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Research paper

The effect of maternal *Strongyloides venezuelensis* infection on mice offspring susceptibility and immune response

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ABSTRACT

Species of *Strongyloides* infect a wide range of hosts worldwide. Due to their complex life cycle, it is hard to control the transmission of these parasites. Several species show evidence of vertical transmission; however, the impact of this transmission route on the susceptibility of the offspring has been poorly investigated. Herein, we used *Strongyloides venezuelensis* infected mice to evaluate transplacental and transmammary parasite transmission and their effect on the susceptibility of offspring. Swiss female mice were infected at the end of the gestation or during the breastfeeding period, and their offspring were examined for the presence of the parasite one week after infection of the mother. Our data showed that female mice infected with *S. venezuelensis* during gestation did not transmit the parasite to their offspring. On the other hand, all newborn mice breastfeeding in *S. venezuelensis* infected females got infected. To evaluate the effect of early exposure to the parasite on susceptibility and immune response of the hosts, the offspring of each experimental group (non-infected, gestation-infected, and breastfeeding-infected mothers) received anti-helminth treatment after parasite evaluation and were subcutaneously infected with *S. venezuelensis* upon reaching adulthood. Mice from the group of breastfeeding-infected mothers showed lower susceptibility to *S. venezuelensis* in adulthood in comparison with mice from non-infected mothers. The low parasite burden was accompanied by earlier eosinophil and neutrophil activation in the gut and higher serum levels of IgE. In contrast, *S. venezuelensis* infection in adult mice born from gestation-infected mothers presented with more worms in the intestine and lower levels of parasite-reactive IgM in serum in comparison with mice born from non-infected mothers, thus suggesting that early exposure to parasite antigens may modulate the protective immune response. Altogether, our data confirmed transmammary, but not transplacental, transmission of *S. venezuelensis* in mice and demonstrated that early exposure to the parasite and/or their antigens has an important effect on host susceptibility to a later infection.

1. Introduction

Nematodes of *Strongyloides* species are intestinal parasites that infect humans and a range of domestic and wildlife animal species worldwide. Human strongyloidosis affects between 30 and 100 million people worldwide, mainly in tropical and subtropical countries lacking adequate sanitary conditions (Siddiqui and Berk, 2001). Nevertheless, several studies indicate that the infection is largely underestimated due to the difficult diagnosis by fecal examination (Paula and Costa-Cruz, 2011; Puthiyakunnon et al., 2014; Schär et al., 2013). In human, *S. stercoralis* infections may remain as a long-lasting asymptomatic condition, but can also turn into a potentially life-threatening disease in immunocompromised individuals, due to uncontrolled autoinfection (Concha et al., 2005; Olsen et al., 2009; Siddiqui and Berk, 2001). *Strongyloides stercoralis* naturally infects humans but has also been found naturally infecting non-human primates (Labes et al., 2011), dogs (Dillard et al., 2007; Jaleta et al., 2017; Nagayasu et al., 2017), and cats (Wulcan et al., 2019), which reinforces the zoonotic importance of this parasite species. The prevalence of *Strongyloides* spp infection range from 0 to 50% in dogs and 0–4 % in cats. Although the infection in these animals is asymptomatic and self-limiting in most cases, dogs infected with high parasite (especially neonates) burden may develop

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severe clinical signs, such as skin lesions, bronchopneumonia, and diarrhea (revised by Thamborg et al., 2017). In livestock, the prevalence of *Strongyloides* spp infection is influenced by climate factors, the management system, and the host species and age group. In cattle and small ruminants, the infection by *S. papillosus* frequently causes transient diarrhea and malnutrition in young animals, but heavily infected calves can suffer sudden cardiac death (Nakanishi et al., 1993; Taira and Ura, 1991; Thamsborg et al., 2017). A similar transmission pattern is observed for *S. westeri* in horses: although the infection is more prevalent in foals of up to five months of age that are probably infected by the lactogenic route, the infection is still transmitted among older animals (Miller et al., 2017).

The complex life cycle of *Strongyloides* species includes alternation between parasitic and free-living generations, allowing amplification of the infective form under specific environmental conditions, thus hindering control measures (Vadlamudi et al., 2006; Viney and Lok, 2007). *Strongyloides* infective larvae generally infect the host by percutaneous penetration. In addition, *S. stercoralis* can also undergo autoinfection in humans, dogs, and cats, which has been associated with long term infection and disseminated hyperinfection in immunocompromised hosts, which is often fatal (Concha et al., 2005; Jaleta et al., 2017; Schad et al., 1989; Vadlamudi et al., 2006; Viney and Lok, 2007; Wulcan et al., 2019). Vertical transmission has also been described in species of *Strongyloides* infecting pigs, horses, rats, human, ewes, and dogs (Brown and Girardeau, 1977; Kawanabe et al., 1988; Lyons et al., 1970, 1969; Moncol and Batte, 1966; Nwaorgu and Onyali, 1990; Shoop et al., 2002; Stewart et al., 1976; Zamirdin and Wilson, 1974). Although transmammary transmission of migrating larvae has been documented for *Strongyloides* species, the migration of infective larvae through the placenta during gestation is still controversial. Moreover, the effect of parasite exposure on the development of offspring protective immune response is yet to be investigated.

