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Research brief

Immunoblotting using *Strongyloides venezuelensis* larvae, parthenogenetic females or eggs extracts for the diagnosis of experimentally infected immunosuppressed rats

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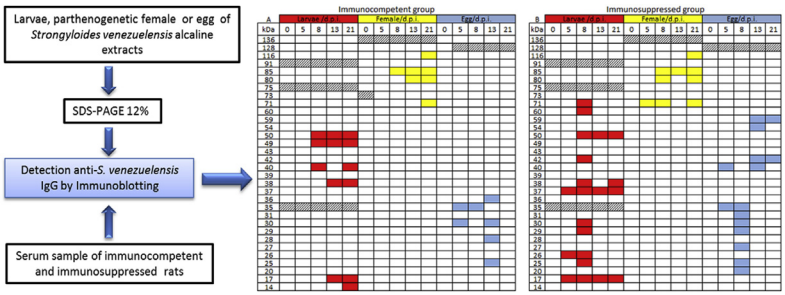
HIGHLIGHTS

- L3 larval extract was more effective in detection of anti-*Strongyloides venezuelensis* IgG.
- IgG was detected early in immunosuppressed group when compared to immunocompetent.
- Fraction of 17 kDa is possible marker of infection in immunosuppressed rats.

GRAPHICAL ABSTRACT



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ABSTRACT

The nematode *Strongyloides stercoralis* is responsible for strongyloidiasis in humans. Diagnosis of infection occurs through detection of larvae in feces, but low elimination of larvae often hampers the detection of disease, particularly in cases of patient immunosuppression. Immunodiagnostic tests have been developed; however obtaining *S. stercoralis* larvae for the production of homologous antigen extract is technically difficult. Thus, the use different developmental forms of *Strongyloides venezuelensis* has become an alternative method for the production of antigen extracts. The aim of this study was to evaluate immunoblotting using alkaline extracts from *S. venezuelensis* L3 larvae, parthenogenetic females or eggs to test detection of experimental strongyloidiasis associated with immunosuppression. Immunocompetent and immunosuppressed male rats were experimentally infected, and serum sample from all animals were obtained at 0, 5, 8 13, and 21 days post infection (d.p.i.). Immunoblotting was evaluated for use in detection of anti-*S. venezuelensis* IgG in both experimental rat groups. The larval extract immunoblotting profile had the most immunoreactive fractions in the immunosuppressed group beginning at 5 d.p.i., while the immunocompetent group reactivity began on 8 d.p.i. Immunoreactive protein fractions of 17 kDa present in larval alkaline extract presented as possible markers of infection in

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immunosuppressed rats. It is concluded that all extracts using immunoblotting have diagnostic potential in experimental strongyloidiasis, particularly larval extract in immunosuppressed individuals.

1. Introduction

Strongyloides stercoralis is widespread throughout tropical and subtropical regions. Geohelminthiasis can occur asymptomatically, as a potentially fatal hyperinfection, or as disseminated infection (Al-Hasan et al., 2007; Puthiyakunnon et al., 2014). Immunosuppression has been associated with cases of hyperinfection syndrome and studies of disseminated disease in transplant patients, asthmatics, and patients with chronic lung or autoimmune diseases have identified corticosteroid therapy as a common denominator for developing serious infection (Mejia and Nutman, 2012; Bollela et al., 2013; Toledo et al., 2015).

Strongyloidiasis is difficult to diagnose because parasite load is low and larval output is irregular (Siddiqui and Berk, 2001; Khieu et al., 2013; Schär et al., 2014). *S. stercoralis* infective larvae are difficult to obtain, and thus so are sufficient quantities of antigens to enable further fractionation and analysis; this technical challenge limits the development and standardization of serological tests with greater sensitivity and specificity (Feliciano et al., 2010; Gonçalves et al., 2012a).

Strongyloides venezuelensis is a nematode that infects wild rodents, and is often used as a model organism for studies of strongyloidiasis. It has also been used to standardize new immunological techniques for improving diagnosis of human strongyloidiasis (Machado et al., 2003; Marra et al., 2011; Gonçalves et al., 2010, 2012a). *S. venezuelensis* release eggs in experimentally infected rats, which in stool culture produce a large quantity infective larvae, simplifying antigen production for accurate and specific recognition of IgG against strongyloidiasis (Machado et al., 2003, 2008; Gonçalves et al., 2008). Other life stages of the parasite, such as parthenogenetic females and eggs may also serve as potential sources of diagnostic antigens. Female parasite and eggs are both found in human intestinal mucosa in infected persons, and contact with host systems may lead to the development of immune response; for this reason it is prudent to test the efficacy of female parasite and egg extracts for use in immunodiagnosis (Gonçalves et al., 2012b).

The aim of this study was to evaluate immunoblotting using alkaline extracts from *S. venezuelensis* L3 larvae, parthenogenetic females or eggs to test detection of experimental strongyloidiasis associated with immunosuppression.

2. Material and methods

2.1. Animals

Male *Rattus norvegicus* (Wistar) rats weighing 100–120 g with age between 6 and 8 week were used in e experiments. Rats were bred in a conventional manner at the *Centro de Experimentação Animal* (CBEA) of the Universidade Federal de Uberlândia (UFU). Rats were kept in cages with a maximum density of four rats per cage lined with bed shavings, with access to water and fed with industrial feed. Colony room temperature was 22 ± 2 °C, and artificial lighting consisted of a 12 h:12 h light–dark cycle. All experiments were conducted in accordance with animal ethics guidelines and were approved by e *Comitê de Ética na Utilização de Animais* of the *Universidade Federal de Uberlândia* (CEUA/UFU 096/10).

2.2. Parasites

The L-2 strains of *S. venezuelensis* used in this study were obtained from feces from the wild rodent of the species *Bolomys lasiurus* (April, 1986), isolated and retained in *Rattus norvergicus* Wistar at the Institute of Biology at Universidade Estadual de Campinas (UNICAMP), São Paulo, Brazil. The strain of *S. venezuelensis* were kindly provided by the Laboratório de Diagnóstico de Parasitoses, Universidade Federal de Uberlândia and maintained in *R. norvergicus*.

S. venezuelensis third-stage infective larvae (L3) were obtained from charcoal cultures of infected rat faeces. The cultures were stored at 28 °C for 48 h, and the infective larvae were collected and concentrated using the Rugai method (Rugai et al., 1954). The pellet (3 mL) from e conical cup were diluted 10 times in distilled water and larvae were counted using stereomicroscopy. For infection, 1.500 *S. venezuelensis* L3 larvae were inoculated subcutaneously into each of the rats.

To recover parthenogenetic females, rats were anesthetized (ketamine 60 mg/kg – Syntec do Brasil Ltda, Cotia, São Paulo and xylazine 7 mg/kg – Syntec do Brasil Ltda, Cotia, São Paulo) and sacrificed on 8 day post-infection (d.p.i.), the small intestines were removed from animals and placed in Petri dishes containing saline solution, longitudinally sectioned and incubated at 37 °C for two hours. Parthenogenetic females were counted following methodology by Sato and Toma (1990).

To obtain eggs, three rats experimentally infected with *S. venezuelensis* were placed on clean, damp paper towel to defecate in 7 and 8 d.p.i. and feces were collected. Feces were recovered moistened with distilled water, macerated and adjusted with distilled water (v/v). The solution was passed in sieve plot 0.300 mm, 0.149 mm and 0.47 mm, respectively. The collected fluid was centrifuged at 13,000 × *g* for 10 min, the supernatant discarded and the pellet resuspended in 0.9% saline. The saline faeces were then centrifuged at 300 × *g* for 5 min and the supernatant collected. To the supernatant was added distilled water v/v and centrifuged at 13,000 × *g* for 10 min. The supernatant was discarded and the pellet resuspended in PBS. The estimated number of eggs per gram of feces was performed using the method of Cornell-McMaster (Gordon and Whitlock, 1939) and eggs were stored at −20 °C for later use.

2.3. Experimental groups

Rats were divided into two groups: immunocompetent (n = 30) and immunosuppressed (n = 30) rats. Prior to infection, immunosuppressed groups received 5 μg/mL of dexamethasone disodium phosphate (Medley Indústria Farmacêutica Ltda, Campinas, São Paulo, Brazil) diluted in water for 5 days, as previously described by Romand et al. (1998). Animals from both groups were inoculated subcutaneously in the abdominal region with 1.500 *S. venezuelensis* larvae, except immunocompetent or immunosuppressed animals of day 0 (negative control of experiments).

At each time point (day 0 and 5, 8, 13 and 21 d.p.i.), 6 rats from each group were anesthetized with ketamine 60 mg/kg (Syntec do Brazil Ltda, Cotia, São Paulo) and xylazine 7 mg/kg (Syntec do Brazil Ltda, Cotia, São Paulo) s.c, and blood samples were collected by