SHORT COMMUNICATION

Strongyloides venezuelensis Alkaline Extract for the Diagnosis of Human Strongyloidiasis by Enzyme-linked Immunosorbent Assay

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The present study was conducted to detected IgG antibodies using Strongyloides venezuelensis *alkaline extract for the diagnosis of human strongyloidiasis by the enzyme-linked immunosorbent assay (ELISA). Sera from 90 subjects were analyzed (30 with strongyloidiasis, 30 with other parasites and 30 healthy individuals). Results were expressed in antibody titers, which were considered as positive when titer was ≥ 80. Sensibility and specificity of the assay were 100% and 96.7%, respectively. It can be concluded that the heterologous alkaline extract could be employed in ELISA as a diagnostic aid in human strongyloidiasis, due to its advantages as easiness of obtaining, practicability in preparing, and high indexes of sensitivity and specificity.*

Key words: *Strongyloides venezuelensis* - strongyloidiasis - diagnosis - enzyme-linked immunosorbent assay

Strongyloides stercoralis infects 30 million people in 70 countries. Infection usually results in asymptomatic chronic disease of the gut, which can remain undetected for decades (Siddiqui & Berk 2001). Due to the fluctuations on the larvae shedding in subjects infected with *S. stercoralis*, the parasitological methods have shown low sensitivity, being necessary repeated stool exams (Dreyer et al. 1996, Uparanukraw et al. 1999). Complementary tests for the diagnosis and the monitoring of the immune response in this parasitosis have been developed. However, the major limitation for the standardization of immunological methods is the difficulty in obtaining large amount of *S. stercoralis* larvae (Sato et al. 1995, Costa-Cruz et al. 1997).

The aim of this study was to diagnose human strongyloidiasis by enzyme-linked immunosorbent assay (ELISA) using alkaline extract of *S. venezuelensis* filariform larvae. The study received approval from the Ethical Committee of the Federal University of Uberlândia.

Strain of *S. venezuelensis* was isolated from feces of the wild rodent *Nectomys squamips* in August 1988 and maintained by experimental infection in *Rattus norvergicus*-Wistar. Infective larvae of *S. venezuelensis* were obtained from the feces of rats experimentally infected and cultured in mineral charcoal for two days at room temperature. Larvae were recovered by the Rugai et al. (1954) method and washed five times in 0.01 M phosphate-buffered saline (PBS) pH 7.2 containing 400 IU/ml of benzyl penicilin and 2 mg/ml of streptomycin sulfate and then stored at -70ºC in PBS until the antigen preparation. Alkaline extract of 100,000 larvae of *S. venezuelensis* was prepared by adding 1 ml of 0.15 M NaOH (Merck, Germany) during 6 h under slow agitation at 4ºC. Subsequently, 0.5 ml of 0.3 M HCl (Merck) was added until reaching the pH 7.0, and this preparation was centrifuged at 10,000 g for 30 min at 4ºC. Protein determination of the supernatant was 240 µg/ml as detected by the Lowry et al. (1951) method.

ELISA was carried out using polystyrene microplates (Difco, São Paulo, Brazil) and the reagents were assayed in 50 µl/well. The plates were coated with alkaline extract at 10 µg/ml in 0.06 M carbonate-bicarbonate buffer, pH 9.6 and incubated overnight at 4ºC. The plates were washed three times for 5 min with PBS containing 0.05% Tween 20 (PBS-T) and incubated with the serum samples, including positive and negative control sera, diluted at 1:80 in PBS-T for 45 min at 37ºC. After new washing as previously described, the conjugate rabbit anti-human IgG (Fc chain specific) labeled with peroxidase (Sigma, US) diluted at 1:2,000 in PBS-T was added and incubated for 45 min at 37ºC. After washing, the enzymatic substrate consisting of H_2O_2 (Merck) plus o-phenylenediamine (OPD) diluted in 0.1 M citrate-Na_2HPO_4 buffer pH 5.5 was added. The reaction was stopped after 15 min with 20 µl/well of 1 M H_2SO_4 and the absorbance values were determined in an

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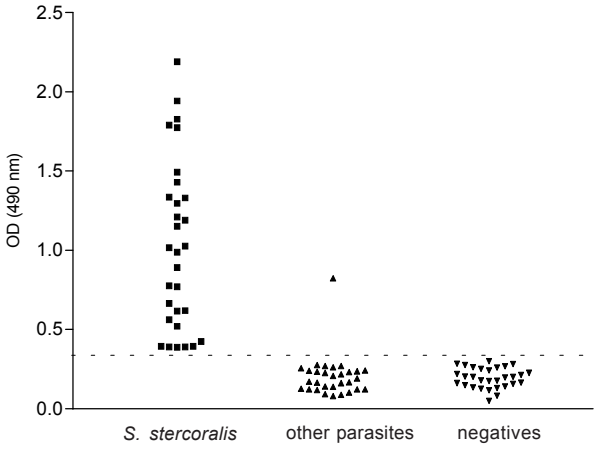
ELISA reader (Metrolab, Argentina) at 490 nm. The cut off was set using the mean absorbance values obtained from 5 non-reactive sera plus two standard deviations, considering titers ≥ 80 as criteria of positivity.

