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Original paper

The protective effect of *Lactobacillus acidophilus* on experimental animals challenged with *Trichinella spiralis*; new insights on their feasibility as prophylaxis in *Trichinella spiralis* endemic area

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ABSTRACT. Trichinellosis is a common parasitic zoonosis. Complications of anthelmintic drugs combined with steroids raise the urge of alternative protective ways. The study aimed to investigate the protective effects of *Lactobacillus acidophilus* probiotic on both *Trichinella spiralis* adults and larvae in experimental animal models. Thirty-six male BALB/c mice were divided into 3 groups: negative control Group (G I); Group (G II) mice were inoculated orally by 500 *Trichinella spiralis* larvae; tested Group (G III) mice were prophylactic by an oral dose of *Lactobacillus acidophilus* in commercially available form for seven consecutive days, before infection. Mature worms and encysted larvae were counted on the 5th and 21st day post-infection (dpi), respectively. IL-1, IL-6, IL-10 and TNF-α concentrations were estimated at 5th and 21st dpi of all groups. Significant reductions in mean worms and larvae burden were detected by 62.1% and 73.5% in the prophylactic group compared to the non-prophylactic group. The cytokine profiles were revealed IL-1 and IL-6 up-regulation compared to IL-10 and TNF-α down-regulation in the tested group compared to other groups. Although *Lactobacillus acidophilus* failed to achieve complete eradication of *Trichinella spiralis* adults and larvae, it showed powerful effects in reducing parasites and cytokines burdens.

Keywords: trichinellosis, probiotics, postbiotics, interleukins, tumor necrosis factor, *Lactobacillus acidophilus*

Introduction

Trichinellosis, an important parasitic zoonosis with worldwide distribution [1] results from infection by a parasitic nematode species of genus *Trichinella* [2]. It is among the top 10 international rankings of foodborne parasitic infections, which causes economic losses and poses an important public health hazard [3].

Human trichinellosis has been reported in 55 countries and currently affects an estimated 10,000 cases per year with a 0.2% death rate [4,5]. Infection is usually acquired by the consumption of raw or undercooked pork meat or products [1]. The currently used treatment for trichinellosis includes benzimidazole anthelmintic (mebendazole or albendazole) combined with steroids [6]. This classical treatment is problematic and far from ideal mainly due to the increasing spread of anthelmintic resistance and the poor susceptibility of migrating and encapsulated muscle larvae to anthelmintic drugs [7]. Moreover, serious coagulopathy disorders as fatal hemorrhages may be provoked due to combinations of anthelminthic with steroids [8]. Therefore, there is a growing interest in developing new effective methods for controlling this disease with lower side effects risk such as the use of the immune-stimulating probiotics [9].

Probiotics are defined as live microbial food supplement microorganisms that confer health benefits when administered in adequate amounts [10]. They have emerged as an alternative therapy both for the prevention and treatment of a wide range of infectious and non-infectious diseases [11]. Modulation of the intestinal environment, enhancement of the mucosal barrier and secretion of active postbiotics, are among the proposed modes of action of probiotics [12].

The immunomodulatory effect of probiotics is attributed to the release of cytokines including interleukins (ILs) and has been characterized by triggered cell production [13]. In recent years, several therapeutic and/or prophylactic efficacy of probiotics have been evaluated as single agents or combination therapies in various infections and diseases; many of them have exerted beneficial properties against different intestinal protozoan such as cryptosporidiosis, giardiosis, coccidiosis. However, the effects of probiotics on helminth infections remain largely unexplored [14]. The few available results relating to their effects on helminth infections are conflicting. For instances, they showed definitive efficacy in reducing the worm burden in mice infected with *Trichinella spiralis* (*T. spiralis)* [15,16], *Toxocara canis* [17,18], *Schistosoma mansoni* [19,20], and *Strongyloides* spp. [21]. In contrast, few studies reported probiotics failure in reducing parasite load in rats [22] and mice [23] infected with *T. spiralis*. Furthermore, Dea-Ayuela et al. [24] reported that oral supplementation of *Lactobacillus casei* increased mice susceptibility to *Trichuris muris*.

