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Saccharomyces boulardii reduces the vertical transmission of *Toxocara canis* larvae in mice

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Research Paper

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Abstract

Probiotics have been shown to reduce the intensity of *Toxocara canis* infection in mice. However, larval transmission of this nematode also occurs via transplacental and transmammary routes. Thus, the aim of this study was to evaluate the effect of the *Saccharomyces boulardii* probiotic on the vertical transmission of *T. canis* in Swiss mice. The mice received 10^7 *S. boulardii* colony-forming units per gram of food. The supplementation began 15 days before mating and was maintained throughout pregnancy and lactation. The animals were inoculated with 300 *T. canis* embryonated eggs on the 14th day of pregnancy. The presence of larvae was examined in the organs of the females and their offspring. The examined organs included the following: brain, liver, lungs, heart, kidneys, spleen, eye, skeletal muscle (carcass) and mammary glands of lactating females. There was a 42% ($P = 0.041$) reduction in the number of larvae transmitted to offspring in the group that received probiotic-supplemented food (GI). Additionally, there was a 50% reduction ($P = 0.023$) in the number of larvae found in the brains of lactating offspring in the GI group. These results reveal the potential of *S. boulardii* probiotic use as an auxiliary method of controlling visceral toxocariasis.

Introduction

Toxocariasis is a worldwide parasitic zoonosis that occurs in both developing and developed countries (Hotez & Wilkins, 2009). However, the prevalence and impact of toxocariasis on public health are underestimated due to the difficultly of diagnosis (Smith *et al.*, 2009). Toxocariasis is a tissue parasitosis; in humans, *Toxocara canis* larvae migrate in the tissues for long periods, which can cause syndromes ranging from asymptomatic to systemic forms (Macpherson, 2013). The clinical presentations of toxocariasis are visceral toxocariasis, ocular toxocariasis and neurological toxocariasis, and subclinical manifestations may occur as a hidden toxocariasis (Magnaval *et al.*, 2001). The current pharmacological treatments have moderate efficiency (Magnaval *et al.*, 2001; Othman, 2012). Therefore, it is necessary to develop treatments to prevent and avoid infection or reinfection (Magnaval & Glickman, 2006).

Since the first registered cases (Beaver *et al.*, 1952), the nematode *T. canis* has been the etiological agent most frequently associated with human toxocariasis (Quattrocchi *et al.*, 2012). This geohelminth presents a complex biological cycle that involves multiple forms of transmission. The definitive hosts of *T. canis* are canids, mostly dogs; all mammals can play the role of paratenic hosts. Humans may be considered as accidental paratenic hosts (Glickman & Schantz, 1981). Thus, the main prophylactic measures for human toxocariasis are based on avoiding the accidental intake of *T. canis* embryonated eggs (Epe, 2009; Amaral *et al.*, 2010). There are also specific measures to prevent infection with larvae present in the meat or viscera of bird and mammal species that have roles as paratenic hosts (Dutra *et al.*, 2013; Cardoso *et al.*, 2020).

However, despite the importance of the route of vertical transmission in dogs, control of this infection route is difficult, in part, because the recommended doses of anthelmintic are not highly effective against dormant somatic larvae (Macpherson, 2013; Overgaauw & Knapen, 2013). This type of transmission has also been observed in paratenic hosts, such as mice (Lee *et al.*, 1976; Reiterová *et al.*, 2003; De Souza Aguiar *et al.*, 2015).

The intestines are a highly complex ecosystem in which nutrients, microbiota and host cells interact in a balance that determines health maintenance. Thus, there is interest in using probiotics to modulate the intestinal microbiota and prevent or treat diseases (Butel, 2014). Trials with probiotics have shown promising results, including tissue parasite control of *Trichinella*

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spiralis (Bautista-Garfias *et al.*, 2001) and *T. canis* (Basualdo *et al.*, 2007; Chiodo *et al.*, 2010). Furthermore, the yeast *Saccharomyces boulardii* has been found to reduce the number of *T. canis* larvae in tissues during the initial and chronic toxocariasis phases (Avila *et al.*, 2012, 2016) and to reduce the intensity of infection in mice caused by the consumption of ser infected with *T. canis* (Cardoso *et al.*, 2020). However, no studies have evaluated the effects of probiotics on larvae vertical transmission.

