

REGULAR ARTICLE

BIO-THERAPEUTIC POTENTIAL OF A COMERCIAL DAIRY PROBIOTIC CONTAINING *Lactobacillus casei* SHIROTA FOR THE CONTROL OF CANINE HOOKWORM

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ABSTRACT

Domestic dogs (*Canis familiaris*) are present in the homes of several families around the world, and are one of the animals with greater contact with humans, either as a guard dog or just as a pet. With this, they can transmit parasites to their owners, since dogs are natural hosts of several parasites, among them the helminths of Ancylostomidae family. Ancylostomiasis presents as main symptoms abdominal pain and anemia. The treatment is performed with anthelmintics, however, it was found that these parasites are acquiring resistance to current drugs, which makes necessary the development of alternative therapies. Thus, this study aims to evaluate the therapeutic effect of a probiotic preparation containing 1x10¹⁰ CFU of *Lactobacillus casei* Shirota, administering 80 mL of this, for a group of four naturally infected dogs during 40 alternate days and, in parallel, administering a same volume during 30 consecutive days for a similar group of dogs. The protective effect was also evaluated by administering 80 ml of the preparation for a group of 5 healthy dogs for 30 consecutive days, which were maintained along with other infected animals. The number of eggs per gram of feces (EPG) was determined every 7 days, noting possible changes. As results, it was observed the potential use of probiotics in the treatment of hookworm in dogs as an inexpensive alternative therapy, since the assessed preparation induced significant reduction of the parasitic load of infected animals (p <0.005) in comparison with the control group, but in the other hand it was not observed protective effect, since the parasitosis have established in the healthy dogs which were pretreated with *L. casei* Shirota.

Keywords: Probiotics; intestinal parasites; hookworm, *Lactobacillus casei* Shirota

INTRODUCTION

Dogs are domestic animals present in the homes of several families, being the animal with which the most people develop an affective bond, even going so far as to consider them as "part of the family". However, this close proximity between people and dogs generates risks of development of zoonotic diseases, since dogs are hosts of several parasites, among them the helminths of the Ancylostomidae family (Coêlho *et al.*, 2013).

These parasites belong to the Animalia kingdom, filo Nematelminthes, sub-filo Nematoda, class Secernentea, order Strongylida, family Ancylostomidae. There are three species that are etiological agents of human hookworms, being *Necator americanus*, *Ancylostoma duodenale* and *Ancylostoma ceylanicum*, and among them the first two species are those that parasitize humans more frequently (Mitreva *et al.*, 2005; Seguel and Gottdenker, 2017).

These parasites can cause intestinal disorders in dogs, leading to the manifestation of symptoms such as anemia, weight loss and abdominal pain, and may even lead to death, and are capable of triggering pathological processes in humans, among them: Larva Migrans Syndrome (LMS) and Eosinophilic Enteritis. They are also causes of inflammatory edema, itching, redness and hemorrhage, being *Ancylostoma braziliense* and *A. caninum* immature larvae the main etiological agents, parasites of the small intestine of dogs and cats (Moro *et al.*, 2008).

To prevent this parasitic disease, it is important to adopt the antiparasitic treatment in dogs. There is a great availability of antiparasitic drugs, however, there is difficulty in controlling the infections caused by Ancylostomidae in dogs, since the available treatments require the administration of several doses throughout the life of the animal, which leads to the abandonment or neglect of the therapy, in addition to the risk of induction of resistance and inherent toxicity to some allopathic antiparasitics (Coêlho *et al.*, 2013).

Another important factor in the spread of this parasite is the large number of abandoned dogs that are not treated. Studies carried out in different Brazilian cities have shown that a large part of dog stool samples collected on the streets was contaminated with hookworms, consisting a great risk of transmission to other animals, such as stray dogs and cats and, consequently, to humans who come into contact with these infected animals (Alves *et al.*, 2016; Moreira *et al.*, 2014; Castro *et al.*, 2005).

Thus, it is necessary to develop new strategies to control this parasitosis, being one of the alternatives the use of probiotic foods, taking into account their benefits and low cost.