Taken together, the above-mentioned data; urge the need to find out appropriate microbial strains with defined characteristics to be incorporated into human health, effective in preventing and treating helminths and also to shed light on the underlying mode of action through which probiotics act. Thus, we aimed in this study to test if the repeated orally administered freeze-dried powder containing *Lactobacillus acidophilus (L. acidophilus)*, can induce preventive effects against the experimental *T. spiralis* infection in mice. *Lactobacillus acidophilus* effectiveness was measured through testing reduction rate of both intestinal adults' burden and larval burden in muscle. As well as to assess the levels of serum cytokines (interleukin-1, IL-6, IL-10, and TNF-α) against the nematode parasite of treated animals as compared with controls.

Materials and Methods

Experimental animals

The experiment was conducted on thirty-six male parasitic free BALB/c mice (6–8 weeks). They weighed 25–30 g and were obtained from the Faculty of Medicine, Assiut University, Assiut, Egypt. Animals were treated according to the national animal ethics guidelines.

Parasite

T. spiralis was originally isolated from the diaphragms of infected pigs from El-Bassatine Abattoir, Cairo. It was routinely maintained in the laboratory of Faculty of Medicine, Assiut University, through the consecutive passage in BALB/c mice according to Gamble [25]. Briefly, heavily infected diaphragms were minced, digested in 1% pepsin-hydrochloride and incubated overnight at 37°C. Larvae were collected by sedimentation method, washed several times in physiological saline (0.85%), and the number of larvae per mL was counted. Infected mice were inoculated orally by 500 larvae [25].

Probiotic strain and dose

L. acidophilus is a commercially available freeze-dried powder containing the probiotic strain (Custom Probiotics Inc., California, 91214). The prepared vehicle powder containing (besides the bacteria) skim milk, sodium glutamate, protease, ascorbic acid, and cornstarch (for the control group, cornstarch powder was given). The powder was dissolved in sterile distilled water, and the viability of the lactobacilli was determined by aerobic culturing of rehydrated powder. The viability of the rehydrated bacteria was over 90%. The suspension concentration was 1.9×10^9 Ufc/ml.

Experimental design

Mice were divided into 3 groups (12 mice each): mice of Group 1 (G I) and Group 2 (G II) received 1 ml of a cornstarch powder suspended into sterile distilled water for seven days. Group 3 (G III) mice were prophylactic by an oral dose of *Lactobacillus* for seven consecutive days, before infection with *Trichinella* larvae. Each dosage was prepared and adjusted to contain 10^9 viable bacteria in 1ml distilled water and was given via oro-gastric gavages [26].

Table 1. Adult worms count in the mice small intestine of different groups

Groups	No.	Mean adult count	SD	Reduction rate	*P*-value
Group I	6	0.00	0.00	0	
Group II	6	330	12.28	0	
Group III	6	125	4.02	62.1%	0.00050**[1]

[1]**P*-value is significant (*P*-value≤0.05); ***P*-value is highly significant (*P*-value≤0.001); Group I: –ve control group; Group II: +ve control group; Group III: tested group

On day 8, mice of both G II and G III were orally inoculated with 500 larvae of *T. spiralis*. While those of G I were kept as a non-infected non-prophylacted control group. Five days post-infection (dpi), six mice of each group were sacrificed and the numbers of adult worms in the intestine were isolated and counted [27]. On the 21st dpi, the remaining six mice of each group were sacrificed for determination of the total number of muscle larvae [28]. The obtained number was divided by the number of grams of muscle mass to obtain the number of larvae per gram. The reduction rate in larval burden was calculated as follows: larval burden reduction rate (%) = (A-B/A×100), where A=no. of worms or larvae extracted from positive control animals and B=no. of worms or larvae extracted from treated animals [29]. Sera from all scarified mice were collected and frozen at -80ºC until use.

