

# Prevention of *Mycobacterium avium* Subspecies *paratuberculosis* (MAP) Infection in Balb/c mice by Feeding *Lactobacillus acidophilus* Strain NP-51<sup>®</sup>

## A.S. Leaflet R2487

Mohamed Osman, graduate research assistant;  
Judith Stabel, lead scientist, Johne's Disease Research Project  
USDA-ARS National Animal Disease Center;  
Jesse Hostetter, associate professor, Veterinary Pathology;  
Daniel Nettleton, professor, Department of Statistics;  
Donald C. Beitz, Department of Animal Science, Iowa State  
University

### Summary and Implications

The immune responses of 390 BALB/c mice fed the probiotic *Lactobacillus acidophilus* strain NP51<sup>®</sup> and infected with *Mycobacterium avium* subspecies *paratuberculosis* (MAP) were evaluated in a 6-month trial. Mice were randomized to nine treatment groups that fed either viable- or heat-killed NP51 and inoculated with either viable- or heat-killed MAP or sterile phosphate-buffered saline. Feeding the NP51 resulted in higher numbers of T lymphocytes in the spleen including the CD8<sup>+</sup> cytotoxic T lymphocytes. In addition, feeding the NP51 lowered the number of immune suppressive T regulatory cells CD4<sup>+</sup>CD25<sup>+</sup> and CD8<sup>+</sup>CD25<sup>+</sup> cells in the spleen. Additionally, feeding the NP51 resulted in higher concentration of interferon-gamma in the supernatant of splenocytes cultured *in vitro*. These results suggest that feeding the NP51 to BALB/c mice might prevent the progression of MAP infection in mice.

### Introduction

*Mycobacterium avium* subspecies *paratuberculosis* the causative agent of Johne's disease (JD) is known to infect domestic ruminants such as cattle, sheep, goats, camelids and other free and captive species worldwide. JD is characterized by development of chronic enteritis, profuse watery diarrhea and progressive weight loss. Prevalence of infection is increasing in countries that do not adopt mandatory control practices. In the US, Johne's disease was present in about 68% of US dairy herds. It has been estimated that losses to the US dairy industry because of JD range from \$200 to \$250 million annually. Economic losses due to JD are manifested as early culling and reduced reproductive efficiency, feed efficiency and milk production and death of cattle. However, milk production losses in subclinically and clinically infected are the most insidious.

### Materials and Methods

Three hundred eighty Balb/c mice (190 females and 190 males) were assigned randomly to one of ten treatment groups: sentinel, control, heat-killed MAP, viable MAP, heat-killed NP51<sup>®</sup>, viable NP51<sup>®</sup>, heat-killed NP51<sup>®</sup> plus heat-killed MAP, heat-killed NP51<sup>®</sup> plus viable MAP, viable NP51<sup>®</sup> plus heat-killed MAP, viable NP51<sup>®</sup> plus viable MAP. Mice were fed  $1 \times 10^6$  CFU of NP-51 • mice<sup>-1</sup> • day<sup>-1</sup> mixed in standard mouse chow starting at day 1 of the study. On day 45, mice were challenged with  $1 \times 10^8$  CFU of MAP intraperitoneally. Apart from the sentinels, ten mice from each treatment group were euthanized on days 45, 90, 135 and 180 of the study. Mice body weights were recorded weekly. Fecal samples were collected on two separate days and composited weekly. Blood samples were withdrawn by cardiac puncture and sera used to quantify IgG and IgA. In addition, spleens, ceca, ilea, livers and mesenteric lymph nodes (MLN) were dissected. Effect of NP51<sup>®</sup> on bacterial burden in tissues was determined by culturing MAP from MLN and liver. Also, effect of NP51<sup>®</sup> on tissue histopathology was examined by staining tissue slides with H & E and Ziehl-Neelsen stains. Splenocyte single cell suspensions were stimulated with MAP antigen for examination of T cell differentiation and cytokines secretion.

### Results and Discussion

Feeding the HK-NP51 to the mice challenged with the viable-MAP increased production of IFN- $\gamma$  by more than 4-fold of that of control mice challenged with the HK-MAP (127.14 vs. 31.69 pg/mL, respectively) and 1.2-fold over that of control mice challenged with the viable MAP (127.14 vs 107 pg/mL, respectively). Similarly, feeding the viable-NP51 to mice challenged with viable-MAP increased IFN- $\gamma$  production by 4-fold of that of control mice challenged with the HK-MAP and by 1.2-fold of mice challenged with the viable-MAP (130.7 vs 31.7 and 107 pg/mL, respectively). Because MAP is an obligate intracellular pathogen, production of IFN- $\gamma$  is crucial for the granuloma formation that prevents the tissue dissemination of MAP and, thus, the progression of JD in animals.

### