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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

TRANSMAMMARY TRANSMISSION OF STRONGYLOIDIASIS IN IMMUNOSUPPRESSED RATS

TRANSMISIÓN TRANSMAMARIA DE ESTRONGILOIDIASIS EN RATAS INMUNOSUPRIMIDAS

Ana Lúcia Ribeiro Gonçalves[1*], Claudio Vieira Silva[1], Edson Fernando Goulart Carvalho[1], Marlene Tiduko Ueta[2] & Julia Maria Costa-Cruz[1]

[1]Instituto de Ciências Biomédicas, Universidade Federal de Uberlândia, MG; [2]Laboratório de Parasitologia, Universidade Estadual de Campinas, SP, Brazil.
E-mail: analuciabio@yahoo.com.br

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Abstract

In order to investigate the levels of antibodies and antigen in sera and in broncho-alveolar lavage fluid (BALF) samples, female rats immunosuppressed or non-immunosuppressed female rats and rats experimentally infected with *Strongyloides venezuelensis* and their young offspring were used. For this, enzyme-linked immune-sorbent assay (ELISA) was conducted using alkaline parasite extracts and anti-L3 polyclonal antibody produced in rabbits. Findings revealed that young offspring from immunosuppressed females presented significantly higher positivity for IgG detection in serum samples and in BALF. In addition, antigen detection in serum samples was also more evident in offspring from immunosuppressed rats. Thus, we reinforce the trans-mammalian route of *Strongyloides* infection in experimental model infection.

Keywords: immunosuppression - rats - *Strongyloides venezuelensis* - strongyloidiasis - transmammary transmission.

Resumen

Con el fin de investigar los niveles de anticuerpos y antígenos en muestras de lavado broncoalveolar (LBA) y suero, ratas hembras inmunosuprimidas o no inmunosuprimidas e infectadas experimentalmente con *Strongyloides venezuelensis* y sus crías fueron utilizadas. Para ello, el ensayo inmunoabsorbente ligado a enzimas (ELISA) se realizó usando extractos alcalinos de larvas y anti-L3 anticuerpo policlonal producido en conejos. Los resultados mostraron que las crías de las hembras inmunosuprimidas fueron significativamente mayores para la detección de IgG en el suero y LBA. Por otra parte, la detección de antígeno en muestras de suero también fue más evidente en las crias de ratas inmunosuprimidas. Así pues, aquí reforzamos la ruta transmamaria de la infección por *Strongyloides* en el modelo experimental de infección.

Palabras clave: inmunosupresión - ratas - *Strongyloides venezuelensis* - estrongiloidiasis - transmisión transmamaria.

INTRODUCTION

Strongyloides venezuelensis Brumpt, 1934 is a rodent parasite that has been extensively used as an experimental model to study human infection (Baek *et al.*, 1998; Gonçalves *et al.*, 2010). In experimental infections, infective larvae from *S. venezuelensis* migrate to lungs before establishing in duodenal mucosa. Thus, its migration in the rodent host is similar to that of *Strongyloides stercoralis* Bavay, 1876 in humans (Negrão-Corrêa *et al*., 2003).

Although transplacental transmission of larvae has not been reported, the transmammary transmission can occur if the female acquire infection between the 1st and 3rd day post-birth. Vertical transmission of larvae is a major pathway in the life cycle of several species of *Strongyloides*, including *Strongyloides ratti* Sandground, 1925 and *S. venezuelensis* in rats (Katz, 1969; Nolan & Katz, 1981). On the other hand, evidence of vertical transmission in human with *S. stercoralis* is absent (Shoop *et al.*, 2002).

In addition, in murine models *Strongyloides* sp. induces the production of cytokines such as IL-3, IL-4 and IL-5, with subsequent secretion of specific IgM, IgG, IgG1, IgA and IgE, essential to the elimination of the parasite (Onah & Nawa, 2000; Rodrigues *et al.*, 2009). However, little attention has been given to immune response in immunosuppressed rats and infected with *S. venezuelensis*. For this reason, IgG antibodies and the antigen detection at vertical transmission can influence the immune response to the parasite during its implantation and/or expulsion from the host.

The present study investigated the levels of antibodies and antigen in sera and bronchoalveolar lavage fluid (BALF) of female rats immunosupressed or not and experimentally infected with *S. venezuelensis* and their offspring.