Cytokine analysis

At the 5th and 21st dpi, the collected sera from all mice groups were tested for IL-1, IL-6, IL-10 and TNF-α concentrations using commercial enzyme-linked immunosorbent (ELISA) kits (BD Pharmingen, USA) according to manufacturer's instructions. All samples were done in triplicate and diluted to 1:2. Tetramethylbenzidine (TMB) was the substrate used and 2 M H2SO4 was added to terminate the reaction. The absorbance was read at 450 nm in a microplate reader (BIO-RAD, USA). Cytokines concentrations were measured using standard curves done with known concentrations of cytokines (BD Pharmingen, USA). Results were reported in picograms per milliliter (pg/ml).

Ethics considerations

Animal experiments were done in the Animal House, Faculty of Medicine, Assiut University. The research work was approved by the Animal House ethical committee, Faculty of Medicine, Assiut University (Approval No. 17300368). Animal handling protocols used meet the standard international guidelines by the National Institutes of Health guide for the care and use of laboratory animals and guidelines used in other Egyptian universities and research centers.

Statistical analysis

Data were reported and expressed as the means ± standard deviation (SD). Student's t-test, using SPSS 13.0 software, was used to provide statistical analysis. P-value≤0.05 was considered to be statistically significant.

Table 2. Muscle larval burden in different groups

Groups	No.	Mean larvae count/g muscle	SD	Reduction rate	*P*-value
Group I	6	0.00	0.00	0	
Group II	6	800	7.07	0	
Group III	6	212	2.82	73.5%	0.00040**[2]

[2]**P*-value is significant (*P*-value≤0.05); ***P*-value is highly significant (*P*-value≤0.001); Group I: –ve control group; Group II: +ve control group; Group III: tested group

Table 3. Inflammatory cytokines profile in different groups in relation to dpi

Groups	No.	dpi	IL-1 Pg/ml	IL-6 Pg/ml	IL-10 Pg/ml	TNF-α Pg/ml
Group I	6	5th	38±2.001	27±0.87	19±0.64	42±1.09
	6	21st	40±1.008	26±0.87	19.7±0.65	40±2.00
Group II	6	5th	95±10.70^{a}	115±3.72^{b}	205±4.23^{c}	360±3.46^{d}
	6	21st	98±3.20^{a}	120±2.87^{b}	232±4.43^{c}	410±9.00^{d}
Group III	6	5th	90±0.76^{a}	380±7.56	115±2.3	201±3.98
	6	21st	450±64.65	570±87.58	211±12.5^{c}	320±7.48^{d}

Values within the column of each cytokines conc. differ significantly (*P*-value< .05) when they are not sharing any common superscript letter. Group I: –ve control group; Group II: +ve control group; Group III: tested group

Results

Lactobacillus acidophilus effects on adult worms in the mice small intestine

On the 5th dpi, 6 mice from each group were sacrificed and adult worms were counted. Significant reduction of adult count (62.1%) was observed in the *Lactobacillus acidophilus* prophylacted group (G III) (125 adults) compared to non- prophylacted animals (G II) (330 adults).

***Lactobacillus acidophilus effects on muscle larval burden* (Table 2)**

Significant reduction of larval counts (73.5%) was observed in the *Lactobacillus acidophilus* prophylacted group (G III) (212 larvae/gm. of muscle) compared to non-prophylacted animals (G II) (800 larvae/g of muscle).

***Inflammatory cytokines profile in different groups* (Table 3)**

Collected sera from the sacrificed mice of all groups at the 5th and 21st dpi were assayed for; IL-1, IL-6, IL-10, and TNF-α. The results showed significance up-regulation for all targeted cytokines in both infected groups (G II and III) compared to the control one (G I) at both time phases (5th and 21st days). The expression of IL-1 was up-regulated on the 21st dpi in (G III) compared to (G II). IL-6 concentration was significantly increased in both 5th and 21st dpi in (G III) compared to (G II). The down-regulation of IL-10 and TNF-α was observed on 5th dpi only in Group III compared to II.

Discussion

Trichinellosis is an important diverse parasitic zoonotic disease. The conventional therapies for trichinellosis are awkward. Therefore, an unusual approach is needed [1–4].

