Summary and Conclusion
SUMMARY AND CONCLUSION

Giardiasis is a diarrheal disease affecting young children, adults, immunosuppressed or malnourished individuals. Due to various adverse effects associated with anti/protozoal drugs and development of antibiotic resistance in pathogenic microorganisms; there is an urgent need for natural interventions that are safe, cheap and effective. One such approach is the concept of live bacteriotherapy using probiotics. Probiotics are the live microbes that transit the gastrointestinal tract and benefit the host health. In the present study, concept of probiotics was employed and its role in modulating murine giardiasis was assessed both in vitro and in vivo. The salient features of the study are as follows:

1. Four standard Lactobacillus strains namely *L. casei*, *L. plantarum*, *L. acidophilus* and LGG employed in the present study were monitored for their probiotic characteristics. All the strains tolerated well the conditions simulating in vivo stresses encountered in the gastrointestinal tract stress i.e. bile tolerance, pH extremes and digestive enzymes.

2. Our data demonstrated that oral inoculation of *Giardia* trophozoites (1x10⁶/ml) to BALB/c mice led to the establishment of *Giardia* infection as monitored by cyst count in mice feces. It was observed that *G. intestinalis* was able to transiently colonize the small intestine and had peak cyst count (306.4±10.57) on day 7 PI, thereafter the cyst count started decreasing and mice became *Giardia* free by day 28 PI.

3. The four lactic acid bacteria; *L. casei*, *L. plantarum*, LGG and *L. acidophilus* were compared for their potential to modulate the *Giardia* cycle in terms of cyst count in mice feces. Amongst the four strains used, LGG was found to be the most effective probiotic in modulating the giardiasis by reducing both the duration and severity of infection when administered seven days prior to *Giardia* infection. It was also observed that the duration of *Giardia* cycle was reduced by day 13 PI in
Summary and Conclusion

LGG fed *Giardia* infected mice compared with other probiotic fed *Giardia*-infected mice.

4. The ability of LGG to adhere to the gastrointestinal mucosa *in vivo* was assessed by monitoring the fecal lactobacilli count. The lactobacilli count increased in LGG fed *Giardia*-infected mice from the beginning of *Giardia* infection and was significantly higher at each point of observation compared with *Giardia*-infected mice.

5. The ability of LGG to inhibit the adherence of *Giardia* trophozoites to the intestine epithelium was assessed by trophozoite count in the intestinal fluid. It was interesting to note that the number of viable trophozoites reduced in the small intestine of LGG fed *Giardia*-infected mice at each point of observation leading to early clearance of the parasite compared with *Giardia*-infected mice.

6. The protective potential of probiotic LGG to combat the oxidative stress mediated tissue injury in *Giardia*-infected mice was assessed by measuring the extent of lipid peroxidation and levels of antioxidants. It was observed that daily administration of probiotic LGG to mice either seven days prior or simultaneously with *Giardia* infection reduced the levels of MDA in contrast with increased levels of anti-oxidants GSH and SOD during each phase of infection compared with *Giardia*-infected mice.

7. The ability of probiotic LGG to modulate giardiasis was also assessed by assaying the intestinal brush border membrane enzymes. It was found that LGG was able to modulate the pathophysiology of murine giardiasis by ameliorating the activities of brush border enzymes. Most notably, oral supplementation of probiotic LGG seven days prior to *Giardia* infection, led to enhanced activities of alkaline phosphatase, sucrase and lactase during each phase of infection compared with *Giardia*-infected mice. However, no significant change in the maltase activity.
Summary and Conclusion

was observed in mice belonging to LGG-Giardia, Giardia-LGG and Giardia-infected groups.

8. Histopathological study further revealed that oral administration of probiotic LGG either seven days prior or simultaneously with *Giardia* infection had a profound effect on the morphological and cellular alterations in the proximal part of small intestine. More specifically, it was observed that LGG feeding to *Giardia*-infected mice decreased the degree of inflammation, infiltration of cells, villi damage and crypts in the small intestine resulting into normal morphometry of jejunal villous enterocytes. These histological observations were confirmed by scanning electron microscopy that also showed normal gut morphology in LGG fed *Giardia*-infected mice compared with dispersed villi, deposition of cellular exudates and ileitis in *Giardia*-infected mice.

9. Immunomodulatory potentials of the probiotic LGG in murine giardiasis were also elucidated and it was found that LGG modulated both arms (humoral and cellular) of the immune system. It was observed that oral feeding of the probiotic LGG to mice either prior or simultaneously with *Giardia* infection resulted in a significant increase in the levels of *Giardia* specific secretory IgA antibody during acute and decline phase of infection compared with *Giardia* infected mice. The enhanced levels of *Giardia* specific secretory IgA antibody levels were further confirmed by increased number of IgA+ cells in the lamina propria of LGG fed *Giardia*-infected mice.

10. It was observed that the probiotic LGG administration to mice either prior or simultaneously with *Giardia* infection modulated the cellular immune response as was evident by the increased percentage of CD4+T cell population and decreased percentage of CD8+T cell population both in the acute and decline phase of infection in the lamina propria compared with *Giardia* infected mice.
Summary and Conclusion

11. In an attempt to ascertain, the role of probiotic LGG in modulating the levels of cytokines pro-inflammatory and inflammatory cytokines were assessed. Interestingly, it was found that LGG feeding either prior or simultaneously with Giardia-infection led to decreased levels of cytokines INF-γ and TNF-α in contrast with the increased levels of cytokines IL-6 and IL-10 at each point of observation compared with Giardia-infected mice. However, no significant change in IL-4 levels was observed in LGG-Giardia, Giardia-LGG and Giardia-infected mice.

12. Further, the colonizing ability of the effective probiotic Lactobacillus rhamnosus GG to murine enterocytes viz-a-viz its ability to inhibit the adherence of Giardia trophozoites to murine enterocytes under conditions simulating the intestinal environment was elucidated in vitro. It was observed that fifty percent of Giardia trophozoites adhered to murine enterocytes at 37°C in Hank’s Balanced Salt Solution, pH 7.8 after 1h of incubation. However, co-incubation of murine enterocytes with probiotic LGG either 30 min. prior or simultaneously with Giardia trophozoites led to 23-27% reduction in the adherence of Giardia trophozoites compared with 46% adherence in the absence of LGG. Scanning electron microscopy further confirmed the in-vitro interactions between Giardia trophozoites, probiotic LGG and murine enterocytes.

In nutshell, data of the present study suggests that continual interactions of LGG with host epithelium contributed towards better gut morphology and priming of the mucosal immune system which is a multi-factorial process. This study provides both in vivo and in vitro scientific evidences for the adjuvant effect of probiotic LGG in modulating gut physiology and mucosal immune response in murine giardiasis. Taken together, it can be concluded that LGG can be used as an alternative live bacteriotherapy for the prevention of giardiasis leading to improved core health, healthier digestion and improved immune system. Moreover, due to entirely different human microflora and metabolic activity the clinical application of probiotic as the microbial interference therapy needs to be further investigated.