Strongyloides infection induces a type-2 immune response related to host protection in both humans (Carvalho and Da Fonseca Porto, 2004) and rodents (Negrão-Corrêa et al., 2006). The protective effect of a type-2 immune response may also be relevant to control *S. papillosus* in infected calves, since animals inoculated with recombinant IFN-γ showed lower eosinophilia rate, delayed production of parasite reactive IgG1, and were more susceptible to experimental infection (Nakamura et al., 2002). In addition, several studies have shown that primary infection with nematode larvae leads to a high rate of protection against reinfection with *S. ratti* or *S. venezuelensis* in mice (Dawkins and Grove, 1981; Fernandes et al., 2008; Schilter et al., 2010). Some studies have shown that eosinophils, neutrophils, complement activation, and parasite-reactive antibodies were associated with the destruction of the parasite larvae (Breloer and Abraham, 2017; El-Malky et al., 2003; Fernandes et al., 2008; Mukai et al., 2017). Indeed, our group demonstrated that in a mouse model of *S. venezuelensis* infection, protection is directed primarily against migratory larvae and is still effective one month after the primary infection (Fernandes et al., 2008). Furthermore, even a small load of parasites in the primary infection can induce a protective immune response against reinfection (Schilter et al., 2010). Nevertheless, the influence of maternal or newborn infection with *Strongyloides* species on the development of a protective immune response in offspring remains unknown.

The present study was carried out to investigate the vertical transmission of *S. venezuelensis* in mice and the possible effect of mother infection and/or newborn transmission on the induction of protective immune response of the offspring at adulthood.

2. Material and methods

2.1. Mice

Male and female Swiss mice of six to nine weeks-old were provided by the Animal Facility of the Biological Sciences Institute of the Universidade Federal de Minas Gerais (CEBIO-ICB, UFMG, Brazil) and were maintained at the Animal Facility for Helminth Infected Animals of the Parasitology Department (ICB, UFMG, Brazil). Animals had free access to rodents' diet (Nuvilab; Colombo, Parana, Brazil) and were provided with tap water *ad libitum*. All experimental procedures received prior approval from the local animal ethics committee (CETEA - UFMG protocol 97/2007).

For the experiments, 27 females were mated with nine males (three females and one male per cage). The mating was confirmed by the observation of a vaginal plug. One week after mating, the pregnant females were randomly separated into three experimental groups, regardless of the male mating, and infected with *S. venezuelensis* as described below. The newborn offspring of each experimental group were examined for parasite infection and used in the experiments upon reaching adulthood, as described in Section 2.3.

2.2. Parasite and infection

S. venezuelensis were initially isolated from *Rattus novergicus* and were maintained by serial passages in Wistar rats. Infective filiform larvae (L_3) were obtained from 72 h vermiculite culture of infected rat feces and isolated using a Baermann apparatus and filtered (Barçante et al., 2003). The recovered L_3 were washed in sterile phosphate buffer saline (PBS - 13.7 mM NaCl, 0.27 mM KCl, 0.14 mM $KH_2SO_4.7H_2O$) and counted. For the experimental infection, each mouse was subcutaneously inoculated with 700 infective larvae in 100 µl of PBS (Negrao-Correa et al., 2004).

Infective larvae were also used for the preparation of the L_3 soluble antigen, as described by Fernandes et al. (2008). Briefly, a suspension of infective larvae was centrifuged and washed several times, resuspended in PBS containing a protease inhibitor cocktail (one tablet in 25 ml of PBS; Boehringer Mannheim, Indianapolis, Ind.), and disrupted by vortexing with glass beads (five cycles, 1 min each). After vortexing, the larvae solution was subjected to sonication (10 cycles, 1 min each) with a cell sonic disrupter (PGC Scientific, Gaithersburg, Md.). Then, the larval homogenate was centrifuged (4000 x *g* for 30 min) and the supernatant was collected, protein concentration was determined, and the aliquots were stored at −20 °C to be used in ELISA assays.

2.3. Experimental procedure

The offspring from the three groups of pregnant mice were used in the following experiments. The first group, called gestation infected group (G/Sv), consisted of nine pregnant females that were infected subcutaneously with 700 L_3 of *S. venezuelensis* one week prior to the expected due date. The second group, breast-feeding infected group (B/Sv), consisted of nine females that were infected with the same parasite load one week after delivery of the offspring, during the breastfeeding period. The remaining nine pregnant females, non-infected group (NI/Sv), were used as controls and were not infected with the parasite (Fig. 1).

