
EFFECT OF ORAL ADMINISTRATION
OF PROBIOTIC AGENTS ON
Trichinella spiralis-INFECTED MICE

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ABSTRACT

This study aims at determining whether or not the oral administration of *Lactobacillus casei* exerts adverse effects on *Trichinella spiralis* (strain BBSC 01)-infected BALB/c mice. Ten mice previously divided into Control Group "C" and Treated Group "T" were used. Group C was fed with a regular diet during 7 days before *T. spiralis* infection. Group T was fed on a daily basis with a regular diet plus a 5 mL of suspension of *L. casei* during seven days before *T. spiralis* infection. On day 8, both Groups were orally inoculated with infecting *T. spiralis* larvae. Five days after infection, mice were sacrificed in order to determine the number of parasites in the intestine and in the intestinal mucosa (females). The average number of recovered adult *T. spiralis* parasites from the intestinal content was 147 and 189 in Group C and in Group T, respectively. Significant differences for $p < 0.05$ were observed between the two groups analyzed. The average number of recovered females in Group C was 81 while in Group T it was 12. Highly significant differences for $p < 0.05$ were observed. Results indicate that the oral administration of *L. casei* to mice produces adverse effects on the penetration of the parasite into the intestinal mucosa.

KEYWORDS: Trichinellosis. Probiotics. *Trichinella spiralis*. *Lactobacillus casei*.

INTRODUCTION

Trichinellosis is a parasitic infection produced by *Trichinella spiralis*. In Argentina, the main endemic area of this disease is Buenos Aires Province, particularly, the region corresponding to Bahía Blanca city (8, 9,10). It is caused by the ingestion of either raw or undercooked pork meat or by the ingestion of its byproducts (sausages and links, cured pork meats, etc.) infected by living *T. spiralis* larvae (11) which, due to the action of the digestive fluids, are released into the intestine. Prevention is the best measure to avoid this disease. The presence of

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gravid females within the intestinal mucosa as well as the expulsion of males into the intestinal lumen after copulation produce a superficial enteritis ending in the gastrointestinal symptoms typical of Trichinellosis. Females enter into the intestinal mucosa where they deposit larvae which later reach the circulatory system, thus disseminating themselves within the organs until they penetrate into the skeletal muscle fibers producing their partial destruction and generating the toxic allergic process typical of Trichinellosis.

To date, no particularly effective treatment against Trichinellosis has been formulated. Immunomodulators, which augment the non-specific immune response, are therefore used as an alternative to control this as well as other types of parasitic diseases (1, 2, 3, 4). *Lactobacillus casei* is a probiotic bacterium used for the production of fermented milk products. Studies reveal that the oral administration of *L. casei* induces an increase in innate immunity and that such increase stimulates the activity of natural killer cells, activates the production of cytokines, prevents certain types of tumors from occurring, and protects the organism either against gastrointestinal pathogens, such as *Escherichia coli*, *Salmonella typhimurium* or against viruses such as *Rotavirus* (13, 14). Our literature retrieval carried out for the present study indicates that the information regarding the protective role of probiotic bacteria in the prevention of parasitic diseases, particularly Trichinellosis, is scarce (5, 6). In view of this, the purpose of this study was to demonstrate that the oral administration of a probiotic protects the host from the invasion of *T. spiralis* gravid females into the intestinal mucosa either by obstructing or interrupting the biological cycle of such larvae.

MATERIALS AND METHODS

Experiments were conducted on 10 sanitarly controlled BALB/c mice which had been grown in the Bioterium of the Department of Biology, Biochemistry and Pharmacy at the *Universidad Nacional del Sur*, Bahía Blanca, Province of Buenos Aires, Argentina. The animals were randomly divided into the following groups of five mice each: the Control Group (Group C), which was normally fed (i.e. with no probiotic agent), and the Treated Group (Group T), which was orally fed with *L. casei*.

The strain of *T. spiralis* (BBSC 01) used in the present research was typified via PCR by Dr. E. Pozzio in Rome, Italy. Probiotic *L. casei* strain ATCC 469 grown in Ellefer broth was used in the present research. The suspension concentration in our experiments was 1.9×10^9 ufc/mL of viable *L. casei*.

A 5 mL supplement of sterile physiological solution was added as placebo to the food corresponding to Group C seven days before *T. spiralis* infection. Group T was daily fed with regular food plus 5 ml of suspension in physiological solution of *L. casei* 1.9×10^9 ufc/mL during seven days before infection with *T. spiralis* larvae.

