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Protective potential of *L. acidophilus* in murine giardiasis

Research Article

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Abstract: This study describes the in vivo activity of *Lactobacillus acidophilus* in *Giardia lamblia* infected BALB/c mice. Experimentally, it was observed that daily administration of lactobacilli 7 days before or in simultaneous inoculation with *Giardia* trophozoites efficiently reduced *G. lamblia* infection in mice. More specifically, excretion of *Giardia* cysts were reduced significantly in probiotic-treated groups, and resolution of infection was observed by day 21 post-inoculation. It was also observed that the lactobacillus count increased tremendously and continuously in faeces of all probiotic-fed mice, and was significantly higher as compared with that in control mice. Histological analysis of microvilli membrane integrity revealed that probiotic administration also protected mice against parasite-induced mucosal damage, whereas *Giardia*-infected mice had severe villous atrophy, oedema, vacuolation and ileitis. Immunologically, the anti-*Giardia* serum IgG level was not stimulated significantly by probiotic treatment administered both prior to and simultaneous with *Giardia* infection, but remained high after the infection peak. Taken together, the data demonstrates the anti-giardial effect of the probiotic in vivo by modulation of the intestinal epithelial cells, inhibiting the colonization of *Giardia* trophozoites and thereby reducing the severity of *Giardia* infection.

Keywords: *Probiotic • Giardiasis • Lactobacillus acidophilus • Murine model*

1. Introduction

Giardia lamblia is one of the most common protozoal infections of the human intestine, and is a leading cause of diarrheal diseases throughout the world. Worldwide incidence is believed to range from 20% to 60%, with an estimated rate of 2.8 x 10^6 people having symptomatic giardiasis [1,2]. Symptomatic infection is usually characterized by diarrhea, epigastric pain, nausea, weight loss, malnutrition and growth retardation, but many infections are asymptomatic. School-age children, malnourished people, common variable immunodeficiency (CVID) patients, hypogammaglobulinemic individuals and persons with human leukocyte antigen (HLA) A_1, A_2, B_8 and B_{12} are highly susceptible to giardiasis [3-5].

Giardia infection is generally treated with antiprotozoal drugs such as metronidazole quinacrine, furazolidone, and nitazoxanide (Alinia). However, in view of the clinical failures, adverse effects of the antigiardial drugs, such as intestinal upsets, a metallic taste, their carcinogenic nature and the evolution of resistant strains, have diverted scientific interest towards the identification of alternative therapeutic strategies that are safe and effective [2,6]. In this context, natural interventions, such as plant extracts and products derived from bees, garlic, long, pepper, pippali, rasayana and probiotics, are being studied [7-9]. Since probiotics as functional food provide health benefits to the host by antagonizing pathogens and modulating both innate and acquired immunity at the local and systemic levels [10-12], these agents could also be used for treatment of parasitic infections. To date, no studies have assessed the protective effect of *Lactobacillus acidophilus* on intestinal parasitosis. The present study, therefore, was specifically addressed to delineate the anti-giardial properties of *Lactobacillus acidophilus* as a probiotic in murine giardiasis.

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2. Material and Methods

2.1. Preparation and inoculation of *G. lamblia* trophozoites

Giardia lamblia trophozoites (Portland 1 strain) were axenically cultured in TYI-S-33 medium [13]. For experimental inoculation, actively growing trophozoites (48–72 hr old culture) were sedimented by centrifugation at 200 g for 10 min and washed with normal saline. Finally, they were resuspended in normal saline at a concentration of $5x10^6$ trophozoites/0.1 ml and were fed intra-oesophageally via catheter [14].

2.2. Bacterial strain, preparation and inoculation

Lactobacillus acidophilus MTCC 447, procured from Institute of Microbial Technology (IMTECH), Chandigarh, India, was grown in De Mann Rogosa Sharpe (MRS) broth and was maintained on MRS agar slants by regular sub-culturing at 15-day intervals. For experimental inoculation, an 18-hr old bacterial culture was sedimented by centrifugation at 8000 rpm for 10 min and washed with normal saline. Finally, the culture was resuspended in normal saline at a concentration of $1x10^9$ lactobacilli/0.1 ml, and was fed intra-oesophageally via catheter [14].