The duration of the experimental study was 29 days, which is considered by many authors ideal to evaluate the course *Lactobacillus acidophilus* prophylaxis on both adult and larvae in mice challenged with *Trichinella spiralis* infection [16,29].

Lactobacillus acidophilus LB was supplied as prophylaxis for seven days preceding *Trichinella spiralis* infection [16]. Although *Lactobacillus acidophilus* failed to achieve complete eradication of *Trichinella spiralis* adults and larvae, they succeeded to reduce both counts by 62.1% and 73.5% respectively, compared to the non-prophylactic group in the experimental animal models. Various cytokine profiles were tracked in trail to declare the possible effect and the protective mechanism that *Lactobacillus acidophilus* acted on the parasite adults and larvae. Results revealed that IL-1 and IL-6 were up-regulated compared to IL-10 and TNF-α that were down-regulated in the tested group compared to both positive and negative control groups. Although the study results agrees with El Temsahy et al. [30], but it differ in experiment depended on the comparison of 3 newly laboratory isolated forms of probiotics regardless of coast and availableness of such strains, as well as testing single different cytokine (IFN-γ).

Although the benefits of *Lactobacillus* *acidophilus LB* supplementation in the reduction of infection burden have been established, mechanisms by which *Lactobacillus acidophilus LB* beneficially affects both adult and larvae load in the current study remain unknown. However, several mechanisms of action could explain the detected action of *Lactobacillus acidophilus* on *Trichinella spiralis* adults; including the production of antimicrobial materials, modulation of the mucosal immune system, alteration of the intestinal microflora, and enrichment of enzymatic activity [31]; however, these theories could not completely explain the detected effects on larvae as well as the altered cytokines profile. Another proposed mechanism may involve the secretion of antimicrobial substances, like bacteriocins, and organic acids such as lactic, acetic, and butyric acid, known products by *Lactobacillus* species that may have a larvicidal effect on parasites [32]. These products or metabolites secreted by living bacteria or released after bacterial lysis were stated to be postbiotics. Postbiotics have become a well-documented strategy for maintaining gut health with physiological benefits to the host. [33,34]. Recently, Lebeer and co-authors proved that postbiotics have the potential to exert protective property in the same way as their parent probiotics [35].

As regards the effect of probiotic administration on the worm burden, it is difficult to compare the current results with the previous works, due to the differences in study design, animal model, probiotic dose and strain, and administration route. *Lactobacillus* strains were the most widely used; anthelmintic efficacy ranges from 75% to 100% protection. This suggested that these *Lactobacillus* strains may be safe to be used as prophylactic or curative probiotics against *T. spiralis* [35].

The altered cytokines profile might be attributed to the ability of the tested bacteria to produce immunostimulation and immunomodulation of either innate or adaptive immune system components [36] which meets with several previous research works explanations for how probiotics exert their action against parasites.

According to D'Souza et al. [37], the non-pathogenic probiotic bacteria interact with the gut epithelial cells and immune cells to start the immune signals, in the form of modulating immunoglobulin production, increasing the number of IgA producing cells on the level of mucosal immunity and increasing in certain cytokines profiles including (TNF-*α,* IFN-*γ*, IL-10, IL-12) to up or downregulate the immune responses and maintain the intestinal homeostasis.

The detected increase in the pro-inflammatory IL-1 on the 21st dpi during *T. spiralis* infection in the prophylactically treated group, compared to infected untreated one, may be responsible for the initiation of the intestinal inflammatory response to this infection [38]. IL-1 organizes the differentiation and function of innate and adaptive lymphoid cells [39].

Our results showed a significant increase in the expression of IL-6 in the tested group compared with controls. In this respect, it has been demonstrated that IL-6 activation by IL-4/IL-13 is required in *T. spiralis* to boost mast cell responses, which in turn induces worm expulsion and intestinal mastocytosis [40]. The essential role of IL-6 in *T. spiralis* infection is done through the enhancement of muscle contractility and epithelial cell fluid secretion [41]; roles probably explain the exerted response on larvae in the muscular stage. Going beyond; several studies discussed the range of biological activities of IL-6 and its pathological role in various diseases particularly immune-mediated diseases, it was anticipated that IL-6 targeting would constitute a novel treatment strategy for that diseases [42].

