CHAPTER 03

Lactobacillus as a One Health Approach for Parasite Infection Control in Animals

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SUMMARY

In the last decade, there has been a lot of interest in the possible uses of *Lactobacillus*, a genus of bacteria that is naturally present in the gastrointestinal tracts of animals, including humans. Recognizing that the health of one is intimately related to the health of others, the notion of One Health highlights the interdependence of human, animal, and environmental health. One promising One Health strategy for controlling parasite infections in animals is the use of lactobacillus-based therapies. It has been discovered that certain Lactobacillus species alter the host immunological response. They have the power to increase the immune system's capacity to identify and eradicate parasites by inducing the synthesis of specific immune cells and materials. Animals who encounter this immune-modulating impact may be less vulnerable to parasite infestations. This chapter mainly elaborates on the research conducted to investigate the anti-parasitic effects of the bacteria.

INTRODUCTION

The use of drugs for controlling parasitic infections has several drawbacks, such as the generation of resistance in the parasites against the chemicals [parasiticides (Waller et al., 1996; Capela et al., 2019)] and the contamination of the environment (Powell et al., 2018; Villada-Bedolla et al., 2019; Domingo-Echaburu et al., 2021). This has led to the need to search for alternative control measures for parasitic infections. On the other hand, focusing on the manipulation of the immune system, and based on the studies of the specific immune response, has had a great impact on the prevention of infectious diseases in man and animals by using specific vaccines (Roth, 2011).

However, a less explored arm of the immune system the non-specific immunity (Janeway & Medzhitov 2002; Gasteiger et al., 2017), can be stimulated to increase the innate resistance of animals and man against pathogenic infections (Jain et al., 2008; Maldonado et al., 2015; Maldonado et al., 2019; Raheem et al., 2021). In this context, probiotics including *Lactobacillus*, activate the innate immune system in such a way that the acquired immune system responds better to different pathogens (Mazziotta et al., 2023).

Based on the previous information, the use of *Lactobacillus casei* for controlling parasitic diseases in different animal species, described in this chapter, shows the results of studies carried out mostly by our research group, due to the fact that there were very scantly studies published on this research topic. Several years ago, we explored the use of Freund´s Complete adjuvant (FCA), which contains Mycobacteria and mineral oil, to generate protection in sheep against parasites such as *Haemonchus contortus* (Bautista-Garfias et al., 1991) and *Fasciola hepatica* (Bautista-Garfias et al., 1992). However, because the use of FCA in animals was banned, we began to evaluate *Lactobacillus casei* as an immunostimulant to generate protection against different parasites. Tab 1. summarizes the cited studies carried out.

MECHANISM OF IMMUNE SYSTEM STIMULATION BY *L. CASEI*

Some researchers are focusing on studying probiotic bacteria, such as *Lactobacillus*, because they present very important health-promoting and immunomodulatory properties (Yan & Polk 2011; Maldonado-Galdeano et al., 2019, Kober et al., 2022; Mazziotta et al., 2023) In this order, Maldonado

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Tab 1. Effect of *Lactobacillus casei* treatment on various hosts against different parasites

Parasite	Host	Treatment	Result after challenge	References
Babesia bovis, B. bigemina	Cattle (*Bos taurus)*	BI along with a bivalent vaccine against bovine babesiosis	SP	Bautista *et al*., 2008; Bautista-Garfias *et al.*,2012
Babesia bovis, B. bigemina	Cattle (*Bos taurus)*	BI along with a bivalent vaccine against bovine babesiosis	SP and increase of specific IgG1 against both species of *Babesia*	Bautista-Garfias *et al*., 2015
Babesia microti	Mouse (*Mus musculus*)	BI	SP	Bautista-Garfias *et al*., 2005; Bautista-Garfias et al. 2008
Eimeria acervulina, Eimeria maxima, Eimeria tenella	Chicken (*Gallus gallus*)	BI	SP	Bautista-Garfias et al., 2003
Plasmodium chabaudi	Mouse (*Mus musculus*)	BI	SP	Martínez-Gómez *et al*., 2006
Toxoplasma gondii	Mouse (*Mus musculus*)	BI	SP	Martínez-Gómez *et al*., 2009
Trichinella spiralis	Mouse (*Mus musculus*)	Before infection (BI)	Significant protection (SP)	Bautista-Garfias *et al*., 1999, 2001, 2004; Martínez-Gómez *et al*. 2011
Trypanosoma cruzi	Mouse (*Mus musculus*)	BI	SP	Bautista-Garfias et al., 2008

Galdeano et al., 2015) demonstrated the activation of macrophages from mouse peritoneum, spleen, and Peyer´s patches, in an experiment in which phagocytic activity, cytokine production (IL-10 and IL-6), TLR2 and TLR4 were assessed. The animals previously drank *L. casei* in the drinking water.

In a related study, *L. casei* was exposed to bovine peripheral blood cells *in vitro* for 72 hours to evaluate if the *Lactobacilli* were able to stimulate blood monocytes. After this time, the monocytes showed a different morphology, similar to that of macrophages, as compared with control non-treated monocytes, which did not change their morphology. This observation was an indicator of the activation of bovine monocytes by *L. casei* (Bautista-Garfias et al., 2016). In this study, it was also demonstrated that the monocytes from bovines (8 and 12 months old), exposed to *L. casei* produced significantly higher Nitric Oxide (NO) than control non-treated monocytes. In Fig 1., we present the hypothesis on the probable protective mode of action of *L. casei* against parasites based mainly on our own observations.

THE IMPORTANT STUDIES WERE CARRIED OUT TO EVALUATE THE PROTECTIVE ABILITY OF *L. CASEI* AGAINST INFECTION WITH DIFFERENT PARASITES

Protection assays in mice with *L. casei* against *Trichinella spiralis* infection

Several experiments were carried out in mice to test the protective effect of *L. casei* against the experimental infection with *Trichinella spiralis*. The general protocol consisted of the intraperitoneal inoculation of *Lactobacilli* (live or dead) in mice seven days before infection with a dose of infective parasites. Five days after infection, some of the mice were sacrificed to determine the number of adults in the intestine, and 21 days after the challenge, in another group of mice, the muscle larvae were assessed by artificial digestion. In all cases, there was a significant reduction in the number of adult worms and muscle larvae in animals treated with *L. case*i as compared with non-treated mice (Bautista Garfias et al., 1999, 2001; Martinez-Gomez et al., 2011). It is worth mentioning that in one experiment *L. casei* was used as adjuvant with *T. spiralis* larvae antigen to vaccinate BALB/c mice. The vaccinated animals acquired significant protection against *T. spiralis* infection, as evaluated by the number of muscle larvae recovered 21 days after infection, in comparison with control non-vaccinated mice (Bautista et al., 2004).

Protection of broilers with *L. casei* against *Eimeria* spp. infection

The protective effect of *L. casei* administered by intraperitoneal route, before an infective challenge dose containing a mixture of *Eimeria acervulina*, *E. tenella*, and *E. maxima* was evaluated in broilers. The results indicated significant protection as compared with the protection conferred by a commercial vaccine against coccidiosis (Bautista-Garfias et al., 2003).

Protection of mice with *L. casei* against *Babesia microti* infection

In this study, it was assumed that those *Babesia* structures known as "crisis forms" (dead parasites) inside parasitized red blood cells after an immune response against the parasite were caused by nitric oxide (NO) produced by activated macrophages in response to *Babesia* infection (Homer et al., 2000). In our study (Bautista-Garfias et al., 2005) we observed that mice

treated with *L. casei* experienced a protective immune response against the infection and showed many crises forms in red blood cells, as compared with non-treated mice. We believe that *L. casei* activated macrophages in treated mice to produce NO, which killed parasites inside red blood cells as depicted in Fig 1.

A related study (Bautista-Garfias et al., 2008) tested the effect of low- and high-molecular-weight components of *L. casei* in NIH mice to determine the resistance to *B. microti* infection. The low-molecular-weight components induce an early protective immune response against the parasite like that of *L. casei* viable. In this context, Skariah et al. (2017) demonstrated that the protective immune response against *B. microti* in mice is dependent on parasite killing inside the red blood cells.

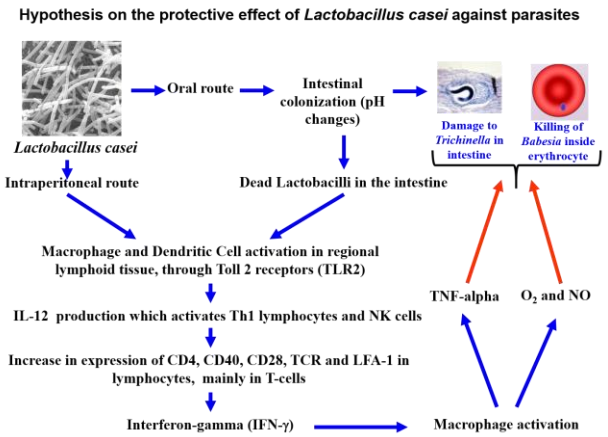

Fig 1. The probable mechanism of action of *L. casei* against *Trichinella spiralis* and *Babesia microti* (figure made by Carlos Ramón Bautista-Garfias, based on data from Bautista-Garfias et al. 2001, Homer et al. 2000, Bautista-Garfias et al. 2005, Maldonado Galdeano and Perdigón 2006, Martínez-Gómez et al. 2006, Turvey and Broide 2010, Martínez-Gómez et al. 2011, Wells 2011, Skariah et al. 2017, Mazziotta et al. 2023). IL-12: Interleukin-12; Th1: Type1 T helper cells; NK: natural killer cells; TNF-alpha: Tumour necrosis-alpha; O2: synglet oxygen; NO: nitric oxide. CD4: helper T-cell; CD40: a cluster of differentiation 40, a protein that functions as a receptor; TCR: T-cell receptor; LFA-1: lymphocyte function-associate antigen 1; IFN-γ: Interferon-gamma.

Protection of mice with *L. casei* against *Plasmodium chabaudi*

Martínez-Gómez et al. (2006) demonstrated that the intraperitoneal administration of *L. casei* to mice induced a non-specific immune response that protected them against *Plasmodium chabaudi* infection. Their results showed reduced parasitemia, reduction in the viability of the parasites recovered from the spleen, and high concentration of nitric oxide (NO) in the mice treated with *L. casei* as compared with non-treated control mice.

Protection of mice with *L. casei* against *Trypanosoma cruzi*

In another research, NIH mice treated orally or intraperitoneally with *L. casei* showed a reduced number of blood parasites after challenge with *Trypanosoma cruzi* (Ninoa strain) as compared with the non-treated control group (Bautista et al., 2008).

Protection of mice with *L. casei* against *Toxoplasma gondii*

Martínez-Gomez et al. (2009) carried out an experiment in which they demonstrated significant protection against *Toxoplasma gondii* brain cyst burden in mice vaccinated with *T. gondii* cytoskeleton proteins using *Lactobacillus casei* as adjuvant.

Use of *L. casei* in the bivalent vaccine against bovine babesiosis

In different assays (Bautista-Garfias et al., 2008, 2012, 2015) it was shown that *L. casei* administered along with the bivalent vaccine against bovine babesiosis, developed by Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) in México, improved the protection conferred to cattle by this vaccine against *Babesia bigemina* and *B. bovis*, both under controlled experimental conditions and in field exposure of bovines to infected ticks. These experiments demonstrated that *L. casei* functioned as an immunological adjuvant too.

OUTCOMES

The information shown in the present chapter (summarised in Tab. 1), through several studies, is indicative of the potential of *Lactobacilli* to improve the immune response of different host species against parasites. These findings are compatible with the beneficial effects of *Lactobacilli* on the host immunity have been indicated by several authors Kerry et al., 2018; Chénard et al., 2020; Ding et al., 2021). The experiments in which *L. casei* was successfully used as an adjuvant are supported by other studies in which *Lactobacillus* was used as an adjuvant in viral vaccines (Ho et al., 2005; Zhao et al., 2012; Liu et al., 2012; Israr et al., 2018). Together, the previous studies are indicative that *Lactobacillus* are beneficial bacteria, and more research should be planned in the future to take advantage of these bacteria for the control of parasitic diseases in man and animals.

CONCLUSIONS

Based on the studies analysed is concluded that *L. casei* (live or dead) (i) stimulates the innate immune system of different animal species when administered orally or intraperitoneally, which improves the acquired immunity and (ii) function as an

immunological adjuvant when applied with antigens of different parasites. The information analysed in this chapter strongly suggests that the immune system can be effectively stimulated, in a non-specific manner, by lactic acid bacteria. The studies published in the last 24 years in different hosts against parasites support this though; however, more studies are needed before implementing the use of *Lactobacilli* as a reliable control measure against parasites of economically important animals.

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