To evaluate sensitivity and specificity of the assay using this extract, 90 serum samples from subjects living in Uberlândia, state of Minas Gerais, Brazil, were studied. This region is considered as hyperendemic area of strongyloidiasis with 13% of positivity rate in children of the urban area, as reported by Machado and Costa-Cruz (1998). Out of 90 sera, 60 samples were from subjects with positive fecal exams by the Ritchie (1948) method performed at the Clinics Hospital of the Federal University of Uberlândia, Brazil. From these, 30 were from subjects shedding only *S. stercoralis* larvae in the feces and 30 were from patients with other parasites: hookworm (5 cases), *Enterobius vermicularis* (5 cases), *Hymenolepis nana* (5 cases), *Trichuris trichiura* (5 cases), *Ascaris lumbricoides* (5 cases) and *Giardia lamblia* (5 cases). The 30 remaining samples were from healthy students of the biomedical area of the same University, who had three negative fecal samples by the Baermann (1917) and Hoffman et al. (1934) parasitological methods, and with no previous history of strongyloidiasis, serving as control group.

Figure gives absorbance values obtained by ELISA using *S. venezuelensis* alkaline extract. Sensitivity and specificity of ELISA were 100% and 96.7%, respectively. The cut-off value was 0.371 for sera initially diluted at 1:80. When considering a gray zone ranging –10% to +10% of cut-off value, values between 0.334 and 0.408 were obtained. Thus, five sera (16.7%) of the *S. stercoralis* positive group, no serum sample (0%) of the group with other parasites, and only one (3.3%) of the negative control group were found in this range. These results still shown high sensitivity and specificity values, such as 83.3% and 93.4%, respectively. The distribution of the specific IgG titers by ELISA is demonstrated in Table. Antibody titers in sera from patients with strongyloidiasis ranged from 80 to 2560.

Once the patients shedding larvae in the feces were considered as gold standard, the detection of circulating IgG antibodies should be expected in all of them. The sensitivity (100%) of this assay for the detection of specific IgG was identical to that found by Conway et al. (1993) when using saline extract of *S. stercoralis* in a sampling of 40 patients, and higher than those reported by Neva et al. (1981) and Gam et al. (1987) that used saline extract of



Results of serum samples (diluted 1:80) from 30 patients with *Strongyloides stercoralis*, 30 with other parasites and 30 negatives obtained by enzyme-linked immunosorbent assay (ELISA) using *Strongyloides venezuelensis* alkaline extract. OD: optical density; ---- cut-off value.

S. stercoralis or *S. ratti.* Using the gray zone criteria, our results were similar to the findings of the two last authors and were going to be more realistic interpreted. Specificity above 90% was demonstrated by Genta (1988) and Lindo et al. (1994) that assayed homologous saline extracts as antigen. A single serum was reactive to other parasites (hookworm) with titer of 160. We are more than 2,500 km far from the endemic area for filariasis in our country (located in the northeastern region) and the conclusions about specificity should be restricted to non-endemic areas to filariasis.

The results showed that the alkaline extract of *S. venezuelensis* filariform larvae could be used as heterologous antigen for the diagnosis of human strongyloidiasis. The advantages include the easiness of maintaining and obtaining *S. venezuelensis* in laboratory as alternative source of antigen, easy preparation and yield of large antigen amounts without requiring sophisticated equipments and expensive reagents, and relatively short time in preparing the alkaline extract. The preparation of alkaline extract of *S. venezuelensis* filariform larvae obtained from experimental infected rats yields a large amount that makes available its utilization in large scale testing. In addition, there is

TABLE

Distribution of the titers from IgG anti-*Strongyloides stercoralis* detected by enzyme-linked immunosorbent assay (ELISA) using *S. venezuelensis* alkaline extract in 30 sera of *S. stercoralis* positivy subjects and 60 controls

	Alkaline extract antigen titers of IgG								Total positive	
Sera	Nr	< 80	80	160	320	640	1280	2560	Nr	%
+/ *S. stercoralis*	30	0	5	8	7	8	1	1	30	100
+/Other parasites	30	29		1					1	3.3
Negatives	30	30							0	0

+ : positive by parasitological methods; Nr: numbers of individuals

the elimination of the risk of infection during the handling of the parasite by the technicians. It can be concluded that ELISA using *S. venezuelensis* alkaline extract showed high sensitivity and specificity for the detection of specific serum IgG, and could be employed as a diagnostic aid in human strongyloidiasis.

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