In consideration of the complexity of the *T. canis* biological cycle and the possibilities of probiotic biotherapeutics (Avila *et al.*, 2012, 2016; Cardoso *et al.*, 2020), this study evaluated the effects of *S. boulardii* probiotic on the vertical transmission of *T. canis* in mice.

Materials and methods

Probiotic S. boulardii *and food administration*

The probiotic *S. boulardii* CNCM I-745 was cultivated in yeast peptone dextrose medium and incubated in a shaker (150 rpm) at 37°C for 72 h. The culture was then centrifuged and washed with sterile phosphate-buffered saline. To add the probiotic supplement to the food, the food was triturated, manipulated and remoulded into pellets. The pellets were then dried in a greenhouse with forced air circulation (Avila *et al.*, 2016).

Collection and incubation of the eggs and larvae of T. canis

Young dogs naturally infected with *T. canis* were treated orally with pyrantel pamoate (12.5 mg/kg) for the recovery of adult worms. Eggs were obtained directly from the uterine tubes of female adults *T. canis* worms and incubated for 30 days in 2% formalin under a humidity greater than 80% and a temperature of 28°C with oxygenation (Avila *et al.*, 2012).

Experimental design

Two groups (G1 and G2) of eight female Swiss mice (outbred) aged five and seven weeks were established, and four males aged ten weeks were used for mating. The animals were housed in a controlled environment at 24°C (±1°C). The light/dark cycle was 12/12 h, and the animals had access to feed and water *ad libitum*. The mice were obtained from the central vivarium of the Federal University of Rio Grande.

The G1 mice received food containing 10^7 colony-forming units of *S. boulardii* per gram of food for 15 days before mating, during pregnancy and through 21 days of lactation. The G2 females received food without probiotics during the same periods.

Each G1 and G2 female was mated with a male mouse, and mating was confirmed by the presence of a vaginal plug. The pregnant females were housed in individual cages and inoculated with 300 *T. canis* embryonated eggs through an intragastric probe on the 14th day of pregnancy. After birth, the females were housed with their offspring for 21 days of lactation. The lactating females and their offspring were then euthanized, and brain, liver, lungs, heart, kidneys, spleen, eyes and carcasses were collected. The mammary glands from the lactating females were also collected. Each organ was macerated, and the tissue was digested with 1% pepsin and hydrochloric acid to recover *T. canis* larvae according to the methodology described by Xi & Jin (1998).

The following data were recorded: (1) larvae transmitted, defined as the number of larvae recovered in the offspring; (2) larvae retained in females, defined as the number of larvae recovered in the females; and (3) total larvae, defined as the number of larvae present in the females plus the number of larvae recovered from the offspring. To determine the transmission rate of *T. canis* larvae to offspring, the total number of larvae was considered 100%. Then, the number of larvae present in the offspring was used to determine the proportion of larvae transmitted – that is, the transmission rate (%).

The Shapiro–Wilk test was initially applied to all data to assess normality. The Mann–Whitney test was used to analyse the non-Gaussian data (recovery of larvae in lactating females and their offspring), and Test T was used to analyse the transmission rate (Gaussian samples). A significance level of 95% was used in both tests performed using GraphPad Prism version 7.0 (GraphPad Software, La Jolla, CA, USA).

Results

The addition of *S. boulardii* probiotic to the food of G1 mice reduced the transmission rate of larvae to offspring by 42% ($P = 0.041$) (table 1). However, there was no significant difference between the groups in the total number of larvae ($P = 0.817$) or the number of non-transmitted larvae (retained larvae) ($P = 0.837$). The examination of *T. canis* larvae distribution in the offspring revealed larval tropism for the brain and carcass. A 50% reduction ($P = 0.023$) in the number of larvae in the brain were observed in G1 offspring relative to G2 offspring (fig. 1).

The distribution of retained larvae in lactating females was evaluated, and tropism for brain was observed. The same average number (6.25) was observed in both groups. There was no statistically significant difference in the number of somatic larvae recovered in lactating females in the G1 and G2 groups ($P = 0.088$). In G1 females, there were larvae in the brain, liver, lungs, heart, kidneys, mammary glands and carcass. In G2, there was larval retention in the brain, liver, kidneys, mammary glands and carcass (fig. 2).

There was no significant difference between the sizes of the offspring between groups G1 and G2 ($P = 0.621$), which presented an average of 7.7 (±2.6) and 8.1 (±1.7) offspring generated per female in the G1 and G2 groups, respectively, yielding a total of 62 offspring in G1 and 65 in G2. The percentage of positive offspring for *T. canis* larvae was 46.77% and 56.96%, respectively, in groups G1 and G2 ($P = 0.492$).