S. venezuelensis infection in pregnant females was confirmed by the presence of parasite eggs in the feces seven days after experimental infection using the sedimentation-concentration method. To verify parasite transmission from the mother to the offspring, two newborn mice from each infected female were euthanized 7 days after infection of the mother for evaluation of the presence of adult worms in the small intestine and/or eggs in feces recovered from the rectum, as described in Section 2.4. After weaning, the offspring of each experimental group (G/Sv, B/Sv, and NI/Sv) were treated with Ivermectin (Chemitec Agro – Veterinária Laboratory, São Paulo, Brazil) in drinking water for seven days, reaching an estimated dose of 4 mg ivermectin/Kg of mouse, as described by Klement et al. (1996) to control pinworm transmission. Since male mice are more susceptible to *S. strongyloides* infection, male offspring from each experimental group (G/Sv, B/Sv, and NI/Sv) were transferred to separate cages and used in the experiments. When the

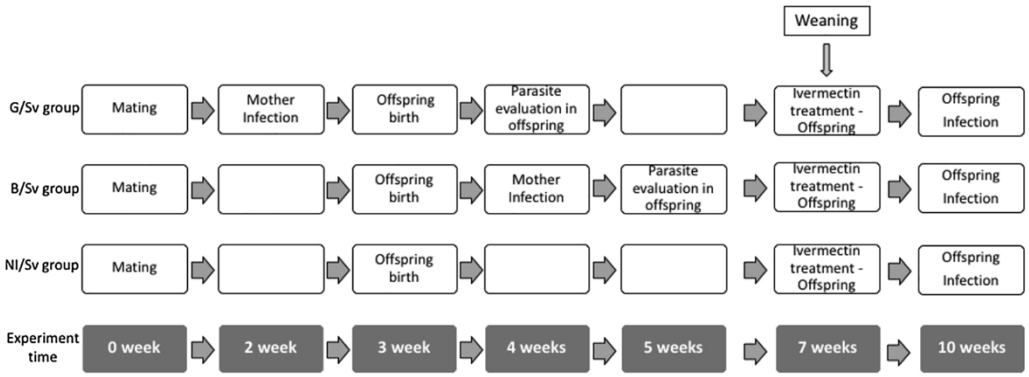

Fig. 1. Schematic representation of the experimental procedure. The gestation group (G/Sv) consisted of female mice infected with *Strongyloides venezuelensis* infective L_3 (700 L_3/mouse) two weeks after mating (i. e., one week before the due date). The breastfeeding group (B/Sv) consisted of mice mothers infected one week after delivery of the offspring. The non-infected group (NI/Sv) consisted of mice mothers that were not infected. Four weeks after birth all offspring were weaned and received ivermectin treatment. At the age of seven weeks-old (10th week of the experimental procedure), the male mice from each experimental group were placed in separate cages for the experiments. For each experimental group, 5–7 offspring males were kept uninfected, while the remaining males were infected with 700 nematode L_3.

offspring reached seven weeks of age, a group of seven male mice from each experimental group were kept uninfected, while the remaining males were subcutaneously infected with 700 L_3 of *S. venezuelensis* (Fig. 1) and evaluated at 2, 7, 10 and 12 days post-infection (dpi). There were 35 males (seven males at each time point - 0, 2, 7, 10 and 12 dpi) in the B/Sv group; 38 males (seven males at 0, 2, 10 and 12 dpi and 10 males at 7 dpi) in the G/SV group; and 44 males (7 males at 0 and 2 dpi and 10 males at 7, 10 and 12 dpi) in the NI/SV group.

At each time point, the infected offspring and control animals from each experimental group were anesthetized via intraperitoneal (i.p.) injection with a mixture of ketamine (88 mg/kg, Dopalen, Sespo Indústria e comércio Ltda, Jacareí, Brazil) and xylazine (16 mg/kg, Kensol, Laboratórios köing S.A., Avellaneda, Argentina), bled via the brachial plexus, and the blood sample was allowed to clot at room temperature for 4 h. Sera were collected after centrifugation (400 × *g* at 4 °C for 10 min) and stored in aliquots at −20 °C for posterior measurement of total IgE concentration and levels of parasite-reactive IgG1, IgG2a, and IgM, as described in Section 2.5. After blood collection, each mouse was euthanized by cervical dislocation, and their small intestines and the recta were removed. The proximal halves of the small intestine from each mouse was longitudinally open for recovery and count of adult worms and eggs eliminated in feces, as detailed in Section 2.4, and the remaining of the small intestine was immediately frozen and used to measure the enzymatic activity of peroxidase of eosinophils (EPO) and myeloperoxidase (MPO), as described in Section 2.6.

2.4. *S. venezuelensis* burden

Euthanized mice from each experimental group had their lungs individually separated at 2 dpi and cut into small pieces. The lung fragments of each animal were placed in a Baermann apparatus and incubated in phosphate-buffered saline (PBS) at 37 °C for 3–4 h. After this period, the free parasite larvae present in the incubation solution of each organ were directly counted under a stereomicroscope (model StemiDV4, Zeiss, Gottingen, Germany). To estimate the number of adult worms, the upper half of the small intestine from each infected mouse was separated at 7, 10 and 12 dpi, longitudinally opened, washed, placed in a Baermann apparatus, and incubated in PBS at 37 °C for 4 h. The worms recovered in PBS solution were directly counted under a stereomicroscope. Remaining intestinal tissue was also examined to count the remaining worms, and the total number of worms was then determined by adding up the number of free worms and tissue-associated worms. At the same time points, well-formed feces were obtained from the rectum of each infected mice, weighed, and homogenized in a known volume of PBS containing 10 % formalin. Parasite eggs were counted in two samples of the fecal solution (100 µl/sample) and used to estimate the number of eggs/per gram of feces (epg) and the fecundity index, as previously described (Fernandes et al., 2008; Negrao-Correa et al., 2004).