2.3. Groups of animals

BALB/c mice aged 5–6 weeks old (18–20 gm), obtained from the Central Animal house, Panjab University, Chandigarh, were housed under standard conditions of light and dark cycle with free access to food and water. Water and food before supplementation to animals were monitored for any bacterial or parasitic contamination by Gram's staining and Lugol's iodine staining techniques [15]. Animals were also screened for protozoal infection via stool examination for three consecutive days. Only parasite-free mice were employed. Care and use of animals were in accordance with the guidelines of the institutional ethical committee. All animals were broadly divided into five groups. **Group I (n=18, control)**: These mice were fed orally with a single dose of 0.1 ml of normal saline for 21 consecutive days. **Group II (n=24, Giardia-Infected):** Mice were fed orally with a single dose of $5x10^6$ *Giardia* trophozoites only. **Group III (n=18, Probiotic):** In this group, animals were administered orally with a single dose of *L. acidophilus* ($1X10^9$ lactobacilli/0.1 ml) daily for 21 consecutive days. **Group IV (n=24, Probiotic-Giardia):** These mice were fed with a single dose of *L. acidophilus* ($1×10^9$ lactobacilli/0.1 ml) for 7 consecutive days. On day 8, these mice were challenged with a single dose of $5x10^6$ *Giardia* trophozoites along with a probiotic dose; only probiotic feeding once a day was continued up to day 21. **Group V (n=24, Giardia-Probiotic):** Mice were challenged with a single dose of *Giardia* trophozoites ($5x10^6$ trophozoites) orally, as well as fed with a single dose of probiotic *L. acidophilus* ($1x10^9$ lactobacilli/0.1 ml); the probiotic treatment once a day was continued up to 21 days.

2.4. Follow up of animals

After the respective treatments, *Giardia* cyst and *Lactobacilli* counts from 8 mice belonging to different groups were studied. The remainder of the mice were bled by the retro-orbital plexus route and sacrificed in batches of 6 on days 7, 14 and 21 post inoculation (PI) for specific anti-*Giardia* serum IgG level and histopathological studies.

2.5. Enumeration of Giardia cysts

Briefly, one gram of freshly voided faecal materials from each mice belonging to groups II, IV and V was thoroughly homogenized in 10 ml of formal saline, and a cyst count was performed in iodine-stained stool samples using a hemocytometer on every third day [10].

2.6. Enumeration of Lactobacilli

To confirm whether the *L. acidophilus* were able to survive the stress within the gastrointestinal tract (GIT) vis-a-vis *G. lamblia* infection, the lactobacilli count was performed in faeces of mice belonging to all groups. An emulsion prepared from freshly voided faeces from each group (1g/mouse) was serially diluted and then spread-plated on MRS agar every 2 days. The plates were incubated at 37ºC for 24 hrs, and colony forming units (cfu) were recorded [10].

2.7. Preparation of *Giardia* antigen

Giardia antigen was prepared for estimation of serum anti-*Giardia* IgG antibodies. Briefly, actively growing *Giardia* trophozoites (48-72 hr old culture) were chilled, centrifuged at 200 g for 10 min and washed thrice with chilled phosphate buffer saline (PBS-7.2). The trophozoites were finally sonicated and centrifuged; the protein concentration was then determined [16].

2.8. Enzyme linked immunosorbant assay (ELISA)

The levels of specific anti-*Giardia* serum IgG were determined by indirect micro-ELISA assay as per Hudson and Hay [17], with minor modifications. Briefly, 50 µl of the *Giardia* antigen (20 µg/ml) diluted in carbonate

bicarbonate buffer (pH 9.6) was added to 96-welled microtitre plates. After overnight incubation at 4˚C, the plates were washed. Nonspecific protein binding sites were blocked by incubating at 37˚C for 2 hours with 1% BSA and the plates were washed again. Test sera were diluted serially and incubated at 37˚C for 2 hours. The plates were washed and 50 µl of diluted HRP conjugated antimouse IgG immunoglobulin (Sigma) was added to each well. After 1-hour incubation at 37˚C, the plates were again washed and 50 µl of substrate solution was added. After 30 minutes of incubation at room temperature, the reaction was terminated by adding 50 µl concentrated sulphuric acid (H_2SO_4); the antibody concentration was determined by ELISA reader (Bio-rad model no 680) at 490 nm.

2.9. Histopathological studies

Mice were sacrificed by retro-orbital plexus bleeding; the upper part of small intestine was removed, fixed in 10% buffered formalin and processed for histological examination. Tissues were dehydrated in different grades of alcohol, i.e., 70%, 80%, 90% and absolute alcohol for 30 min, 40 min and 1 hour, respectively, followed by washing in xylene for 1 hour each at room temperature. Finally, the tissues were dipped in molten paraffin wax and were quickly cooled to prevent crystallization. Thin sections of tissue were cut; embedded tissue sections were kept in a water bath at 50˚C to remove the wax. Sections were mounted on separate clean glass microscope slides and stained with haematoxylin and eosin stain (H & E stain). The slides were blot-dried, mounted with di-styrene plasticizer xylene (DPX), and examined by light microscopy.

2.10. Statistical Analysis

Results are expressed as mean ± standard error (SE). The inter-group variation was assessed by one-way analysis of variance (ANOVA); statistical significance was set at $p < 0.05$.