Discussion

This study is the first to demonstrate the potential of probiotics to reduce the vertical transmission rate of *T. canis* larvae. A reduction of 42% ($P = 0.041$) in the transmission rate of *T. canis* larvae to offspring was observed in G1 group relative to G2 group, and 50% reduction in the number of larvae in the brains of the offspring was observed in G1 relative to G2 ($P = 0.023$). These results corroborate previous studies demonstrating promising results of probiotics *Enterococcus faecalis* CECT7121 (Basualdo *et al.*, 2007; Chiodo *et al.*, 2010), *Lactobacillus rhamnosus* (Walcher *et al.*, 2018) and *S. boulardii* (Avila *et al.*, 2012, 2016; Cardoso *et al.*, 2020) in altering *T. canis* larvae infection intensity.

Despite a reduction in the transmission rate observed in offspring in the probiotic-treated females, no significant difference in the total number of larvae recovered was observed between the *S. boulardii*-treated and control females. Similarly, there was no significant difference between groups in the total number of

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Table 1. Distribution of larvae recovered from lactating females (retained larvae), offspring (transmitted larvae), total larvae recovered and percentage of larvae transmission from lactating to offspring.

	Larvae number			
	Retained larvae	Transmitted larvae	Total larvae	Transmission (%)
G1 group	14	10	24	41.67
	28	6	34	17.65
	5	12	17	70.59
	5	0	5	0.00
	3	8	11	72.73
	4	1	5	20.00
	7	6	13	46.15
	9	1	10	10.00
Total	**75**	**44**	**119**	
Media	**9.4**	**5.5**	**14.90**	**34.85[a]**
SD	**±8.3**	**±4.5**	**±9.9**	**±27.35**
G2 group	25	8	33	24.24
	14	24	38	63.16
	1	16	17	94.12
	0	2	2	100.00
	5	10	15	66.67
	2	6	8	75.00
	7	7	14	50.00
	4	3	7	42.86
Total	**58**	**76**	**134**	
Media	**7.3**	**9.5**	**16.8**	**64.40[b]**
SD	**±8.4**	**±7.3**	**±12.6**	**±25.61**

G1, group supplemented with probiotic *Saccharomyces boulardii*; G2, control group; SD, standard deviation.
Different letters in the same column indicate a significant difference ($P < 0.05$).

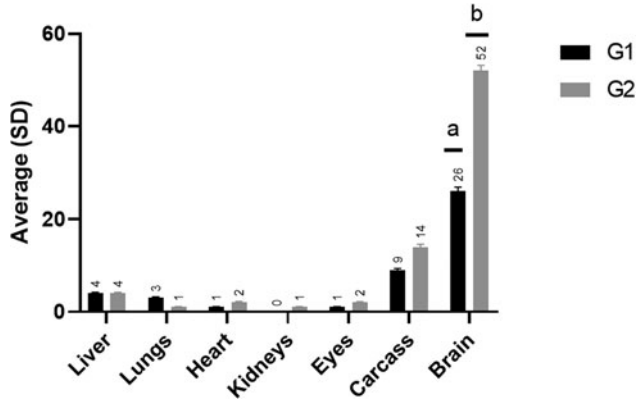

Fig. 1. *Toxocara canis* larvae distribution in the offspring after vertical transmission in the G1 (*Saccharomyces boulardii*, $n = 62$) and G2 (control, $n = 65$) groups. SD, standard deviation. Different letters indicate significant differences.

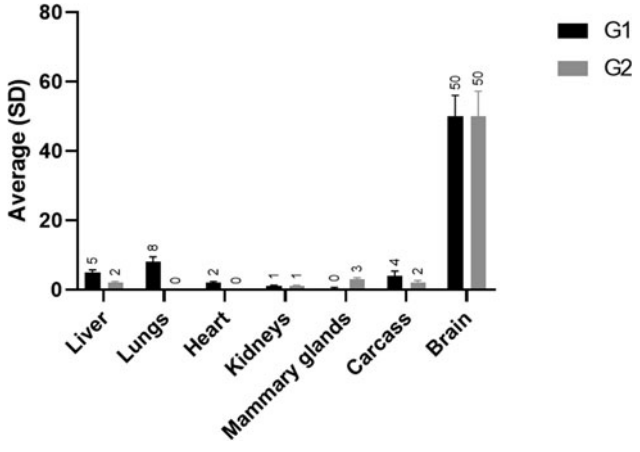

Fig. 2. *Toxocara canis* larvae distribution in G1 (*Saccharomyces boulardii*) and G2 (control) lactating females ($n = 8$). To investigate potential tropism and the difference in somatic larvae distribution, liver, lungs, heart, kidneys, mammary glands and skeletal muscle (carcass) were analysed. SD, standard deviation.