IL-10 expression might have a crucial role in the regulation of inflammation intensity caused by *T. spiralis* infection. IL-10 is named cytokine synthesis inhibitory factor that limits the production of IFN-γ; IL-12 and other cytokines that prevent the expulsion of the worm [43]; as IL-12 increases the muscular parasite burden and delays the adult worm expulsion from the intestine. This contradicted with Beiting et al. [44] conclusion of IL-10 which restricts the initial inflammatory response to muscle infection by *T. spiralis* with no effect on the survival of intestinal *T. spiralis*. The sustained control of inflammation during chronic muscle infection is independent of IL-10 and is accompanied by a shift to a Th2 response following completion of parasite development in the muscle, thus IL-10 neither endorses nor compromises the survival of larvae in the muscle.

TNF-α was documented to have a crucial role in the induction of T helper cell-mediated resistance to infection with gastrointestinal helminth parasites [45]. TNF-α acts through the activation of intestinal mucosal mast cells, thus promoting local inflammation. One of the properties of TNF-α is the induction of nitric oxide (NO), which finally acts mainly as an active molecule against both extracellular and intracellular parasites [46]. Nevertheless, several studies have suggested that TNF-α and NO inflammatory response may be injurious to the host, through favoring the development of enteropathy [47].

In conclusions, the dramatic reduction in both *Trichinella spiralis* adults and larval burden in animal models caused by *Lactobacillus acidophilus* supplement strongly suggests its use as a prophylactic agent in *Trichinella spiralis* endemic areas. The changes in the inflammatory cytokines caused by the used probiotic, may present new insights on their feasibility as prophylaxis in *Trichinella spiralis* endemic areas in a safe, cheap and far from serious complications manner. These changes also, may flare up the question; could it affect the cytokines storm in new pandemic COVID 19 virus assumed to be responsible for severe complications and even deaths. Results need further confirmation studies in the near future.

References

[1] Pozio E., Zarlenga D.S. 2005. Recent advances on the taxonomy, systematics and epidemiology of *Trichinella. International Journal for Parasitology* 35: 1191-1204. doi:10.1016/j.ijpara.2005.07.012

[2] Osten-Sacken N., Solarczyk P. 2016. *Trichinella spiralis* in road-killed raccoon dogs (*Nyctereutes procyonoides*) in western Poland. *Annals of Parasitology* 62: 77-79. doi:10.17420/ap6201.36

[3] Sviben M., Meštrović T., Čičmak Smirnjak L. 2019. The value of systematic screening for *Trichinella* antibodies among individuals with eosinophilia in recognizing outbreak events: a seroprevalence study from Croatia. *Annals of Parasitology* 65: 177-189. doi:10.17420/ap6502.199

[4] Pozio E., Darwin Murrell K. 2006. Systematics and epidemiology of *Trichinella*. *Advances in Parasitology* 63: 367-439. doi: 10.1016/S0065-308X(06)63005-4

[5] Darwin Murrell K., Pozio E. 2011. Worldwide occurrence and impact of human trichinellosis, 1986-2009. *Emerging Infectious Diseases* 17: 2194-2202. doi: 10.3201/eid1712.110896

[6] Dupouy-Camet J., Bruschi F. 2007. Management and diagnosis of human trichinellosis. FAO/WHO/OIE Guidelines for the Surveillance, Management, Prevention and Control of Trichinellosis. 2007 Jan 1; 37-68.

[7] Gottstein B., Pozio E., Nöckler K. 2009. Epidemiology, diagnosis, treatment, and control of trichinellosis. *Clinical Microbiology Reviews* 22: 127-145. doi:10.1128/CMR.00026-08

[8] Ozeretskovskaia N.N., Sergiev V.P. 1994. The specific and biological actions of chemical preparations and their combination with pathogenic agents in trichinosis. *Meditsinskaia Parazitologiia* 4: 9-14.