3. Results

3.1. Giardia cysts in faeces

Giardia-Infected mice (Group II) voided significantly ($p < 0.05$) large numbers of cysts in faeces and had a rapid increase in cyst count from day 0 to 9 post-inoculation (PI). Thereafter, the infection began to resolve, and the mice became *Giardia*-free by day 21PI (Figure 1). Interestingly, the administration of lactobacilli significantly ($p < 0.05$) reduced the excretion of *Giardia* cysts in faeces of Probiotic-Giardia (Group IV) and

Figure 1. Giardia cysts in faeces of mice belonging to different groups employing *L. acidophilus* as the probiotic. Values are mean ± standard error (SE), *$p < 0.05$ v/s Giardia-Infected (days 3, 5, 7, 9, 11, 13, 15, 18, 21).

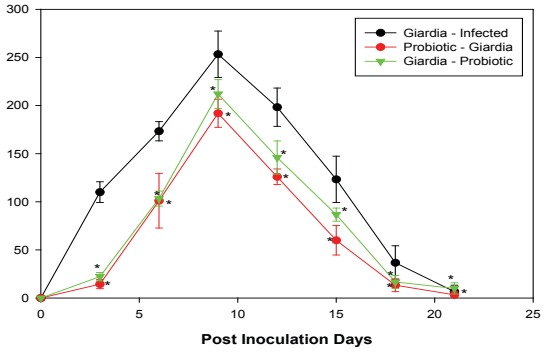
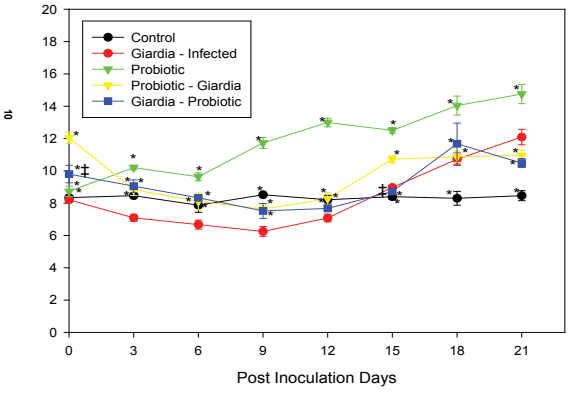


Figure 2. *Lactobacilli* count in faeces of mice belonging to different groups using *L. acidophilus* as the probiotic. Values are mean ± SE, *$p < 0.05$ v/s Giardia-infected (days 0, 3, 5, 7, 9, 11, 13, 15 , 18 ,21).



Giardia-Probiotic (Group V) mice compared to Giardia-Infected mice (Group II). It was observed that the pattern of cyst excretion in probiotic-fed but infected mice (Groups IV and V) was similar to Giardia-Infected mice (Group II).These mice also had peak *Giardia* infection on day 9 PI and were free from *Giardia* infection by day 21 PI (Figure 1).

3.2. *Lactobacilli* count in faeces

The endogenous microbiota, comprising mostly lactobacilli, were enumerated by spread-plating on selective MRS media. A significant ($p < 0.05$) increase in the faecal *Lactobacillus* count was observed in probiotic-fed (Group III, IV and V) mice as compared with that in control mice (Group I, Figure 2). However, Giardia-Infected mice had a significantly ($p < 0.05$) reduced *Lactobacillus* count as compared even with control mice. Interestingly, oral feeding of lactobacilli along with, or 7 days prior to, *Giardia* infection resulted in reduced *Giardia* infection and an enhanced faecal *Lactobacillus* count (Figure 2) as compared with Giardia-Infected mice (Group II, Figure 1).

Figure 3. *Anti-Giardial* IgG levels in serum of different groups of mice on day 7, 14 and 21 PI. Values are mean ± SE, *$p < 0.05$ v/s Giardia- infected group (days 14 and 21).

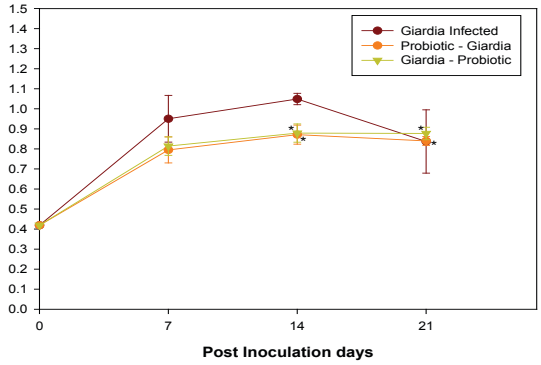


Figure 4. Photomicrograph of the small intestine of a control (Group I) mouse showing a healthy muscle coat (MC), intact mucosal epithelial lining (ME),basal crypts (BC) and normal morphology of microvilli, (H & E stain, 100 X).