larvae, which represents the number of larvae expected in females in the absence of pregnancy or lactation. These results are inconsistent with the findings of previous studies, which demonstrated a reduction in the number of *T. canis* larvae in mice *S. boulardii* supplementation (Avila *et al.*, 2012, 2016; Cardoso *et al.*, 2020). However, in this study, unlike previous ones, the females were in the reproductive period, which can alter the immune system

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(Mor & Cardenas, 2010; Nauta *et al.*, 2013; Alijotas-Reig *et al.*, 2014). In this study, inoculation of embryonated eggs occurred at a time (the final third of pregnancy) favourable for nematode survival (Oshima, 1961; Glickman & Schantz, 1981; Strube *et al.*, 2013). Therefore, the physiological and immunological conditions during pregnancy and lactation and the parasite characteristics might have influenced the difference in infection intensity between G1 and G2 females ($P > 0.05$).

However, we revealed an influence of *S. boulardii* probiotic use on the vertical transmission of *T. canis* larvae in mice. The decrease in the transmission rate of larvae in offspring, despite the absence of a larval reduction in G1 females, indicates that there may have been a delay or decrease in larval migration in the G1 group, resulting in a lower level of larval transmission to offspring. However, there was no significant difference between the number of somatic larvae recovered in females in groups G1 (25 larvae) and G2 (eight larvae) ($P = 0.088$).

This microorganism has been revealed capable of modulating the mucosal response in mice (Moura *et al.*, 2017). When present in the intestine, probiotics can stimulate specialized cells of the epithelium associated with lymphoid follicles (M cells) and dendritic cells located in Peyer's patches. These cells are important components of the gut-associated lymphoid tissue that functions as a trigger to activate immunological systemic responses (Jung *et al.*, 2010). This capacity may allow the probiotics to function as immunomodulators in distant anatomic sites and induce responses beyond the gut mucosa (Clancy, 2003). Although these systemic effects are probably insufficient to eliminate *T. canis* larvae in pregnant females, they can interfere with larval migratory capacity and reduce their transmission to offspring. These effects may derive from the ability of *S. boulardii* to increase the levels of pro-inflammatory interleukins IL-12 and IFN-γ (Avila *et al.*, 2016; Moura *et al*, 2017), which can favour the formation of granulomas and thus decrease larval migration capacity; the increase of IL-12 is pointed out as the basis for the protective mechanism of this probiotic against *T. canis* (Avila *et al.*, 2016).

The two groups of lactating females exhibited the same average number of larvae in brain (6.25), which is an anatomic site with immunological weakness. In the brain, *T. canis* larvae are protected from host immune system action, and larvae in the chronic phase of infection are commonly present in the brain (Oshima, 1961; Glickman & Schantz, 1981; Jin *et al.*, 2008; Strube *et al.*, 2013), likely promoting their persistence until their transfer to the next generation (Dunsmore *et al.*, 1983).

Our results highlight the importance of somatic larvae to vertical transmission and revealed high heterogeneity in larval distributions in different tissues between the two groups. In both groups, larvae were found in the mammary glands, with slightly higher numbers being observed in G1 than G2. This finding can be attributed to the ability of the probiotic to interfere with larval migration. This result has significance, given the importance of transmammary transmission observed in BALB/c mice with chronic toxocariasis (De Souza Aguiar *et al.*, 2015). *Saccharomyces boulardii* yeast exhibits potential for decreasing the vertical transmission of visceral toxocariasis. The use of this probiotic reduced the transmission rate of *T. canis* larvae transmitted to the offspring of females infected during pregnancy and the number in the brains of offspring. Additional studies should be conducted to elucidate the mechanisms and the impacts of probiotic use on the biological cycles and survival strategies of *T. canis*.

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Conflicts of interest. None.

Ethical standards. This study was approved by the Ethics Committee on Animal Use of Federal University of Rio Grande (report number 055/2011), and all experiments were performed according to Brazilian legislation on animal care.

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