2.5. Evaluation of total IgE concentration and levels of parasite-reactive IgG1, IgG2a, and IgM in the serum

Total IgE concentration in the serum of *S. venezuelensis*-infected and non-infected offspring from each experimental group was measured by ELISA using a commercial kit (Bethyl Laboratories Inc, Montgomery, Texas), according to the instructions supplied by the manufacturer. Known concentrations of the recombinant mouse IgE were used to create a standard curve to convert optical density readings into IgE concentrations, expressed as ng/ml (Negrão-Corrêa et al., 2006).

Parasite-reactive IgM, IgG1, and IgG2a in the serum were also estimated by ELISA, based on a previous protocol (Fernandes et al., 2008). Briefly, 96-well plates (Nunc Maxisorp, Nagle Nunc International, Rochester, NY, USA) were coated with 100 µL of *S. venezuelensis* L_3 antigen (10 µg/ml) in 0.1 M carbonate buffer (pH 9.6) and blocked with PBS containing 1 % of bovine serum albumin (BSA) at room temperature. Serum samples were diluted 1:100 in PBS containing 0.5 % Tween-20 (PBS-T20) and 0.1 % BSA (Sigma-Aldrich, St. Louis, MO, USA) and were added to the plate. Then, the plates were washed with PBS-T20, and the bound immunoglobulins were detected using affinity-purified goat anti-mouse IgG1 (Bethyl) or affinity-purified goat anti-mouse IgG2a (Bethyl) diluted 1:1000 in PBS, followed by incubation with horseradish peroxidase-conjugated rabbit anti-goat IgG (Bethyl). Parasite-reactive IgM was detected with anti-IgM conjugated with peroxidase (Goat anti-Mouse IgM, µ-chain-specific, Sigma) diluted 1:10000 in PBS. The presence of reactive antibodies was revealed by adding a substrate solution (4 mM o-Phenylenediamine containing hydrogen peroxide in 0.05 M phosphate-citrate buffer pH 5.0). After 30 min of incubation, the reaction was interrupted with 4 N H_2SO_4 (50 µl/well). The absorbance was measured in a microplate reader (Status-Labsystems Multiskan RC) at 492 nm.

2.6. Eosinophil peroxidase (EPO) and myeloperoxidase (MPO) assays

One-hundred mg of the distal portion of the small intestine recovered from each euthanized mouse were homogenized in 1 ml of PBS containing protease inhibitors (0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzethonium chloride, 10 mM ethylene diamine tetra-acetate, 20 KI aprotinin A, and 0.05 % Tween 20; all reagents from Sigma) using a tissue homogenizer (Power General 125; Fisher Scientific, Pittsburgh, PA) and centrifuged at 3000 x *g* for 10 min. The supernatant was discarded, and the pellet was further processed for estimation of the enzymatic activity characteristic of eosinophil (EPO) or neutrophil (MPO) infiltration/activation. The activity level of EPO was measured in intestine homogenates, as described by Strath et al. (1985), and the myeloperoxidase (MPO) activity was measured using the method

originally described by Ivey et al. (1995). Both methodologies were adapted to be used in mouse models and are described in detail by Fernandes et al. (2008).

2.7. Statistical analysis

Normally distributed data were recorded as the mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA) for comparisons within each time point, or two-way ANOVA for comparisons within two or more time points. *P* values were assigned using Tukey's post-test. $P < 0.05$ were considered significant.

3. Results

3.1. Vertical transmission of S. venezuelensis in mice

The examination of the offspring one week after the infection of the mother revealed the presence of eggs in the feces and adult worms in the small intestines of all the mice born from mothers infected during breastfeeding (B/Sv) that were examined. In contrast, no mice born from mothers infected at the end of the gestation period (G/Sv) showed adult worms in the small intestine or eggs in the feces seven days after infection (Table 1). The data shows that *S. venezuelensis* can be transmitted by transmammary route in mice, although no transplacental transmission was detected.

3.2. S. venezuelensis susceptibility in offspring born from non-infected and infected females

The data in Fig. 2**A** reveals that, at 2 dpi, the number of *S. venezuelensis* larvae recovered from the lungs of offspring born from uninfected (NI/Sv), gestation infected (G/Sv), and breastfeeding infected female mice (B/Sv) were statistically similar. In contrast, mice born from breastfeeding infected mothers (B/Sv) showed significantly lower ($P < 0.001$) numbers of adult worms recovered from the small intestine (Fig. 2**B**), lower numbers of parasite eggs in feces (Fig. 2**C**), and lower fecundity index (Fig. 2**D**) at 7 dpi, compared with offspring from NI/Sv and G/Sv. Although the number of adult worms recovered from the small intestine of mice at 10 dpi was lower than that observed at 7 dpi in all experimental groups, the number of worms in the G/Sv group was significantly higher than those in the NI/Sv ($P = 0.008$) and the B/Sv ($P = 0.02$) groups, and only animals in the G/Sv group still showed adult worms in the small intestine at 12 dpi (Fig. 2**B**).