[9] Markowiak P., Ślizewska K. 2017. Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients* 9. doi:10.3390/nu9091021

[10] Morelli L., Capurso L. 2012. FAO/WHO guidelines on probiotics: 10 years later. *Journal of Clinical Gastroenterology* 46 (Suppl. 1): 10-11. doi:10.1097/MCG.0b013e318269fdd5

[11] Britton R.A., Versalovic J. 2008. Probiotics and gastro-intestinal infections. *Interdisciplinary Perspectives on Infectious Diseases*: 1-10. doi:10.1155/2008/290769

[12] Howarth G.S., Wang H. 2013. Role of endogenous microbiota, probiotics and their biological products in human health. *Nutrients* 5: 58-81. doi:10.3390/nu5010058

[13] Hummel S., Veltman K., Cichon C., Sonnenborn U., Schmidt MA. 2012. Differential targeting of the E-cadherin/β-catenin complex by gram-positive probiotic lactobacilli improves epithelial barrier function. *Applied and Environmental Microbiology* 78: 1140-1147. doi:10.1128/AEM.06983-11

[14] Reda A.A. 2018. Probiotics for the control of helminth zoonosis. *Journal of Veterinary Medicine* 2018: 1-9. doi:10.1155/2018/4178986

[15] Martínez-Gómez F., Santiago-Rosales R., Ramón Bautista-Garfias C. 2009. Effect of *Lactobacillus casei* Shirota strain intraperitoneal administration in CD1 mice on the establishment of *Trichinella spiralis* adult worms and on IgA anti-*T. spiralis* production. *Veterinary Parasitology* 162: 171-175. doi: 10.1016/j.vetpar.2009.02.010

[16] Bautista-Garfias C., Ixta-Rodríguez O., Martínez-Gómez F., López MG., Aguilar-Figueroa BR. 2001. Effect of viable or dead *Lactobacillus casei* organisms administered orally to mice on resistance against *Trichinella spiralis* infection. *Parasite-Journal* 8: S226-228. doi:10.1051/parasite/200108s22

[17] De Avila L.F.D.C., De Leon P.M.M., De Moura M.Q., Berne M.E.A., Scaini C.J., Leivas Leite F.P. 2016. Modulation of IL-12 and IFNγ by probiotic supplementation promotes protection against *Toxocara canis* infection in mice. *Parasite Immunology* 38: 326-330. doi:10.1111/pim.12314

[18] De Avila L.F. da C., Telmo P. de L., Martins L.H.R., Glaeser T.A., Conceição F.R., Leite F.P.L., Scaini C.J. 2013. Eyfeito protetor do probiótico *Saccharomyces boulardii* na infecção por *Toxocara canis* não se deve à ação direta sobre as larvas. *Revista do Instituto de Medicina Tropical de Sao Paulo* 55: 363-365 (in Portuguese). doi:10.1590/S0036-46652013000500012

[19] Zowail M.E.M., Osman G.Y., Mohamed A.H., El-Esawy H.M.I. 2012. Protective role of *Lactobacillus sporogenes* (Probiotic) on chromosomal aberrations

and DNA fragmentation in *Schistosoma mansoni* infected mice. *Egyptian Journal of Experiemental Biology (Zoology)* 8: 121-130.

[20] Mohamed A.H., Osman G.Y., Zowail M.E.M., El-Esawy H.M.I. 2016. Effect of *Lactobacillus sporogenes* (probiotic) on certain parasitological and molecular aspects in *Schistosoma mansoni* infected mice. *Journal of Parasitic Diseases* 40: 823-832. doi.org/10.1007/s12639-014-0586-4

[21] Oliveira-Sequeira T.C.G., David É.B., Ribeiro C., Guimarães S., Masseno A.P.B., Katagiri S., Sequeira J.L. 2014. Effect of *Bifidobacterium* animalis on mice infected with *Strongyloides venezuelensis*. *Revista do Instituto de Medicina Tropical de Sao Paulo* 56: 105-109. doi:10.1590/S0036-46652014000200003