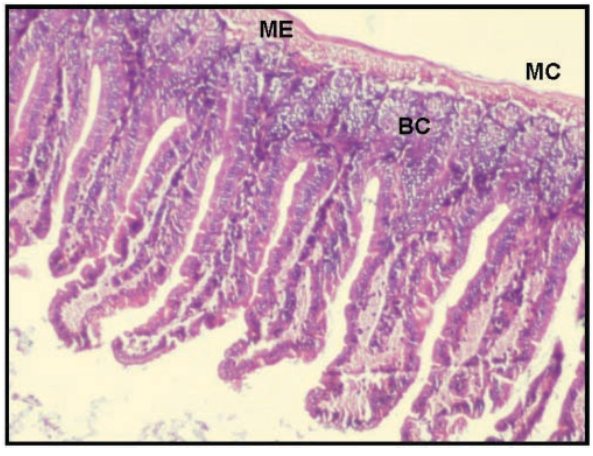
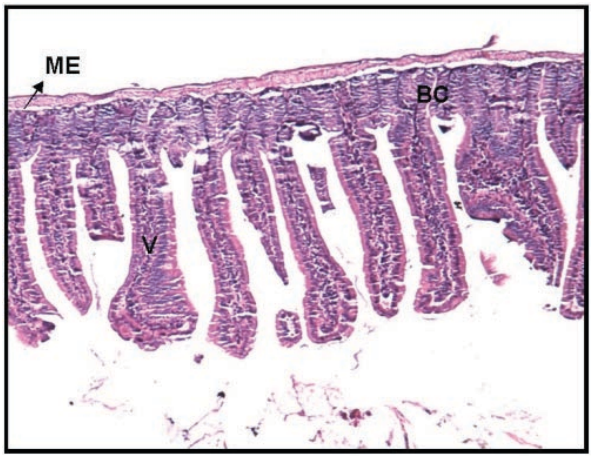


Figure 5. Photomicrograph of the small intestine of a mouse fed with probiotic (Group III) showing an intact mucosal epithelium (ME), well-preserved morphology of the basal crypts (BC) and microvilli (V), (H & E stain, 100 X).



3.3. Anti-*Giardia* Serum IgG Levels

Total specific anti-*Giardia* serum IgG levels increased significantly ($p < 0.01$) in Giardia-Infected mice (Group II) on day 7 and 14 PI and thereafter, it decreased by day 21 PI (Figure 3). However, the level of total anti-*Giardia* serum IgG level increased in both probiotic fed and challenged mice (Group IV and V) on day 7, 14, and remained constant thereafter (day 21 PI), but the level of anti-*Giardia* serum IgG were significantly ($p < 0.01$) lower compared with Giardia-Infected mice (Figure 3). Interestingly, it was observed that once the antibody level increased in probiotic-treated and infected mice, it remained high in spite of reduced infection (Figure 3).

3.4. Histopathological Studies

Histopathological examination of the small intestines of control mice (Group I) showed healthy muscle coats and intact mucosal epithelial linings, basal crypts and normal villi (Figure 4). In contrast, the small intestine of Giardia-Infected mice (Group II) revealed profound effects on the structure of intestinal mucosa showing varying degrees of villous atrophy, inflammation, lymphocytic infiltration, and lymphocytic hyperplasia resulting from an increased numbers of inflammatory cells (Figure 6) as compared with control mice (Group I, Figure 4). The villi were swollen, disrupted at tips and inflammation extended up to the muscle coat and the crypts, indicating severe ileitis in Giardia-Infected mice (Group II) on day 14 PI (Figure 6), followed by complete disruption of intestinal villi and high inflammation on day 21 PI (Figure 6). Interestingly, probiotic-fed mice (Group III) had intestinal structures identical to controls (Group I) with clearly defined and increased villous length and width (Figure 5). However, probiotic-fed but *Giardia*-challenged mice (Group IV and V) had less intestinal damage or mild inflamed villi (Figure 7 and 8), than Giardia-Infected mice (Group II). A novel finding of the present study is that mice belonging to the Probiotic-Giardia (Group IV) had the least damage to the villi, and inflammation, and had almost restored normal mucosal architecture (Figure 7) compared with that of the Giardia-Probiotic (Group V, Figure 8) and Giardia-Infected mice (Group II, Figure 6).

4. Discussion

The importance of *Lactobacillus* as a probiotic has been recognized because of its fermentative ability, nutritional and health benefits, and its wide range of antimicrobial activities that help to tone-up the intestinal environment [18]. Thus, the main thrust of the

Figure 6. Photomicrograph of the small intestine of a *Giardia*-infected mouse (Group II); **a)** on day 7 PI showing hypertrophy and severely damaged microvilli (DV) tips along with heavy lymphocytic infiltration (LI); **b)** On day 14 PI showing severely damaged, inflamed intestinal mucosa with complete disruption of the microvilli (DDV), increased vacuolation (Va) of epithelial cells and oedema of lamina propria, a severe ileitis; **c)** on day 21 PI showing heavy lymphocytic infiltration (LI) in the microvilli (V) and lamina propria (LP). The villi are swollen with dissolved edges (DV) and increased vacuolation of epithelial cells along with swelling and infiltration of the basement membrane (SBM), (H & E stain, 100 X).