3.3. Humoral response and granulocyte infiltration in infected offspring born from non-infected and infected females

In mice born from uninfected females (NI/Sv) or from females infected during gestation (G/Sv), the nematode infection significantly increased the serum concentration of IgE after 12 days of infection (Fig. 3**A**). However, in mice born from breastfeeding infected females (B/Sv), *Strongyloides* infection induced an earlier and more intense increase of serum IgE compared with other infected groups (Fig. 3**A**).

Antibody reactivity against larvae soluble antigens was also evaluated during *S. venezuelensis* infection in the serum of offspring mice from the different experimental groups. IgG1 and IgG2 reactivity were low and their levels were not significantly different ($P > 0.05$) among the experimental groups during the nematode infection (data not shown). The level of parasite-reactive IgM significantly increased ($P < 0.05$) in the serum of mice from the groups NI/Sv and B/Sv after 12 dpi. On the other hand, mice from the G/Sv group showed no significant increase ($P = 0.93$) in parasite-reactive IgM during the infection. Therefore, at 12 dpi the level of parasite reactive IgM was significantly lower in mice from the G/Sv group in comparison with mice from the groups NI/Sv and B/Sv ($P = 0.01$; $P = 0.03$ respectively) (Fig. 3**B**).

At 10 dpi, only the tissue homogenates from infected offspring born from breastfeeding infected females (B/Sv) showed a significant increase in EPO (Fig. 4**A**) and MPO (Fig. 4**B**) activities ($P = 0.003$; $P = 0.009$ respectively) compared with the enzymatic activity detected before the infection in the same experimental group. Moreover, at the same time point, the EPO and MPO activity levels in B/Sv were significantly higher than in NI/Sv and G/Sv ($P < 0001$. At 12 dpi, all infected mice showed a significant increase $P < 0.05$) in EPO and MPO activity compared with uninfected mice of the same experimental group (Fig. 4).

Table 1
Presence of *Strongyloides venezuelensis* worms in the small intestine and presence of eggs in the feces of mice born from females infected during gestation (G/Sv) or breastfeeding (B/Sv).

Experimental groups	Worms in the intestine[a]	Eggs in feces[b]
G/Sv	0/18	0/18
B/SV	18/18	18/18

[a] Number of offspring with *S. venezuelensis* adult worms in the small intestine at 7 days after the nematode infection of the mothers with 700 infective larvae/mouse among the total of offspring tested.

[b] Number of offspring with *S. venezuelensis* eggs in feces at 7 days after the nematode infection of the mothers with 700 infective larvae/mouse among the total of offspring tested.

4. Discussion

Our data demonstrated that female mice infected with *S. venezuelensis* during the breastfeeding period can transmit the nematode to their offspring. In contrast, mice infected with the nematode two weeks after mate did not transmit the parasite to the progeny. Therefore, our data confirmed transmammary, but not transplacental, transmission of *S. venezuelensis* in mice.

Among the nematodes of medical and veterinary importance, vertical transmission has been reported in *Toxocara* sp infecting cats, dogs, and mice, and this transmission route has a great impact on parasite epidemiology (de Souza Aguiar et al., 2015; Jacobs, 1987; Parsons, 1987; Swerczek et al., 1971). Transmmamary transmission of Ancylostomidae larvae has also been reported in mice, humans, and sea lions (Setasuban, 1975; Setasuban et al., 1980). Among the species of the genus *Strongyloides*, transmammary transmission has been reported in *S. ratti* and *S. venezuelensis* infecting rats (Nolan and Katz, 1981; Wilson et al., 1976; Zamirdin and Wilson, 1974), *S. papillosus* infecting sheep and cattle (Lyons et al., 1970; Nwaorgu and Onyali, 1990), *S. westeri* infecting horses (Lyons et al., 1969), *S. ransoni* infecting pigs (Moncol and Batte, 1966; Stewart et al., 1976), and *S. stercoralis* in experimentally infected dogs (Shoop et al., 2002). *Strongyloides* larvae were also found in the milk of *S. fuelleborni*-infected lactating women resident in Zaire, thus suggesting the occurrence of vertical transmission in the human population (Brown and Girardeau, 1977). As reported in the current experimental study using *S. venezuelensis* infection in mice, no evidence of prenatal parasite transmission has been observed in *S. westeri*-infected horses (Lyons et al., 1973) and no signs of infection were found in lambs born from *S. papillosus*-infected ewes that were euthanized just after birth, prior to breastfeeding (Nwaorgu and Onyali, 1990). However, pigs infected with *S. ransoni* were able to transmit the parasite to their offspring by both the transmammary and the prenatal routes (Stewart et al., 1976).

