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Short communication

**Saccharomyces boulardii** reduces infection intensity of mice with toxocariasis

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**A B S T R A C T**

Several studies have shown the benefit of the use of probiotics in the prevention and treatment of diseases; however, few of them have investigated the effect of probiotics on parasitosis. In this study, the effect of *Saccharomyces boulardii* on the intensity of infection of mice with toxocariasis was evaluated. The animals were fed with a diet supplemented with *S. boulardii* for 15 days before inoculation with *Toxocara canis* eggs and for 2 or 60 days post-inoculation. *S. boulardii* promoted a reduction of approximately 36% in the average number of recovered *T. canis* larvae, suggesting that it can be used as an alternative to help control toxocariasis.

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Toxocariasis is a chronic tissue parasitosis that is distributed worldwide. It is a neglected disease and presents high prevalence levels mainly in developing countries. This parasitosis is characterized by the migration and permanence of larvae of helminthes in humans, and the etiologic agent most frequently associated to the disease is the nematode *Toxocara canis*. These parasites develop in the intestine of dogs, definitive hosts that contaminate the soil with parasite eggs. Humans and other paratenic hosts, such as mice, rats, chickens, lambs, pigs, among other, acquire toxocariasis by ingesting *T. canis* eggs containing the infective-stage larvae (Glickman and Schantz, 1981). The lack of a standard protocol for clinical and laboratory diagnosis makes it difficult to record the clinical forms and accurately determine the prevalence rates of *T. canis*. This zoonosis is mainly controlled by administering anti-helminthic drugs to dogs, which are important sources of infection of *T. canis* for man and other hosts. Nevertheless, treatment is difficult due to the occurrence of different clinical forms of human toxocariasis; therefore, the search for new alternatives of control is necessary (Smith et al., 2009).

Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits to the host. They have been successfully used in the prevention and treatment of diseases (Htwe et al., 2008; Canonici et al., 2011). The most commonly used probiotics are lactic acid bacteria, such as lactobacilli and bifidobacteria, and *Saccharomyces boulardii* yeast. The administration of *S. boulardii* reduces diarrhea and hospitalization (Htwe et al., 2008) and promotes immunostimulant and anti-inflammatory actions in the intestinal mucosa (Jawahra and Poulain, 2007).

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Few studies with probiotics have been conducted to evaluate the effect on parasitic diseases. Among the protozoa, the beneficial effects of Enterococcus faecium SF68 and Lactobacillus johnsonii La1, including a reduction of the number of trophozoites were observed, on Giardia intestinalis infection (Benyacoub et al., 2005; Humen et al., 2005). A protective effect was also demonstrated with the administration of the probiotic Lactobacillus casei, which reduced parasitemia in animals infected with Plasmodium chabaudi AS (Martínez-Gómez et al., 2006). In helminthiasis, Randazzo and Costamagna (2005) observed that the administration of L. casei in BALB/c mice inhibited the infection of Trichinella spiralis and the invasion of the intestinal mucosa.

Regarding toxocariasis, only two studies with probiotics have been performed. In both of them, a reduction in the number of larvae recovered from mice administered Enterococcus faecalis was observed during the initial phase of the infection (Basualdo et al., 2007; Chiido et al., 2010). However, considering that visceral toxocariasis is characterized as a chronic parasitic disease, it is important to conduct studies to investigate the effects of probiotics during the chronic phase of the infection. The aim of this study was to evaluate the effects of S. boulardii probiotic on the intensity of the infection of mice with toxocariasis in the initial and the chronic phases. Mice were chosen as experimental model for presenting larval migration similar to that occurring in humans (Glickman and Schantz, 1981).

Four groups of eight Swiss female mice aged between 6 and 7 weeks were used. The animals were kept in an acclimatized environment at 22 °C (± 2 °C) with a bright-dark cycle of (12 h); food (Labcol®) without antibiotics and water were available ad libitum. This study was approved by the Research Ethics Committee of the Universidade Federal do Rio Grande, and all of the experiments were performed according to the Brazilian legislation for animal experimentation.

T. canis eggs were collected from female adult parasites after treating young dogs with pyrantel pamoate (15 mg/kg). Then, the eggs were incubated in 2% formalin solution at 28 °C with a humidity higher than 90% and oxygenation for 30 days. The S. boulardii yeast was cultivated in YPD (Yeast Peptone Dextrose) medium, which was incubated in an orbital agitator (150 rpm) at 37 °C for 72 h. Then, the culture was centrifuged at 4000 × g for 10 min at 4 °C, and the pellet was washed with phosphate buffered saline (PBS) and submitted to lyophilization. The dried yeast was stored at −20 °C and quantified by determining colony forming units (CFU) in Agar YPD spread with serial dilutions in PBS from the lyophilized powder.

Groups I and III received daily milled ration and were supplemented with S. boulardii at a concentration of 107 CFU/g of ration for 15 days prior to inoculation by an intragastric probe containing 100 embryonated eggs of T. canis. Group I continued receiving the same diet for 2 days after infection, and Group III for 60 days after infection. Groups II and IV, controls of the Groups I and III, respectively, were also inoculated with 100 eggs of T. canis and receiving daily milled ration without probiotics during the same periods. Groups I and II were euthanized 2 days following infection and Groups III and IV, 60 days following infection. The animals were examined for larvae by digesting the carcass, brain, liver, lungs, kidneys, heart, eyes and spleen in a solution of 1% hydrochloric acid and 1% pepsin, according to Wang and Luo (1998). Data regarding the recovery of larvae from the organs and carcasses of mice were subjected to an analysis of variance, and the means were compared by Student’s t-test, with a significance level of 95%.