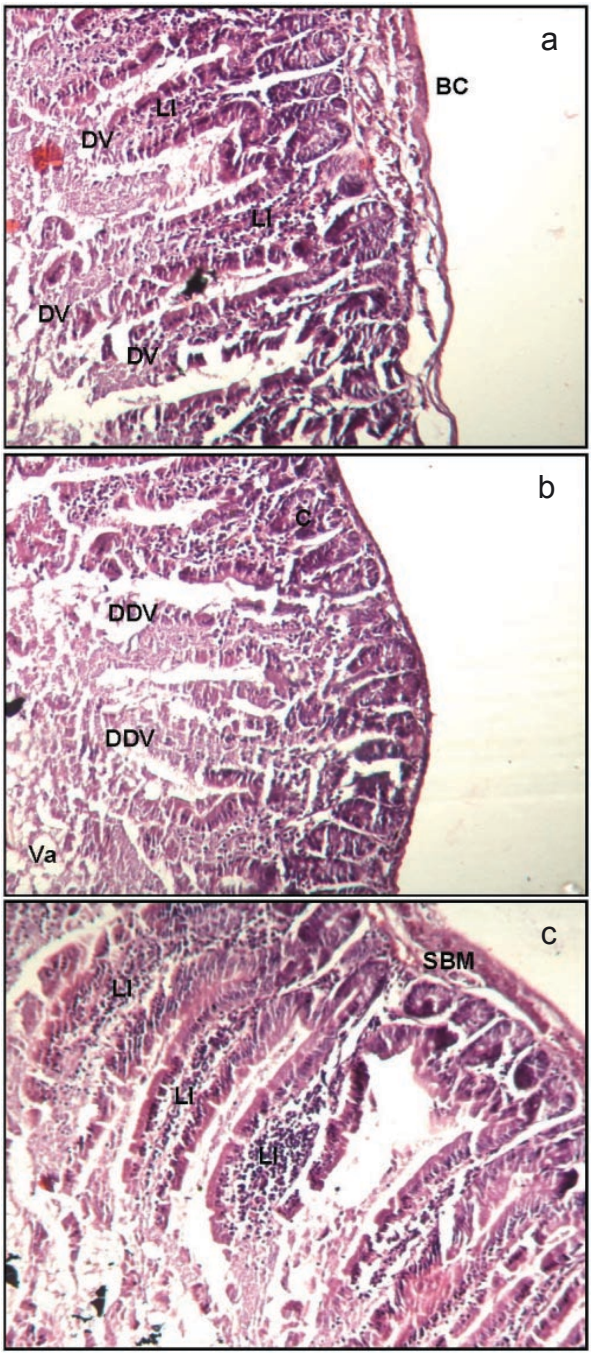


Figure 7. Photomicrograph of the small intestine of a Probiotic-*Giardia* mouse (Group IV); **a)** on day 7 PI showing a preserved architecture of microvilli (V) with mild inflammation (MI); **b)** on day 14 PI showing a large number of lymphocytes in the microvilli and in the lamina propria. Note the less-dissolved villi tips (DV); **c)** on day 21 PI showing mild cellular disruption of the mucosal epithelial lining and the large number of lymphocytes in the microvilli, (H & E stain, 100 X).