MATERIALS AND METHODS

Animals

Two female Wistar rats weighing 100-120g, bred at the Instituto de Ciências Biomédicas, Universidade Federal de Uberlândia (ICBIM-UFU), were used in the experiments. During this study, the rats were maintained at the animal facilities of the Centro de Experimentação e Utilização de Animais, UFU, MG, Brazil, fed with laboratory ration, and tap water to drink *ad libitum*. All experiments were conducted in accordance with animal ethics guidelines and were approved by the Comitê de Ética na Utilização de Animais of the Universidade Federal de Uberlândia (CEUA-UFU 096/10).

Parasites

Strongyloides venezuelensis third-stage infective larvae (L_3) 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 (3mL) from the conical cup were diluted 10 times in distilled water and larvae were counted using stereomicroscopy. Larvae were quantified according to the following formula: N x 10 x 60 (N = the number of larvae counted, 10 = dilution factor and 60 = correction factor when using pipettes of 50 *u*L). For each infection, 1500 *S. venezuelensis* L_3 larvae were inoculated subcutaneously in rat abdominal cavity.

Immunosuppression and infection of female rats before conception

Before the infection, the immunosuppressed female rat received 5 ug·mL^{-1} of dexamethasone disodium phosphate, in water, for 5 days as described previously (Romand *et al*., 1998). For the infection the two female rats were inoculated subcutaneously in the abdominal cavity with 1500 *S. venezuelensis* L_3, after conception.

Serum and brochoalveolar lavage fluid samples

On the 76th day post-infection, two female rats and their offspring (six of female non immunosuppressed and six of female immunossuppressed) were anesthetized with 60

mg·Kg^{-1} ketamine and 7 mg·Kg^{-1} xilazine s.c, and blood samples were collected by cardiac puncture. Blood was then centrifuged, and the serum was stored at -20°C. Subsequently, the chest cavity of each animal was carefully opened, and the trachea was exposed and catheterized. The catheter was tied in place, and sterile phosphate buffered saline (PBS)/sodium citrate (0.5%) was infused in three 1 mL aliquots. The BALF was collected and placed on ice, and then the aliquots were stored at -20°C.

Alkaline parasite extracts

Alkaline extracts were prepared using 300,000 *S. venezuelensis* larvae, as previously described (Machado *et al.*, 2003). The protein content of the supernatant was determined by the Lowry method (Lowry *et al.*,1951).

Production of immune serum and conjugate

Two rabbits were immunized for anti-S. venezuelensis immune serum production. Immunization and anti-*S. venezuelensis* specific IgG purification and horseradish peroxidase conjugation was carried out as previously described (Gonçalves *et al.*, 2010).

Measurement of specific IgG in serum and BALF samples

For serum samples, polystyrene microplates were coated overnight, as previously described (Gonçalves *et al.*, 2012). Results were arbitrarily expressed as ELISA index (EI), previously reported in research on human strongyloidiasis according to the following formula: EI = OD sample/cut off, where the cut off was established as the mean OD of three negative control sera plus two standard deviations. Sera with EI > 1.0 were considered positive.

For BALF samples, high-binding microtitre plates (Corning-Costar) were coated overnight at 4 oC with 50 µL·well^{-1} of *S. venezuelensis* alkaline extract (10 µg·mL^{-1}) in 0.06 M carbonate-bicarbonate buffer (pH 9.6). Plates were washed three times with PBS-T. After washing, plates were incubated with undiluted BALF samples for 45 min at 37ºC and subsequently with the secondary antibody consisting of peroxidase-labeled goat anti-rat IgG (Sigma, Chemical Co., St Louis, MO) at the ideal dilution of 1:8000 for 45 min at 37ºC. The reaction was revealed by adding the enzyme substrate (0.03% H_2O_2 and o-phenylenediamine [OPD] in 0.1M citrate-phosphate buffer, pH 5.0) and incubated for 15 min at room temperature. The reaction was stopped by adding 2N H_2SO_4 and the optical density (OD) was determined at 492 nm in a plate reader (Titertek Multiskan; Flow Laboratories, McLean VA). BALF with EI > 1.0 were considered positive.