On day 8 (day 0), both Group C and Group T were orally inoculated with 500 *T. spiralis* infecting larvae obtained following the artificial digestion method (15). Larvae subsequently continued living normally. Five days after infection, mice were sacrificed in accordance with national animal ethics guidelines for the determination of the number of adult parasites present not only in the intestine (males and females) but also in the intestinal mucosa (females). Intestinal parasites were obtained by washing the intestinal content in 5%-formolated physiological solution while females from the intestinal mucosa were obtained following the artificial digestion method, which is habitually used to detect the presence of larvae in raw meat and cured meat (15). The male and female infecting larvae found in each place were subsequently counted.

Statistical Analysis

Statistically significant differences ($p < 0.05$) were determined via the t-test by comparing the presence of parasites in the intestinal mucosa in Group C as well as in Group T with the presence of adult parasites (males and/or females) in the intestinal lumen.

RESULTS

Analysis of intestinal content

The quantification of the parasites found in the intestinal content indicated that the total number of recovered adult *T. spiralis* parasites in group C after washing the intestinal content in 5%-formolated solution was 735. Average value was 147 and standard deviation was 18.07.

The quantification of the parasites found in Group T after washing the intestinal content in 5%-formolated solution indicated that the total number of recovered adult *T. spiralis* parasites was 945. Average value was 189 and standard deviation was 26.71. The t calculated value was 2.91 (8 degrees of freedom) and it exceeded t critical value. Significant differences for $p < 0.05$ were therefore observed between the two populations. Results are shown in Table 1.

After comparing the results obtained from the processing of the intestinal mucosa in Group C and Group T, it was observed that the total number of recovered female parasites in Group C was 405. Average value was 81 and standard deviation was 14.04, respectively.

The quantification of the parasites found in the intestinal mucosa in Group T indicated that the total number of recovered male parasites was 60. Average value was 12 and standard deviation was 6.0, respectively. The t calculated value was 10.1 (8 degrees of freedom) and it exceeded t critical value. Significant differences for $p < 0.05$ were registered between the two populations. Results are shown in Table 1.

Table 1. *T. spiralis* recovered adult parasites after washing the intestinal and the intestinal mucosa content 5 days following infection (t distribution used)

<i>T. spiralis</i> recovered adult parasites after washing the intestinal content				
Group	Treatment	Total Number of Adults	Average Value	Standard Deviation
C (n:5)	Sterile Solution	735	147	18.07
T (n:5)	<i>L. casei</i>	945	189	26.71
<i>T. spiralis</i> recovered adult parasites from the intestinal mucosa				
C (n:5)	Sterile Solution	405	81	14.04
T (n:5)	<i>L. casei</i>	60	12	6.0

Results indicate that the oral administration of viable *L. casei* to mice during seven days before *T. spiralis* infection induces a significantly protective response ($p < 0.05$) against Trichinellosis. Results from the present research also demonstrate that the number of females that penetrated into the intestinal mucosa was considerably lower when treatment with the probiotic bacterium was followed. This indicates that the evolution cycle of *T. spiralis* seems to be interrupted as a result of the cyclic and continuous administration of *L. Casei*, thus minimizing the invasion of larvae into the host.

DISCUSSION

Probiotic agents, particularly, lactic bacteria, have beneficial effects on the health of the host (13, 14). They activate the production of IgA, they have immunostimulatory effects as well as antitumoral effects, and they play a key role in the prevention of a large variety of enteric infections (16, 17). Such effects result from a cyclical and prolonged treatment with probiotic bacteria. Both the search for effective antiparasitic agents and the increase in the resistance to anthelmintic agents make it necessary to look for new alternative treatments for the prevention and control of parasitic diseases.

Our results indicate that the oral administration of *L. casei* seems to produce adverse effects on the penetration of the parasite into the intestinal mucosa and induces a significantly protective response against the establishment of *T. spiralis* (5, 7) through a mechanism that has not been fully elucidated to date. In addition, it has been demonstrated that the protection induced by *L. casei* exclusively operates on adult parasites and that the consumption or inoculation of either *L. casei* or other probiotic agents should be continuous and close to the stage of infection. It has also been reported that protection is partly due to the production of interferon generated by the treatment with the lactobacillus (5, 6, 12). The oral administration of probiotic bacteria may generate resistance to infection directly related with the innate immune response (7). As stated above, trichinellosis is a parasitic zoonosis transmitted through food. It represents a cause of morbi-mortality particularly in endemic areas. In the absence of either a specific or an effective