Our results also showed that transmammary transmission of *S. venezuelensis* in mice occurs during systemic migration of the larvae in lactating mice since females infected during gestation still had adult worms in their intestines at the time of birth and breastfeeding of the offspring but did not transmit the parasite to their neonates. Indeed, published studies showed that *S. ratti* larvae reach the host's mammary glands around 48 h after infection and remain there for a short period of

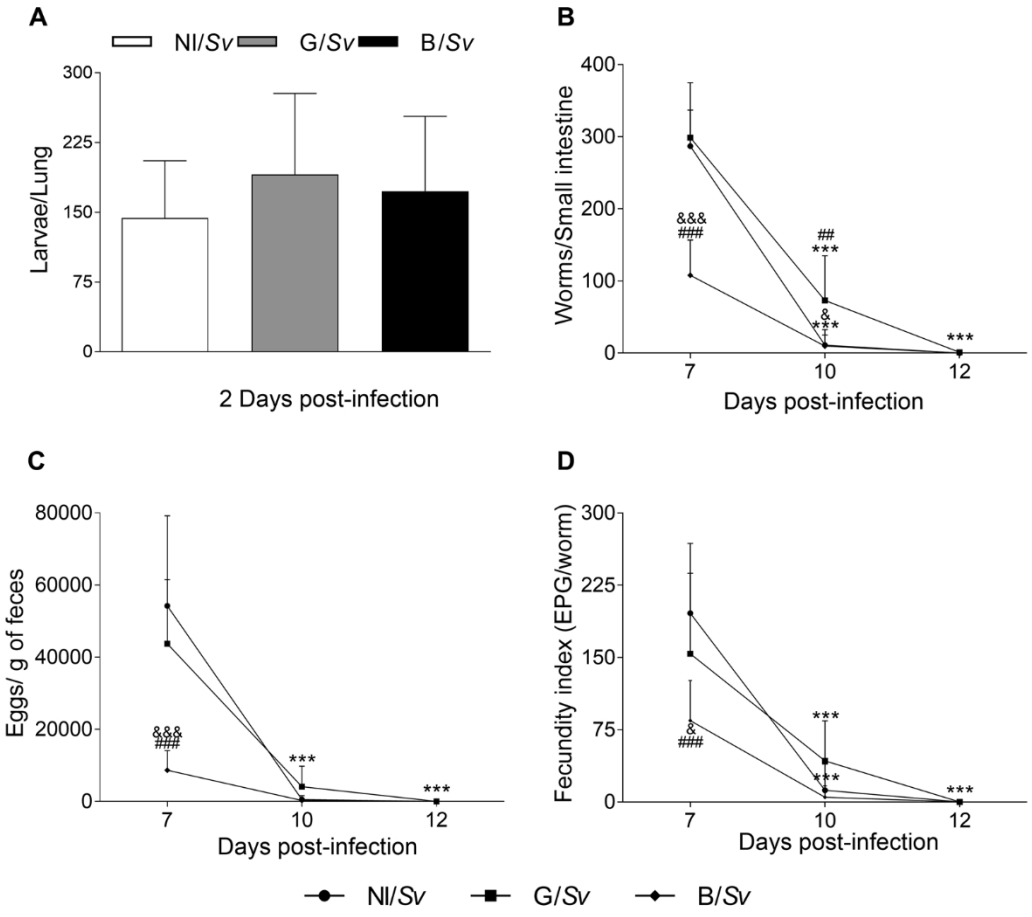

Fig. 2. *Strongyloides venezuelensis* burden in mice born from non-infected or infected mothers. (**A**) Number of parasite larvae recovered from the lungs at 2 days post-infection (dpi). (**B**) Number of adult worms recovered from the small intestine. (**C**) Number of parasite eggs eliminated in mice feces. (**D**) Eggs eliminated per gram of feces for each adult worm recovered in the small intestine (fecundity index). Infected offspring born from non-infected females (NI/Sv), from females infected during gestation (G/Sv), and from females infected during breastfeeding (B/Sv) were evaluated at 0, 2, 7, 10 and 12 days after *S. venezuelensis* infection with 700 infective larvae/mouse. The values are represented as the mean ± SD of 6–10 Swiss mice per group at each period and analyzed by two-way ANOVA with Tukey's post-test; *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ in comparison with the mice from the same experimental group at 7 dpi. ### $P < 0.001$, ## $P < 0.01$, # $P < 0.05$ in comparison with the infected offspring from the NI/Sv group at the same time point. &&& $P < 0.001$, && $P < 0.01$, & $P < 0.05$ in comparison with the infected offspring from the G/Sv group at the same time point.

time. As suggested by Wilson (1977) and by Zamirdin and Wilson (1974), breastfeeding may stimulate the migration of *S. ratti* larvae from the lungs to the mammary glands.

