

# Evaluation of *Lactobacillus rhamnosus* GG Heat-Stability During Infant Formula Preparation

Rosita Aitoro BS<sup>1</sup>, Lorella Paparo BS<sup>1</sup>, Rita Nocerino RN<sup>1</sup>, Carmen di Scala LDN<sup>1</sup>, Gaetano Polito MLT<sup>1</sup> and Roberto Berni Canani MD, PhD<sup>1,2,3\*</sup>

<sup>1</sup>Department of Translational Medical Science, Pediatric Section, University of Naples-Federico II, Italy

<sup>2</sup>European Laboratory for the Investigation of Food Induced Diseases, University of Naples-Federico II, Italy

<sup>3</sup>CEINGE-Advanced Biotechnologies, University of Naples-Federico II, Italy

## Abstract

*Lactobacillus rhamnosus* GG survives in adequate amount after reconstitution of infant formulas according to the Food and Agriculture Organisation (FAO) and World Health Organisation (WHO) recommendations. Our results suggest that an infant formula containing this probiotic could be efficiently used in Countries where FAO/WHO recommendations are mandatory.

**Keywords:** Cow's milk allergy; Formula preparation; Hypoallergenic formula

**Abbreviations:** CMA: Cow's Milk Allergy; EHCF: Extensively Hydrolyzed Casein Formula; LGG: *Lactobacillus rhamnosus* GG; IgE: Immunoglobulin E; PIF: Preparation of Infant Formulas.

## Introduction

Cow's milk allergy (CMA) is among the most common food allergies in children [1]. In children with CMA, extensively hydrolysed casein formula (EHCF) supplemented with the probiotic *Lactobacillus rhamnosus* GG (LGG) has been shown to induce higher tolerance rates compared to EHCF without LGG and other formulas [2,3]. These results are consistent with the findings of another study that comparatively evaluated the use of EHCF supplemented with LGG with EHCF or amino-acid-based formula in the management of CMA in the US. The authors have demonstrated that more infants fed with EHCF supplemented with LGG were successfully managed by 12 months than those who were fed either of the other two formulas [4]. Multiple mechanisms may be responsible for the observed clinical effects. Preliminary data suggest that the dietary intervention with EHCF+LGG has positive effects on gut dysbiosis, short chain fatty acids production [5], and epigenetic regulation of Th1 and Th2 cytokine genes expression [6,7]. Such mechanisms suggest a possible long-term effect on the immune system of children with CMA treated with EHCF+LGG. In a randomised controlled trial (RCT) we demonstrated that a dietary intervention with EHCF supplemented with LGG could influence the occurrence of other atopic manifestations in children with immunoglobulin E (IgE)-mediated CMA [8]. Concerns have been raised regarding LGG's stability during powdered infant formula reconstitution according to the Food and Agriculture Organisation (FAO) and World Health Organisation (WHO) recommendations. FAO/WHO recommend the following procedure for the preparation of infant formulas (PIF): 1) to boil a sufficient volume of safe water; 2) to pour the appropriate amount of boiled water into a cleaned and sterilised feeding bottle and allow to cool it slightly; 3) to dilute the powdered formula in water at a temperature of not below 70°C; 4) to cool bottle quickly to feeding temperature by holding under a running tap; and 5) to consume formula soon after each preparation [9]. Similar recommendations have been put forward by the UK Department of Health, the Finnish Food Safety Authority, Health Canada [10] and the European Food Safety Authority [11]. FAO/WHO recommendations outline the best practice for the safe preparation of infant formula in order to reduce the risk of infection in that developing country

where recommendations are mandatory. In this study, we evaluated whether the LGG contained in EHCF could survive during the formula preparation procedure indicated by FAO/WHO.

## Materials and Methods

According to FAO/WHO recommendations, we boiled drinking water for 10 min. Water was left at room temperature until a temperature of 70°C was achieved, then EHCF containing LGG powder (Nutramigen LGG, Mead Johnson Nutrition, Evansville IN, US) was dissolved in the bottle. The bottle was immediately cooled to feeding temperature by holding the bottom under a cold running tap. EHCF supplemented with LGG dissolved in water at room temperature served as the control. Samples were diluted 1:1000 in distilled water, and 100 µl of each sample was spread on the MRS agar plates as previously described [12]. The plates were incubated under anaerobic conditions for 72 h at 37°C. All experiments were performed in triplicate using two random selected batches.

## Results

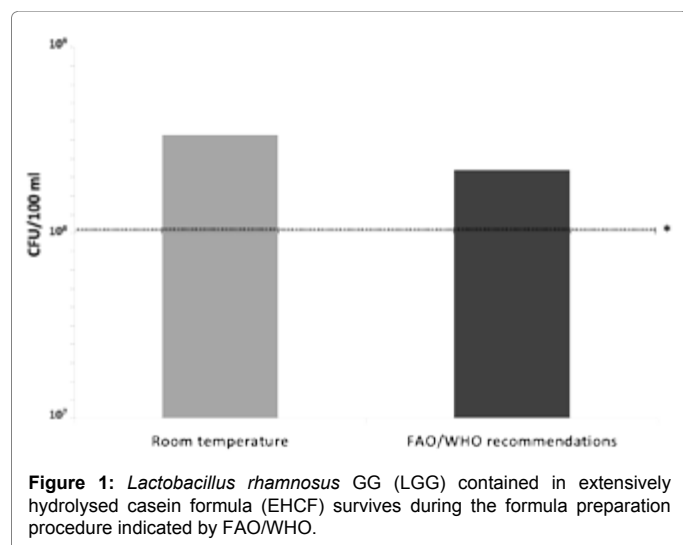
Manufacturer's specification indicates an LGG concentration from  $2.5 \times 10^7$  to  $5 \times 10^8$  CFU/gr with a guaranteed level of  $1.46 \times 10^7$  CFU/100 ml (approximately  $1 \times 10^6$  CFU/gr). In line with our previous observation [2], we found a LGG concentration within this range in the control sample ( $5.3 \times 10^8$  CFU/100 ml). After EHCF containing LGG preparation according to FAO/WHO recommendations, the median total LGG count was  $3.4 \times 10^8$ , which exceeded the guaranteed level of CFU/100 ml (Figure 1). LGG concentration in EHCF at room temperature is  $5.3 \times 10^8$  CFU/100 ml. After preparation according to FAO/WHO recommendations, LGG concentration is  $3.4 \times 10^8$  CFU/100 ml. Guaranteed level ( $0.14 \times 10^8$  CFU/100 ml).

\*Corresponding author: Roberto Berni Canani, Director, Pediatric Allergy Program, Department of Translational Medical Science, Pediatric Section, University of Naples "Federico II", Via S Pansini 5 80131 Naples, Italy, Tel: +390817462680; E-mail: [bemi@unina.it](mailto:bemi@unina.it)

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## Conclusions

Reconstitution of EHCF+LGG according to FAO/WHO recommendations for infant formula preparation allows an adequate degree of probiotic survival. Our results suggest that this dietary approach could also be efficiently adopted in Countries where FAO/WHO recommendations are mandatory.

## Conflicts of Interest and Source of Funding

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