The administration of S. boulardii reduced the total average number of larvae recovered from animals analyzed 2 days post-inoculation by 36.7% (p < 0.0002). In addition, it promoted a reduction of 41.9% (p = 0.0001) in the average number of larvae recovered from the liver (Table 1). In the other organs and in the carcass, there was no significant difference (p > 0.05) (data not shown). In the group treated with S. boulardii and analyzed 60 days post-inoculation, there was a reduction of 35.9% in the total average number of larvae recovered (p = 0.06). There was also a reduction of 34.0% (p = 0.04) in the average number of larvae recovered from the brain. But, in the other organs or in the carcass, there was no significant difference (data not shown). Therefore, S. boulardii treatment reduced the intensity of the infection of mice with toxocariasis and is an alternative treatment for the control of this neglected zoonosis.

Until now, the effect of S. boulardii in the infection by T. canis had not been studied. In addition, no study had evaluated the effect of probiotics in chronic toxocariasis. Basualdo et al. (2007) and Chiido et al. (2010) observed a significant reduction in the number of larvae of T. canis in mice in the first 72 h after administration of probiotic CECT7121 of E. faecalis. Although the beneficial effects of

Table 1
Mean number of recovered Toxocara canis larvae of mice supplemented with Saccharomyces boulardii and controls (n = 8). Other organs were not shown because the number of recovered larvae was not significant.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean number of larvae (liver)</th>
<th>Mean number of larvae (brain)</th>
<th>Mean number of larvae (all organs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control – 2 days</td>
<td>15.5 ± 2.6</td>
<td>4.7 ± 2.1</td>
<td>16.6 ± 2.7</td>
</tr>
<tr>
<td>S. boulardii – 2 days</td>
<td>9.0 ± 1.6**</td>
<td>3.1 ± 1.3***</td>
<td>10.5 ± 2.3**</td>
</tr>
<tr>
<td></td>
<td>Reduction of 41.9%</td>
<td>Reduction of 34.0%</td>
<td>Reduction of 36.7%</td>
</tr>
<tr>
<td>Control – 60 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. boulardii – 60 days</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.0001,
**p < 0.0002,
***p < 0.04.
the administration of *S. boulardii* in several diseases are evident (Qamar et al., 2001; Htwe et al., 2008), there is still a gap in the understanding of the mechanism of action of this and other probiotics.

It is well documented that *S. boulardii* modulates adaptive immune responses (Jawhara and Poulain, 2007). However, this does not seem to have been the mechanism used by the yeast in this study because, only after 2 days of infection, we observed a reduction in the number of larvae of *T. canis* in mice treated with *S. boulardii*. Moreover, the reduction of larvae in the animals 60 days post infection (35.9%) was similar to the reduction observed in animals examined after 2 days (36.7%). To assess whether the mechanism is a direct effect of the probiotics on the parasite, we incubated *T. canis* larvae with *S. boulardii* in RPMI medium at 37 °C and 5% of CO2 and at the same concentration used in vivo. We observed that there was no larvicidal activity in vitro (data not shown). It can be inferred that the interaction between the yeast and the host is necessary for the development of the protective effect. In contrast, Chiodo et al. (2010) showed that the probiotic strain CECT7121 of *E. faecalis* presented direct activity on *T. canis* because it caused death of larvae in vitro after 48 h of incubation.

Considering the lack of participation of the adaptive immune response or the direct action of the yeast on *T. canis* larvae, it is possible that the reduction of the number of larvae in mice supplemented with *S. boulardii* could have occurred by increasing the integrity of the intestinal mucosa (innate immunity), hindering the penetration of *T. canis* larvae, making the animals more resistant to infection. According to Foligné et al. (2010), one of the effects of *S. boulardii* is related to maintaining the integrity of the intestinal mucosa. This mechanism was also suggested by Canonici et al. (2011), who found an increase in the secretion of collagen receptor α2B1 related to an increase in the recovery of the intestinal mucosa. Dahan et al. (2003) showed that *S. boulardii* has the ability to modulate the signal transduction pathways involved in controlling the tight-junction structure.

In a previous study, the supplementation of mice with *S. boulardii* increased the Kuffer cell number (Rodrigues et al., 2000). This phenomenon could help to explain the reduction of 41.9% in the mean number of larvae recovered from the livers of animals inoculated 2 days before probiotic treatment. However, although these cells play an important role in the destruction of pathogens (Beattie et al., 2010), the elimination of helminth larvae in just 2 days is unlikely, suggesting that Kuffer cells are not involved in the protective mechanism.

The effects of the administration of available drugs for the treatment of toxocarasis are generally unsatisfactory (Fernando et al., 2011) and require case control studies to evaluate the efficacy of existing, as well as new classes, of antihelmintic drugs (Smith et al., 2009). The combination of probiotics with conventional medications for the treatment of some parasitic diseases has shown promising results. In *Entamoeba histolytica* infection, patients receiving *S. boulardii* with metronidazole treatment showed significant results in reducing the duration of diarrhea and presence of cysts in the feces (Dinleyici et al., 2009). According to Besirbelloioglu et al. (2006), this combination also increased the efficacy of giardiasis treatment. Thus, the use of *S. boulardii* associated with antihelminthic drugs could also enhance the control of toxocarasis.

This was the first study to evaluate the effects of *S. boulardii* in toxocarasis, showing that such probiotics promoted an important reduction in the intensity of the infection by *T. canis* larvae. Therefore, this probiotic may constitute a novel alternative for the control of this parasitosis.

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