The current experimental study also evaluated *S. venezuelensis* susceptibility and immune response in offspring from mothers infected during the gestation (G/Sv) or during breastfeeding (B/Sv). Our data demonstrated that the offspring from the B/Sv group showed a 40 % lower parasite burden and faster nematode elimination compared with offspring from G/Sv or NI/Sv (Fig. 2). These data suggest that infection through the transmammary route can induce a protective immune response against future infection. Indeed, previous studies have demonstrated that a primary infection by *S. venezuelensis* or immunization in BALB/c mice induced an immune response that was able to promote early (7 dpi) and complete elimination of the adult worms upon a secondary infection performed 15 or 45 days after the primary infection or the immunization (Fernandes et al., 2008). The protective immune response against a secondary infection was induced even when the primary infection was performed with a low infective dose (Dawkins and Grove, 1982; Schilter et al., 2010). Transmammary transmission and the early development of a long-lasting protective immunity could explain the most frequent occurrence of strongyloidosis clinical disease among young animals of livestock (Thamborg et al.,

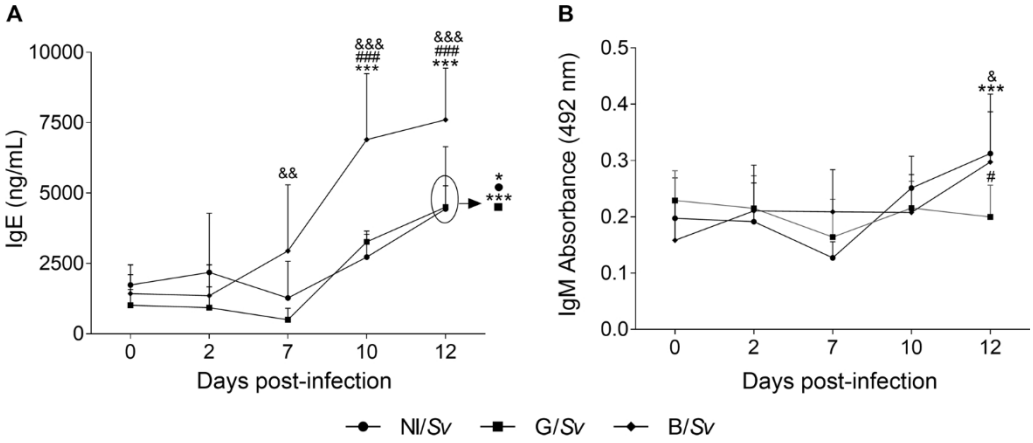

Fig. 3. Antibody response in the serum during *Strongyloides venezuelensis* infection in mice born from non-infected or infected mothers. (**A**) Serum concentration of total IgE and (**B**) level of IgM-reactivity to *S. venezuelensis* soluble infective larvae (L_3) in the serum of infected offspring from non-infected females (NI/Sv), from females infected during gestation (G/Sv), and from females infected during breastfeeding (B/Sv). *S. venezuelensis* infection was performed with 700 infective larvae/mouse and 6–10 mice from each experimental group were evaluated at 0, 2, 7, 10 and 12 dpi. The values are represented as the mean ± SD and analyzed by two-way ANOVA with Tukey's post-test; *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ in comparison with the uninfected mice from the same experimental group. ### $P < 0.001$, ## $P < 0.01$, # $P < 0.05$ in comparison with the infected offspring from the NI/Sv group at the same time point. &&& $P < 0.001$, && $P < 0.01$, & $P < 0.05$ in comparison with the infected offspring from the G/Sv group at the same time point.

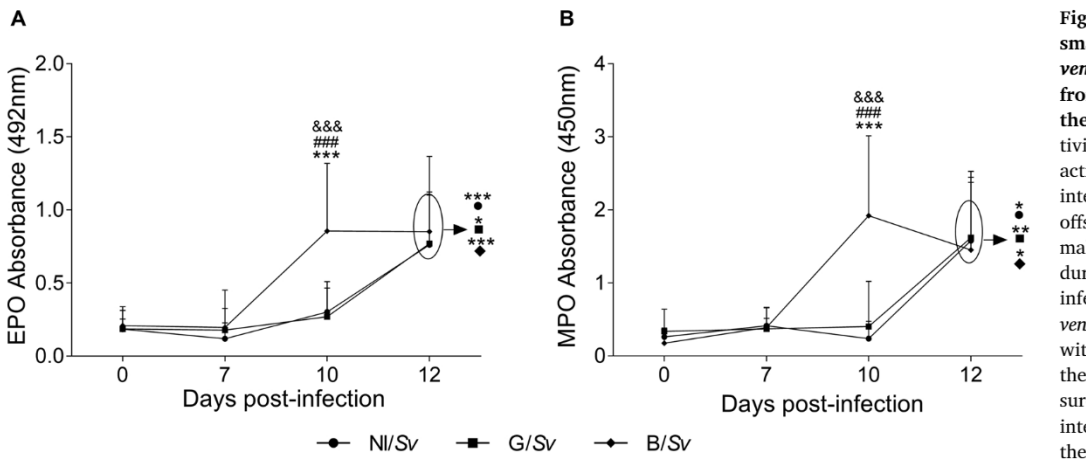

Fig. 4. Granulocyte infiltration in the small intestine during *Strongyloides venezuelensis* infection of mice born from non-infected or infected mothers. (**A**) Eosinophil peroxidase activity (EPO) and (**B**) myeloperoxidase activity (MPO) were measured in small intestine homogenates from infected offspring born from non-infected females (NI/Sv), from females infected during gestation (G/Sv) or from female infected during breastfeeding (B/Sv). *S. venezuelensis* infection was performed with 700 infective larvae/mouse and the EPO and MPO activities were measured at 0, 7, 10 and 12 dpi in small intestine homogenates from mice from the different experimental groups. The values are represented as the mean ± SD of 6–10 Swiss mice per group at each period and analyzed by two-way ANOVA with Tukey's post-test; *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ in comparison with the uninfected mice from the same experimental group. ### $P < 0.001$, ## $P < 0.01$, # $P < 0.05$ in comparison with the infected offspring from the NI/Sv group at the same time point. &&& $P < 0.001$, && $P < 0.01$, & $P < 0.05$ in comparison with the infected offspring from the G/Sv group at the same time point.