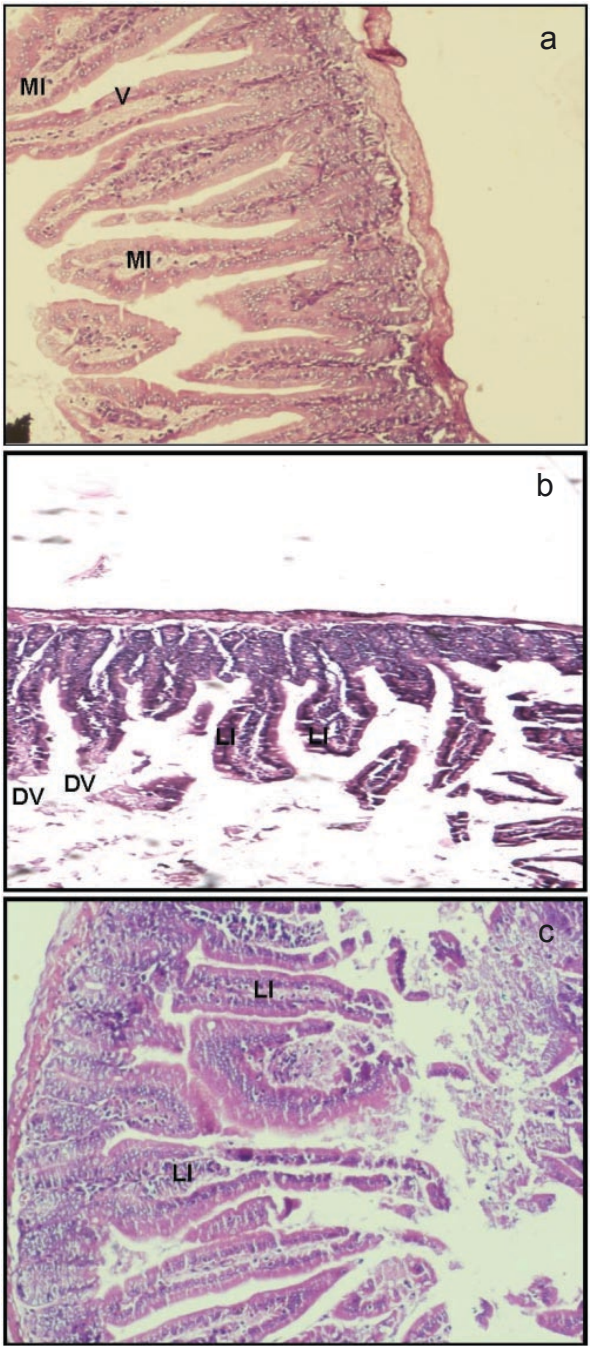
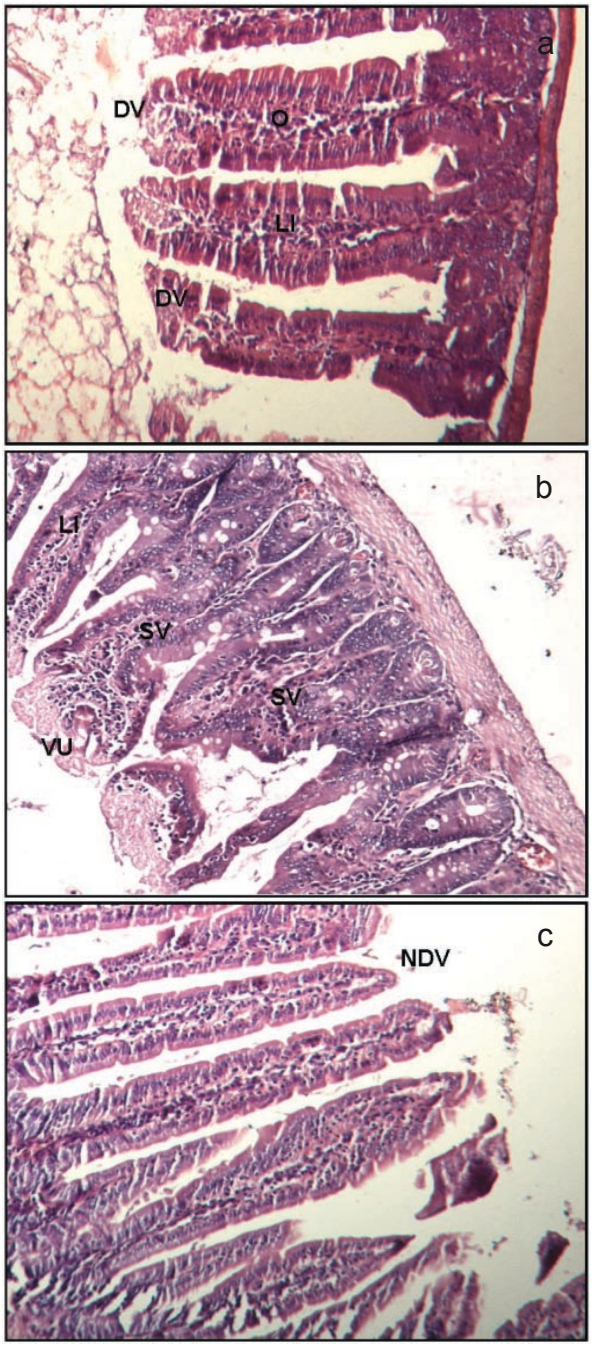


Figure 8. Photomicrograph of the small intestine of a *Giardia*-Probiotic mouse (Group V); **a)** on day 7 PI showing mild dissolutions of microvilli tips (DV), moderate hypertrophy of microvilli ,lymphocytic infiltration (LI) with thickened lumen (O); **b)** on day 14 PI showing broader swollen villus (SV), oedema, lymphocytic infiltration (LI) and dissolved microvilli: a case of villus ulcer (VU); **c)** on day 21 PI showing no disruption of the microvilli (NDV): normal microvilli structure. A high degree of lymphocytic infiltration (LI) in the microvilli with a minor number of lymphocytes extending into lamina propria, (H & E stain, 100 X).



present study was to monitor the protective potential of *L. acidophilus* in *Giardia*-infected BALB/c mice.