Measurement of antigen in serum and BALF samples

For serum samples, polystyrene microplates were coated overnight, as previously described (Gonçalves *et al.*, 2012). Sera with EI > 1.0 were considered positive.

For BALF samples, high-binding microtitre plates (Corning-Costar; Laboratory Sciences Company, New York, NY) were coated overnight at 4ºC with 50µL·well^{-1} of IgG anti-S. venezuelensis (40µg·mL^{-1}) in 0.06 M carbonate-bicarbonate buffer (pH 9.6). Plates were washed three times for 5 minutes with PBS containing 0.05% Tween 20 (PBS-T). After washing, plates were incubated with undiluted bronchoalveolar lavage fluid samples for 2 h at 37ºC and subsequently with the secondary antibody consisting of peroxidase-labeled rabbit anti-*S. venezuelensis* IgG at the optimal dilution of 1:40 for 45 min at 37ºC. The reaction was revealed as described above. BALF with EI > 1.0 were considered positive.

Statistical analysis

Each experiment was performed twice. Statistical variations were analyzed using Student't test followed by Mann-Whitney test. The criterion for statistical significance was set at $P < 0.05$.

RESULTS

Parasite-specific IgG was measured in serum samples from infected immunosuppressed female rats and offspring on the 76th day post-infection, and these values were then compared

with those found for infected non immunosuppressed female rats and offspring (Fig. 1A). Serological assays demonstrated that 2/6 (33.3%) offspring of a non immunosuppressed female rat and 5/6 (83.3%) offspring of an immunosuppressed female rat were seropositive to *S. venezuelensis.* Levels of IgG antibodies to *S. venezuelensis* were higher in immunosuppressed female rat (Fig. 1A). Levels of IgG antibodies to *S. venezuelensis* in BALF samples demonstrated that 1/6 (16.7%) offspring of a non immunosuppressed female rat and 6/6 (100%) pups of immunosuppressed female rat were positive. These results were significantly higher in the offspring of an immunosuppressed female rat ($P < 0.01$) (Fig. 1B).

When ELISA was used for the detection of *S. venezuelensis* antigen in serum samples (Fig. 1C), 3/6 (50%) offspring of a non immunosuppressed female rat and 3/6 (50%) offspring of an immunosuppressed female rat were seropositive. In BALF samples, levels of antigen were detected only in immunosuppressed female rat and in an offspring from a non immunosuppressed female (Fig 1D).