2017).

The reduction of parasite load observed in mice born from breastfeeding infected mothers was accompanied by early induction of IgE production and granulocyte infiltration. IgE production and eosinophilia are hallmarks of the systemic Th-2 response since IL-4 stimulation is required for the activation and the IgE-class switching of B cells (Mandler et al., 1993) and IL-5 is required for eosinophil differentiation and activation (Rothenberg and Hogan, 2006). Data from a *Strongyloides*-infected population (Porto et al., 2001) and murine models (Negrão-Corrêa et al., 2006; Sasaki et al., 2005) indicate that the Th-2 response is necessary for parasite control. Similarly, Fernandes et al. (2008) showed that the protection observed in *S. venezuelensis*-challenged mice was associated with a Th-2 predominant immune response and intense IgE and reactive IgG1 production. Indeed, Matsumoto et al. (2013) demonstrated that adoptive transfer of serum-derived IgG and IgE from *S. venezuelensis*-infected mice restored the ability of activation-induced cytidine deaminase-deficient ($AID^{-/-}$)mice, which cannot switch IgM to other isotypes, to promptly expel *S. venezuelensis* from the intestinal mucosae, mainly through mast cell activation via FγcRIII and FcεRI engagement, respectively. In addition, experimental data demonstrated that adoptive transfer of immune serum-derived IgE led to a significant reduction of *S. venezuelensis* load in infected mice, thus confirming the participation of IgE in *Strongyloides* control (Matsumoto, 2016).

Nevertheless, some recent data indicate that although IgG, IgE, and mast cells have an important role in worm elimination during the primary infection, the control of *Strongyloides* infection is even more complex and involves multiple elements of the immune response, including TCD4+ cells, ILC2, basophils, and eosinophils (Breloer and Abraham, 2017; Mukai et al., 2017). The participation of eosinophils in *Strongyloides* elimination from the host intestine was also reported, with the earlier elimination of adult worms transferred to the intestine of mice that overexpressed IL-5 compared with the controls (El-Malky et al., 2003). Moreover, cytotoxicity mediated by activated neutrophil, eosinophil or macrophage and activation of complement-mediated by IgG and IgM have been associated with *Strongyloides* migrating larvae control (Brigandi et al., 1996; Galioto et al., 2006; Herbert et al., 2000; Ligas et al., 2003; O'Connell et al., 2011; Yasuda et al., 2014).

Our data also showed that infected offspring from the groups N/Sv and B/Sv, but not from G/Sv, had increased levels of parasite-reactive IgM during worm elimination. Interestingly, offspring from G/Sv also showed more worms in the intestine at 10 dpi and delayed elimination compared with the other experimental groups. The protective role of parasite-reactive IgM in *Strongyloides* larvae control was also confirmed by the adoptive transfer of parasite-reactive IgM to experimentally infected mice (Brigandi et al., 1996). Later, Nouir et al. (2012) also showed that monoclonal IgM specific for *S. ratti* HSP60 was able to eliminate migrating larvae. Therefore, the absence of parasite-reactive IgM induction in offspring from G/Sv could explain the delay of parasite elimination in this experimental group. Moreover, the data suggest that parasite exposition during the prenatal period may induce modulatory mechanisms, which should be better evaluated.

5. Conclusion

In summary, the current data confirmed that *S. venezuelensis* migrating larvae can be transmitted to mice offspring by the transmammary route. Additionally, it demonstrated the lack of prenatal transmission of the nematode. Surprisingly, infected offspring born from mice infected during gestation had more worms in the intestine and low parasite-reactive IgM levels in the serum, thus suggesting that mother infection may modulate the protective immunity of the offspring. In contrast, transmammary infection induced protective immunity in the offspring and led to a lower parasite burden, which was accompanied by an earlier increase of serum IgE concentration and infiltration of eosinophil and neutrophils in the small intestine during challenge infection. Thus, our data suggest that early exposure to the parasite and/or their antigens in neonate mice has an important effect on host susceptibility to a later infection.

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Authors' individual contributions

Negrão-Corrêa conceived the idea. Costa, de Rezende and Rodrigues-Oliveira performed the experiments. Costa, Rodrigues, Coelho and Negrão-Corrêa designed and analyzed the data. Coelho and Negrão-Corrêa supervised the overall project. Rodrigues and Negrão-Corrêa wrote the manuscript with suggestion from all the other co-

authors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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