treatment against this type of parasitosis, prevention measures and the use of natural immunomodulators to increase the immune non-specific response are, in the meantime, the best alternative against this disease. Our results demonstrate that the oral administration of viable *L. casei* to mice seven days before *T. spiralis* infection induces a significantly protective response ($p < 0.05$) against the establishment of parasites. Our study also reveals a significant increase in the number of adult parasites in the intestinal content that were later eliminated through fecal matter as well as a significant decrease in the number of females that penetrated into the intestinal mucosa at the moment of conducting the treatment with the probiotic agent. This may be indicative of the fact that the evolution cycle of *T. spiralis* is obstructed through the cyclical and continuous administration of *L. casei* because the entrance of females into the intestinal mucosa is interrupted, thus minimizing the penetration of larvae into the host.

RESUMO

Efeito da administração oral de probióticos em camundongos infestados com *Trichinella spiralis*

Objetivou-se determinar se a ingestão oral de *Lactobacillus casei* exerce uma ação antagonica na infestação de camundongos com larvas de *T. spiralis* (cepa BBSC 01), protegendo o hospedeiro da invasão à mucosa intestinal das fêmeas grávidas do parasito. Foram utilizados dez camundongos BALB/c, distribuídos ao acaso em dois grupos: Grupo C ou grupo controle e Grupo T ou grupo tratados com *L. casei*. Ao alimento do grupo C, foram inclusos 5 mL de solução fisiológica durante sete dias antes da infestação com larvas de *T. spiralis*. O Grupo T recebeu alimentação normal, diariamente, acrescida de 5 mL de suspensão em solução fisiológica de *L. casei* durante sete dias, com larvas infestantes de *T. spiralis*. Ao oitavo dia, ambos os grupos (C e T) foram inoculados via oral com larvas infestantes de *T. spiralis*. Aos cinco dias após infestação, os animais foram sacrificados para determinação da carga parasitaria presente no intestino e na mucosa intestinal (fêmeas). O número médio de parasitos adultos de *T. spiralis* recuperados do conteúdo intestinal no grupo controle foi de 147, enquanto que no grupo tratados foi de 189, apresentando diferenças significativas para $p < 0,05$ entre ambos os grupos analisados. O valor promédio de fêmeas recuperadas no Grupo C foi de 81 enquanto no grupo T foi de 12 fêmeas, apresentando diferenças altamente significativas para $p < 0,05$. Os resultados obtidos indicam que o *L. casei* administrado pela via oral produz um efeito antagonico sobre a penetração do parasito na mucosa intestinal.

DESCRITORES: Trichinelose. Probióticos. *Trichinella spiralis*. *Lactobacillus casei*.

RESUMEN

Efecto de la administración oral de probióticos en ratones infestados con *Trichinella spiralis*

Nuestro objetivo fue determinar si la administración oral de *Lactobacillus casei* ejerce una acción antagonica en la infestación de ratones con larvas de *T. spiralis* (cepa BBSC 01), protegiendo al hospedador de la invasión a la mucosa intestinal de las hembras grávidas del parásito. Se utilizaron, para la experiencia, 10 ratones BALB/c. Los ratones fueron distribuidos al azar en dos grupos: Grupo C o Grupo Control y Grupo T o Grupo Tratados con *L. casei*. Al alimento del grupo C se le incluyó 5 ml de solución fisiológica durante siete días previos a la infestación con larvas de *T. spiralis*. El grupo T se alimentó diariamente, además del alimento normal, con 5 ml de suspensión en solución fisiológica de *L. casei* durante siete días, previo a los desafíos con larvas infestantes de *T. spiralis*. Al octavo día ambos grupos (C y T) fueron inoculados vía oral con larvas infestantes de *T. spiralis*. A los cinco días post-infestación, los animales se sacrificaron para determinar la carga parasitaria presentes en el intestino y en la mucosa intestinal (hembras). El número promedio de parásitos adultos de *T. spiralis* recuperados del contenido intestinal en el grupo Control fue de 147 mientras que en el grupo Tratados fue de 189; presentándose diferencias significativas para $p < 0,05$ entre ambos grupos analizados. El valor promedio de hembras recuperadas en el grupo C fue de 81 mientras que en el grupo T fue de 12 hembras, presentando diferencias altamente significativas para $p < 0,05$. Los resultados obtenidos indican que el *L. casei* administrado vía oral produce un efecto antagonico sobre la penetración del parásito en la mucosa intestinal.

PALABRAS CLAVE: Trichinelosis. Probióticos. *Trichinella spiralis*. *Lactobacillus casei*.

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