Probiotics are products composed of living and non-pathogenic microorganisms that can promote a favorable balance of the gastrointestinal tract microbiota as well as a positive modulation of the immune response, thus contributing to the prevention and treatment of pathologies (Coêlho *et al.*, 2013).

Bacteria present in probiotic foods are gram-positive, usually catalytic and producing lactic acid. Such microorganisms are tolerable to the pH of the gastrointestinal tract, and can compete in different ways with other microorganisms, including those that are considered pathogenic (Badaró *et al.*, 2008; Fleisch *et al.*, 2014; Siqueira *et al.*, 2008; Varavallo *et al.*, 2008).

Bacteria of *Lactobacillus* genus are able to tolerate the acidic pH, surviving the passage through the gastrointestinal tract. These microorganisms are producers of lactic acid and, unlike most obligate anaerobes, are able to grow in the presence of oxygen. The production of lactic acid, as result of carbohydrates fermentation, is capable of inhibiting the growth of competing microorganisms, and can control several infectious processes. Such microorganisms may also enhance the immune response, and these properties may be particularly useful in assisting in the control of various infectious processes, including hookworm in dogs (Coêlho *et al.*, 2013; Loukas *et al.*, 2001).

Some species of *Lactobacillus* belonging to the "casei group" are considered probiotic, that is, capable of positively affecting the health of the individual when administered in correct amounts. These microorganisms are highly used in the food industry in the manufacture of fermented milks or in the improvement of the quality of cheeses acting as initiators of the fermentation (Buriti *et al.*, 2007). According to Moal *et al.* (2014), such a beneficial effect may be due to competition for mucosal and nutrient binding sites, the production of antimicrobial compounds or the reduction of intestinal pH regarding the production of lactic acid and other short chain organic acids, a factor that also may provide a reduction in the incidence of pathogens.

The aim of this study was to evaluate the beneficial effects of the ingestion of a commercially available probiotic food containing 1x10¹⁰ colony forming units (CFU) of *Lactobacillus casei* Shirota as a mean of preventing in healthy dogs or treating hookworm infection in naturally infected dogs.

MATERIAL AND METHODS

This study was approved by the Animal Research Ethics Committee of FUNVIC - Faculty of Pindamonhangaba, protocol number 009/2009.

For the selection of the animals fecal samples were collected from a total of 150 dogs from residences or kennels, of which 24 were selected to compose the study.

The fecal samples were collected and sent under refrigeration to the Laboratory of Parasitology and Malacology - LAPAM of the Christian Life University Foundation/Faculty of Pindamonhangaba - FUNVIC, and analyzed according to the Willis method (flotation in NaCl d=1.2). After initial diagnosis, quantitative analysis was performed to determine the number of eggs per gram of feces (EPG) using the McMaster method.

To perform the experiment, the animals were distributed into four groups, as follow:

Group 1 - Six animals naturally infected with hookworm, which were treated on alternate days, during 30 days, with 80 mL of dairy probiotic containing 1×10^{10} CFU of *Lactobacillus casei* Shirota.

Group 2 - Six animals naturally infected with hookworm, treated continuously during 30 days, with 80 mL of of dairy probiotic containing 1×10^{10} CFU of *Lactobacillus casei* Shirota.

Group 3 - Six healthy animals, which were submitted to the same treatment of group 2.

Group 4 - Six infected animals, which were not submitted to any treatment (control).

The evaluation of the treatments was done by performing fecal sample collection every 7 days during the period of time of each experiment. To determine the therapeutic efficacy of the fermented milk in study, the samples were analyzed regarding the determination of EPG (Eggs Per Gram of fezes). The reduction of the parasite load of the animals was calculated according to the following formula (Pereira-Junior et al., 2017):

$$\text{Reduction (\%)} = \frac{\text{EPG average of initial day} - \text{EPG average of day n} \times 100}{\text{EPG average of initial day}}$$

Statistical methods were used to evaluate the results, been chosen according to the characteristics of the sample distribution. The Kruskal-Wallis test was used at a significance level of 5% and Student-Newman-Keuls test to evaluate differences between means, using BIO ESTAT software 5.0.