[22] De Waard R., Garssen J., Snel J., Bokken G.C.A.M., Sako T., Huis in 't Veld J.H.J., Vos J.G. 2001. Enhanced antigen-specific delayed-type hyper-sensitivity and immunoglobulin G2b responses after oral administration of viable *Lactobacillus casei* YIT9029 in Wistar and Brown Norway rats. *Clinical and Diagnostic Laboratory Immunology* 8: 762-767. doi:10.1128/CDLI.8.4.762-767.2001

[23] Verdú E.F., Bercík P., Bergonzelli G.E., Huang X-X., Blennerhasset P., Rochat F., Fiaux M., Mansourian R., Corthésy-Theulaz I., Collins S.M. 2004. *Lactoba - cillus paracasei* normalizes muscle hypercontractility in a murine model of postinfective gut dysfunction. *Gastroenterology* 127: 826-837. doi:10.1053/j.gastro.2004.06.007

[24] Dea-Ayuela M.A., Rama-Iñiguez S., Bolás-Fernandez F. 2008. Enhanced susceptibility to *Trichuris muris* infection of B10Br mice treated with the probiotic *Lactobacillus casei*. *International Immunopharmacology* 8: 28-35. doi:10.1016/j.intimp.2007.10.003

[25] Gamble H. 1996. Detection of trichinellosis in pigs by artificial digestion and enzyme immunoassay. *Journal of Food Protection* 59: 295-298.

[26] Randazzo V., Costamagna S.R. 2005. Effect of oral administration of probiotic agents on *Trichinella spiralis*-infected mice. *Revista de Patologia Tropical* 34: 129-135.

[27] Denham D., Martinez A. 1970. Studies with Methyridine and *Trichinella spiralis* 2. The use of the drug to study the rate of larval production in mice. *Journal of Helminthology* 44: 357-363. doi:10.1017/S0022149X00022021

[28] Wang Z.Q., Cui J., Wei H.Y., Han H.M., Zhang H.W., Li Y.L. 2006. Vaccination of mice with DNA vaccine induces the immune response and partial protection against *T. spiralis infection*. *Vaccine* 24: 1205-1212. doi:10.1016/j.vaccine.2005.08.104

[29] Basyoni M.M.A. 2013. Therapeutic potential of myrrh and Ivermectin against experimental *Trichinella spiralis* infection in mice. *Korean Journal of Parasitology* 51: 297-304.

[30] El.Temsahy M.M., Ibrahim I.R., Mossallam S.F., Mahrous H., Bary A.A., Salam S.A.A. 2015. Evaluation of newly isolated probiotics in the protection against experimental intestinal trichinellosis. *Veterinary Parasitology* 214: 303-314. doi:10.1016/j.vetpar.2015.08.029

[31] Baajagi Y.S., Klieve A.V., Dart P.J., Bryden W.L. 2016. Probiotics in animal nutrition. 179th ed. (Ed. Harinder P.S. Makkar). FAO, Rome.

[32] De Keersmaecker S.C.J., Verhoeven T.L.A., Desair J., Marchal K., Vanderleyden J., Nagy I. 2006. Strong antimicrobial activity of *Lactobacillus rhamnosus* GG against *Salmonella typhimurium i*s due to accumulation of lactic acid. *FEMS Microbiology Letters* 259: 89-96. doi:10.1111/j.1574-6968.2006.00250.x

[33] Aguilar-Toalá J.E., Garcia-Varela R., Garcia H.S., Mata-Haro V., González-Córdova A.F., Vallejo-Cordoba B., Hernández-Mendoza A. 2018. Postbiotics: An evolving term within the functional foods field. *Trends in Food Science & Technology* 75: 105-114. doi:10.1016/J.TIFS.2018.03.009