It has been very well demonstrated that composition of intestinal microflora affects the colonization of the mouse gut by *Giardia* trophozoites [19]. In present study, *Giardia* trophozoites were found to effectively colonize the small intestine of Giardia-Infected mice, as was evident by enhanced cyst excretion in faeces but was also self-limiting. However, oral administration of probiotic *L. acidophilus* in probiotic-treated but *Giardia*-challenged mice modified the composition of gut flora that lead to reduced excretion of giardial cysts in faeces. This may be attributed to better colonizing ability of the lactobacilli in the intestinal epithelial cells, thereby inhibiting the adherence of *Giardia* trophozoites. This observation corroborates very well with an earlier study where use of *Enterococcus faecum* SF 68 as a probiotic cleared the *Giardia* infection in C57BL/6 mice [14].

The viability and activity of probiotic bacteria in a host are important considerations, as these bacteria must be able to survive within the gastrointestinal tract. Therefore, to evaluate a strain as a promising probiotic, investigation of its survival in the digestive tract is needed. In present study, it has been demonstrated that oral administration of *L. acidophilus* can survive, can travel through the gastrointestinal tract of mice and can be detected in faeces. It was observed that probiotic-fed mice, irrespective of *Giardia* infection, had a significantly enhanced level of lactobacilli count in faeces compared with control mice. This could result from effective colonization of lactobacilli in the intestine and better interaction with enterocytes, thus altering the intestinal microbiota or composition of endogenous bacteria. However, modification of other key components of the intestinal microbiota can not be excluded. The better colonization in the small intestine and enhanced number of lactobacilli in faeces may well explain the reduced ability of *Giardia* trophozoites to colonize in probiotic-fed mice. This observation is in agreement with earlier studies where an increased lactobacilli count in the faeces of probiotic-treated rats was observed [18,20].

The possible mechanism of probiotic therapy have been hypothesized to include the normalization of increased intestinal permeability and altered gut microbiota, or could result from improvement of the intestine's immunological barrier and alleviation of the intestinal inflammatory response [21-23]. In this preliminary study, we have observed that Probiotic-treated and Giardia-Infected mice (Group IV and V) suffered neither from severe *Giardia* infection nor had high levels of specific serum anti-*Giardia* IgG. This may result from binding of lactobacilli to the epithelial lining, which may prevent the antigen recognition by

intra epithelial or lamina-propria–mediated T and B lymphocytes. However, the enhanced specific anti-*Giardia* serum IgG level in *Giardia*-infected mice is in agreement with previous studies where it was documented that giardiasis enhanced the immune response to allergens in the mucosa [24]. This suggests that *L. acidophilus* modulates *Giardia* infection, probably by inhibiting the colonization of *Giardia* trophozoites in intestinal epithelial cells or by secreting anti parasitic substances. Moreover, Perdigon et al. [21] have also documented that *L. acidophilus* induced gut mucosal activation by interaction with epithelial cells without increase in the immune cells associated with bronchus. Based on this preliminary observation, we suggest that more emphasis should be put on an understanding the intricate protective mechanism of probiotics, employing more sensitive immunological techniques.

Histopathological studies showed mild cellular injury and inflammatory process in small intestine of probiotic-fed mice both before and after *Giardia* challenge, as inflammatory status of the intestinal mucosa is one of the key determinants of the outcome of infection. The present study clearly demonstrates the low inflammatory process in the small intestine of probiotic-treated but *Giardia*-Infected mice. It also reveals that the probiotic also protected mice against parasite-induced mucosal damage, as was evident from reduced cellular injury and infiltration compared with severely inflamed and damaged small intestines in *Giardia*-Infected mice, suggesting that *L. acidophilus* may either be colonizing efficiently or may have an adjuvant effect on the modulation of the immune response *in vivo* [20].

Taking all evidence into account, the present study clearly highlights the protective potential of *Lactobacillus acidophilus* in murine giardiasis. It can clearly be deduced from the results that lactobacilli colonize the intestinal tract and inhibit the binding of *Giardia* trophozoites, leading to a decrease in cyst count, enhanced faecal *L. acidophilus* count and fewer architectural alterations in the small intestine. These observations should provide an impetus to further research on the use of probiotics for treatment or modulation of common intestinal infections, especially in developing countries, and particularly in the case of infections with emerging resistance. However, the precise mechanism by which probiotic *L. acidophilus* antagonizes *Giardia* infection needs to corroborate results with clinical studies, especially in humans, who have an entirely different gastrointestinal physiology as compared with mice.