RESULTS AND DISCUSSION

As observed in Table 1, the evaluation of the mean EPG values of dogs belonging to group 1 (animals infected with hookworm which were treated on alternate days with the probiotic drink evaluated) showed that there was no significant reduction ($p < 0.005$) of these values during the four week treatment and the followed two weeks experiment extension. It is observed that after the fifth and sixth week of treatment the values related to EPG reduction were

Table 1 Mean values of eggs per gram of feces (EPG) and percentage of EPG reduction in dogs infected with hookworm and treated with a probiotic preparation containing *Lactobacillus casei* Shirota administered on alternate days (Group 1)

	Initial Day	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week
EPG Average	36825	3612,5	2437,5	1525	1112,5	862,5*	375*
% EPG reduction	-	90,19%	93,38%	95,86%	96,98%	97,66%	98,98%

* = significant reduction in parasite load ($p < 0.005$) compared to day 0.

Table 2 Mean values of eggs per gram of feces (EPG) and percentage of EPG reduction in dogs infected with hookworm and treated with a probiotic preparation containing *Lactobacillus casei* Shirota daily (Group 2)

	Initial Day	1 st week	2 nd week	3 rd week	4 th week
Average	13130	9465	6050	740*	390*
% reduction	-	27,91%	53,92%	94,36%	97,03%

* = significant reduction of parasite load ($p < 0.0001$) in relation to day 0.

After evaluating the mean EPG values of the uninfected dogs belonging to Group 3 which were treated with *Lactobacillus casei* Shirota, it was observed that there was a significant increase ($p < 0.005$) in EPG in the fourth week of treatment when compared to the initial day values (one day before the start of the experiment), as shown in Table 3.

It's important to highlight that this group was designed to evaluate the protective effect of the probiotic preparation, in order to prevent the animals to the risk of natural infection.

These results showed that the use of *L. casei* Shirota in the experimental conditions evaluated in the present study, did not induce protective activity for uninfected dogs, since despite the previous treatment with the probiotic preparation they subsequently developed the parasite in question. In this context,

significant, when compared to the values obtained at the initial day (one day before the beginning of the experiment).

The efficacy of the probiotic dairy consumption was also observed after evaluating the mean EPG values of dogs infected with hookworm belonging to Group 2 (which were treated with *Lactobacillus casei* Shirota daily). It was observed that there was a significant reduction ($p < 0.0001$) of this parameter in the third and fourth week of treatment when compared to values obtained at the initial day (one day before the beginning of the experiment).

These results ara according to those presented by Coêlho et al. (2013) that demonstrated the potential use of probiotic preparations in the control of hookworm in dogs belonging to a group of 10 naturally infected animals which were treated with 5 mL of a probiotic preparation with three different strains of *Lactobacillus* containing 1×10^6 CFU / mL for 28 days. At the end of the treatment, it was observed a parasite load reduction of 88,83%, which was statistically significant ($p < 0.05$) regarding the control group.

The benefits of using probiotics in dogs parasitized with hookworm were also demonstrated in a study by Mussi et al. (2008), in which 19 dogs infected with this parasite were treated with anthelmintics such as febantel, pirantel pamoate and praziquantel concomitantly with a commercial probiotic food, the preparation being administered for 15 days on alternate days. The results showed that the mentioned probiotic promoted a beneficial effect influencing positively in the health of the animals.

It should be noted, however, that the species of *Lactobacillus* evaluated in the present study had already shown promise for the control of other parasitic diseases, among which giardiasis and triquinellosis. Coêlho et al. (2016) evaluated the use of a commercially available dairy drink containing $1,6 \times 10^6$ live *Lactobacillus, L. casei* Shirota species for the control of giardiasis in naturally infected children, and observed that after 21 days of treatment, evolutionary forms of this parasite were not detected, with improvement of the clinical condition and the consistency of the feces.

