect. Dis. 76 (1), 31–35.
- Bosqui, L.R., Gonzaga, H.T., Gonçalves-Pires M do R de, F., de Paula, F.M., Almeida, R.S., Pavanelli, W.R., et al., 2017. Avidity as a criterion for diagnosis of human strongyloidiasis increases specificity of IgG ELISA. Diagn. Microbiol. Infect. Dis. 89 (4), 262–264.
- Ruantip, S., Eamudomkarn, C., Techasen, A., Wangboon, C., Sithithaworn, J., Bethony, J.M., 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.
- Marcos, L.A., Terashima, A., Canales, M., Gotuzzo, E., 2011. Update on strongyloidiasis in the immunocompromised host. Curr. Infect. Dis. Rep. 13 (1), 35–46.
- van Doorn, H.R., Koelewijn, R., Hofwegen, H., Gilis, H., Wetsteyn, J.C.F.M., Wismans, P.J., et al., 2007. Use of enzyme-linked immunosorbent assay and dipstick assay for detection of *Strongyloides stercoralis* infection in humans. J. Clin. Microbiol. 45 (2), 438–442.
- Schur, P.H., 1988. IgG subclasses. A historical perspective. Monogr. Allergy 23, 1–11.
- Vidarsson, G., Dekkers, G., Rispen, T., 2014. IgG subclasses and allotypes: from structure to effector functions. Front. Immunol. 5.
- González-Quintela, A., Vidal, C., Gude, F., 2002. Alcohol-induced alterations in serum immunoglobulin e (IgE) levels in human subjects. Front. Biosci. 7, e234–244.
- Lomholt, F.K., Nielsen, S.F., Nordestgaard, B.G., 2016. High alcohol consumption causes high IgE levels but not high risk of allergic disease. J. Allergy Clin. Immunol. 138 (5), 1404–1413 e13.
- Gonçalves, A.L.R., Rocha, C.A., Gonzaga, H.T., Gonçalves-Pires M do R de, F., Ueta, M.T., Costa-Cruz, J.M., 2012. Specific IgG and IgA to larvae, parthenogenetic females, and eggs of *Strongyloides venezuelensis* in the immunodiagnosis of human strongyloidiasis. Diagn. Microbiol. Infect. Dis. 72 (1), 79–84.
- Silva, L.P., Barcelos IS da, C., Passos-Lima, A.B., Espindola, F.S., Campos, D.M.B., Costa-Cruz, J.M., 2003. Western blotting using *Strongyloides ratti* antigen for the detection of IgG antibodies as confirmatory test in human strongyloidiasis. Mem. Inst. Oswaldo Cruz 98 (5), 687–691.