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References

[1] Eckmann L., Mucosal defences against Giardia, Parasite Immunology, 2003, 25, 259-270
[2] Upcroft P., Upcroft J.A., Drug targets and mechanisms and of resistance in the anaerobic protozoa, Clinical Microbiol. Rev., 2001,14, 150-164
[3] Eckmanm L., Gillin F.D., Microbes and microbial toxis: paradigms for microbial-mucosal interactions. Pathophysiological aspects of enteric infections with the lumen dwelling protozoan Giardia lamblia, Am. J. Physio. Gestroenterology, 2001, 280, G1-G6
[4] Khanna R., Vinayak V.K., Mehta F.S., Kumkum R.K., Nain C.K., Giardia lamblia infection in immunosuppressed animals causes severe alterations to brush border membrane enzymes, Dig. Dis. Sci., 1998,33, 1147-1152
[5] Walia B.N., Ganguly N.K., Mahajan R.C., Kimar D., Madan I.J., Gambhir S.K., Kanwar S.S., Morbidity in pre school giardia cyst excretors, Trop. Geograp. Med.,1986, 38(4), 367-370
[6] Gardner T.B., Hill D.R., Treatment of Giardiasis, Clin. Microbiol. Rev., 2001,14: 114-128
[7] Calzada F., Meckes M., Cedillo-Rivera R., Antiamoebic and antigiardial activity of plant flavonoids, Planta. Med., 1999, 65, 78-80
[8] Aggarwal A.K., Singh M., Gupta N., Management of giardiasis by an immuno-modulatory herbal drug Pippali rasayana, J. Ethropharmacol., 1994, 44,143-146
[9] Kaneda Y., Torii M., Tanaka T., Aikawa M., In vitro effects of berberine sulphate on the growth and structure of Entamoeba histolytica, Giardia lamblia and Trichomonas vaginalis, Ann. Trop. Med. Parasitol., 1991, 85, 417-425
[10] Oyetayo V.O., Adetuyi F.C., Akinyosoye F.A., Safety and protective effect of Lactobacillus acidophilus and Lactobacillus casei used as probiotic agent in vivo, Afr. J. Biotechnol. 2003, 2 (11), 448–452
[11] Aattouri N., Bouras M., Tome D., Marcos A., Lemonnier D., Oral ingestion of lactic acid bacteria by rats increases lymphocyte proliferation

and interferon – production, British. J., 2001, 87:367–373
[12] Koop-Hoolihan L., Prophylactic and therapeutic uses of probiotics: A review, J. Am. Dietetic. Assoc., 2001, File: //G:/ Intern 1
[13] Diamond L.S., Technique of axenic culture of E. histolytica schaudinn, 1903 and E. histolytica like amoebae, J. Parasitol., 1963, 64, 1047-1056
[14] Benyacoub J., Perez P.F., Rochat F., Saudan K.Y., Reuteler G., Antille N., Humen M., De Antoni G.L., Cavadini C., Blum S., Schiffrin E.J., Enterococcus faecium SF68 enhances the immune response to Giardia intestinalis in mice, J. of Nutrition, 2005, 135, 1171-1176
[15] Tiwari R.P., Hoondal G.S., Tewari R., Laboratory Techniques in Microbiology and Biotechnology, 2001, Abhishek Publication
[16] Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J., Protein measurement with folin's phenol reagent, J. Bio. Chem. 1951, 193, 265-275
[17] Hudson L., Hay F.C., The lymphocyte: its role and function, Practical Immunology, 1980, Third edition, Blackwell Scientific Publication, Oxford, London
[18] Oyetayo V.O., Oyetayo F.L., Potential of probiotics as biotherapeutic agents targeting the innate immune system, Afr J Biotechnol 2005, 4 (2), 123-127
[19] Singer S.M., Nash T.E., T-cell dependent control of acute Giardia lamblia infections in mice, Infect. Immunity, 2000 , 68, 170-175
[20] Humen M.A.L., De Antoni J., Benyacoud M.B., Costas M.I., Cardozo L., Kozubsky K., Saudan A., Doenzli-Bruand S., Blum E.J., Schiffrin P.F., Perez Lactobacillus johnsonii La1 antagonizes Giardia intestinalis in vivo, Infect. Immun., 2005, 73, 1265–1269
[21] Perdigon G., Vintini E., Alvarez S., Medina M., Medici M., Study of the possible mechanisms involved in the mucosal immune system activation by lactic acid bacteria, J. Dairy Sci., 1999, 82, 1108-1114
[22] Salminen S., Bouley C. Boutron-Ruault M.C., Functional food science and gastrointestinal physiology and function, Br. J. Nutr., 1998, (suppl) s, 147-71
[23] Isolauri E., Kalia M., Arvola T., Diet during rotavirus enteritis affects jejunal permeability to macromolecules in suckling rats, Pediatr. Res., 1993, 33, 548-53
[24] Farthing M.J.G., Diarrhoeal disease: current concepts and future challenges (pathogenesis of giardiasis), Trans. R. Soc. Trop. Med. Hyg., 1993, 87, S17- S21