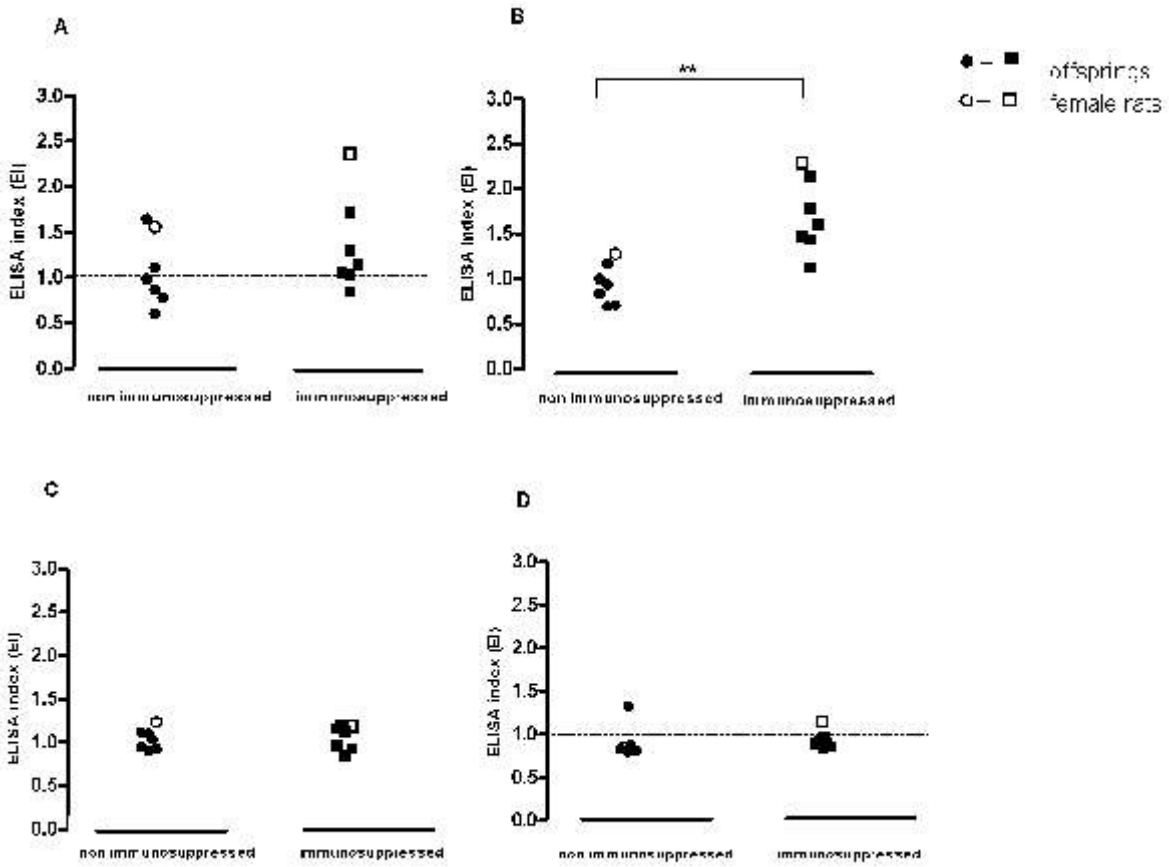

Figure 1. Levels of *S. venezuelensis* specific IgG detection in serum (A) and BALF (B) samples, using alkaline parasite extract and levels of *S. venezuelensis* antigen detection in serum (C) and BALF (D) samples from non immunosuppressed or immunosuppressed female rats and their offspring. Dashed lines indicate the detection limit for the antibody or antigen. ** $p<0.01$.

DISCUSSION

Vertical transmission of larvae, especially through the transmammary route, is a major pathway in the life cycle of several species of *Strongyloides*, including *Strongyloides ransomi* Schwartz & Alicata,1930; in swine (Moncol & Batte, 1966; Gomes, 2009), *Strongyloides papillosus* Wedl,1856 in cattle and sheep (Lyons *et al.*, 1970; Andrade, 2010), *Strongyloides westeri* Ihle, 1917 in horses (Lyons *et al.*, 1969), and *S. ratti* and *S. venezuelensis* in rats (Katz, 1969; Nolan & Katz, 1981; Pereira, 2008). Moreover, many studies verified that vertical transmission through prenatal pathways does not occur in dogs infected with *S. stercoralis*. On the other hand, vertical transmission of this parasite through transmammary routes is possible in dogs (Shoop *et al.*, 2002).

Our approach was meant to disclose the possibility of *S. venezuelensis* vertical transmission through transmammary route in non immunossupressed or immunosuppressed rats. Our findings revealed that the offspring from immunosuppressed females presented significantly higher positivity for IgG detection in serum samples. In addition, antigen detection in serum samples was also more evident in offspring from immunossupressed rats. Previous studies showed that antigen detection in serum samples may not be a good approach for strongyloidiasis diagnosis, because the detection rates were below the cut-off limits for normal and immunocompromised rats during the kinetics of infection (Gonçalves *et al.*, 2012).

When BALF samples were used, it was observed that the levels of specific IgG may be an alternative to study vertical transmission through transmammary route in rats. However, the antigen detection may not be a good assay for these analyses.

Previous studies showed that continued cycling of larvae during disseminated strongyloidiasis could have the same effect as those parasitic species that use arrested development as a tactic to keep larvae available for the time period when the host becomes gravid (Shoop *et al.*, 2002). The limitation of the work focuses on control of clutches making it difficult to extend the results to different hosts. Commonly the dissemination occurs in immunosuppressed host that reinforce the approach of the present study. In conclusion, we reinforce the importance of transmammarian route of *Strongyloides* infection in experimental animal infection.

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Correspondence to author/ Autor para correspondencia:
Ana Lúcia R. Gonçalves

Av. Pará 1720, Uberlândia, 38400-902, Minas Gerais, Brazil. Tel.: +55 34 3218 2600.

E-mail / Correo electrónico:
analuciabio@yahoo.com.br