According to Fleisch et al. (2014), the use of probiotics stimulates phagocytosis, since such microorganisms induce an increase in the count of circulating lymphocytes and cytokines. Thus, the intestinal immune system is optimized, decreasing the incidence of infections and favoring the intestinal microbiota, promoting the local immune response and at a systemic level.

Regarding the immune humoral response, Martínez-Gómez et al. (2009) carried out a study that evaluated the therapeutic effect of a probiotic preparation containing 1×10^8 CFU of *L. casei* Shirota on 60 CD1 mice infected with *Trichinella spiralis*, a parasite belonging to the Nematoda class. Therefore, it was observed that intraperitoneal administration of such preparation, which was administered once a week for 3 weeks, caused a significant ($p < 0.05$) decrease of adult parasites found in the intestines of the animals. In addition, is was shown a statistically significant ($p < 0.05$) increase in IgA levels in the intestinal mucosa of the animals, in relation to the control group.

it should be emphasized that the frequency of probiotic preparation consumption is relevant.

The lack of ability of probiotics to induce protection against the establishment of a hookworm infection may be related to immunity mechanisms involved in both the colonization process of the intestinal mucosa and in the process of inducing an effective immune response against the above mentioned parasite.

It is known that probiotics exert a significant influence on the modulation of the function of dendritic cells in the intestinal mucosa. Although dendritic cells are components of the innate immune system, being able to phagocytose pathogens, their most important function is to process antigenic material and present it to specialized cells of the immune system. Due to its great plasticity and maturation capacity in response to local danger signals derived from innate immunity,

dendritic cells are a key element in the connection between innate immunity and responses of adaptive immunity (You et al., 2014; Bernardo, 2013). Thus, it is possible to infer that the time elapsed between colonization of the intestine by the parasite and the development of an effective immune response induced by *L. casei* Shirota and controlled by immune cells, such as dendritic

cells, was not sufficient to determine the control of the infectious process in this group, unlike the groups in which the animals were infected before starting treatment.

Table 3 Mean values of eggs per gram of feces (EPG) and percentage of EPG reduction in uninfected dogs treated with a probiotic preparation containing *Lactobacillus casei* Shirota daily (Group 3)

	Initial Day	1 st week	2 nd week	3 rd week	4 th week
Average	0	0	0	0	730*

* = significant reduction in parasite load (p <0.005) compared to initial day

Finally, regarding the results of the mean EPG values of the naturally infected animals that did not undergo any treatment (group 4 - control) shown in Table 4, it is observed that there was no significant reduction during the period of the experiment. Note that this group was performed aiming to evaluate the progression of the disease, in the absence of any treatment, particularly with regard to the number of EPG. The persistence of hookworm eggs in the feces of

the animals belonging to group 4 reinforces the evidence that the significant reduction of EPG observed in groups 1 and 2 was due to the action of *Lactobacillus* present in the milk beverage evaluated, not being a reduction that could happen naturally.

Table 4 Mean values of eggs per gram of feces (EPG) and percentage of EPG reduction in dogs infected with hookworm and not treated (Group 4)

	Initial Day	1 st week	2 nd week	3 rd week	4 th week
EPG Average	5630	5950	5500	5650	5580
% EPGreduction	-	-5,7	2,3	-0,3	0,8

Therefore, it was demonstrated the feasibility of using a commercially available probiotic food containing *Lactobacillus casei* Shirota for the control of such parasitosis, since the consumption of the evaluated probiotic food induced a significant reduction of the parasite load in feces of dogs naturally infected with hookworm.

CONCLUSION

These results showed that the evaluated probiotic containing 1x10¹⁰ CFU of *Lactobacillus casei* Shirota, exerted therapeutic effect in dogs naturally infected with hookworm. This treatment resulted in a significant reduction of EPG in the faeces of the animals when administered daily, as well as on alternate days. The results also revealed that the administration of the studied dairy probiotic in healthy dogs, kept in the same environment as parasitized dogs, did not promote a protective action, since the infection prevention was not observed. Therefore, the use of probiotics in the control of intestinal parasitoses can be seen as a potential alternative treatment, taking into account its low cost and the health benefits provided.

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