[34] Tsilingiri K., Barbosa T., Penna G., Caprioli F., Sonzogni A., Viale G., Rescigno M. 2012. Probiotic and postbiotic activity in health and disease: Comparison on a novel polarised ex-vivo organ culture model. *Gut* 61: 1007-1015. doi:10.1136/gutjnl-2011-300971

[35] Lebeer S., Bron P.A., Marco M.L., Van Pijkeren J-P., O'Connell Motherway M., Hill C., Pot B., Roos S., Klaenhammer T. 2018. Identification of probiotic effector molecules: present state and future perspectives. *Current Opinion in Biotechnology* 49: 217-223. doi:10.1016/j.copbio.2017.10.007

[36] Dvorožňáková E., Bucková B., Hurníková Z., Revajová V., Lauková A. 2016. Effect of probiotic bacteria on phagocytosis and respiratory burst activity of blood polymorphonuclear leukocytes (PMNL) in mice infected with *Trichinella spiralis*. *Veterinary Parasitology* 231: 69-76. doi:10.1016/j.vetpar.2016.07.004

[37] D'Souza A.L.D., Rajkumar C., Cooke J., Bulpitt C.J. 2002. Probiotics in prevention of antibiotic associated diarrhoea: *British Medical Journal* 324: 1-6.

[38] Stadnyk A.W., Kearsey J.A. 1996. Pattern of proinflammatory cytokine mRNA expression during *Trichinella spiralis* infection of the rat. *Infection and Immunity* 64: 5138-5143.

[39] Mantovani A., Dinarello C.A., Molgora M., Garlanda C. 2019. Interleukin-1 and related cytokines in the regulation of inflammation and immunity. *Immunity* 50: 778-795. doi:10.1016/j.immuni.2019.03.012

[40] Urban J.F., Schopf L., Morris S.C., Orekhova T., Madden K.B., Betts C.J., Gamble G.R., Byrd C., Donaldson D., Else K., Finkelman F. 2000. Stat6 signaling promotes protective immunity against

Trichinella spiralis through a mast cell- and T cell-dependent mechanism. *The Journal of Immunology* 164: 2046-2052. doi:10.4049/jimmunol.164.4.2046

[41] Finkelman F.D., Shea-Donohue T., Goldhill J., Sullivan C.A., Morris S.C., Madden K.B., Gause W.C., Urban J.F.1997. Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. *Annual Review of Immunology* 15: 505-533. doi:10.1146/annurev.immunol.15.1.505

[42] Tanaka T., Ogata A., Shima Y., Narazaki M., Kumanogoh A., Kishimoto T. 2013. IL-6 targeting strategy for various: Immune-mediated diseases other than rheumatoid arthritis: an update review. *Proteomics Research Journal* 4: 269-317.

[43] Subramanian Iyer S., Cheng G. 2012. Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. *Critical Reviews in Immunology* 32: 23-63. doi:10.1038/jid.2014.371

[44] Beiting D., Bliss S., Schlafer D., Roberts V., Appleton J. 2004. Interleukin-10 limits local and body cavity inflammation during infection with muscle-stage *Trichinella spiralis*. *Infection and Immunity* 72: 3129-3137. doi:10.1128/IAI.72.6.3129-3137.2004

[45] Ierna M.X., Scales H.E., Mueller C., Lawrence C.E. 2009. Transmembrane tumor necrosis factor alpha is required for enteropathy and is sufficient to promote parasite expulsion in gastrointestinal helminth infection. *Infection and Immunity* 77: 3879-3885. doi:10.1128/IAI.01461-08

[46] James S.L. 1995. Role of nitric oxide in parasitic infections. *Microbiological Reviews* 59: 533-547. doi:10.1128/mmbr.59.4.533-547.1995

[47] Muñoz-Carrillo J.L., Muñoz-Escobedo J.J., Maldonado-Tapia C.H., Chávez-Ruvalcaba F., Moreno-García M.A. 2017. Resiniferatoxin lowers TNF-α., NO and PGE2 in the intestinal phase and the parasite burden in the muscular phase of *Trichinella spiralis* infection. *Parasite Immunology* 39: 1-14. doi:10.1111/pim.12393

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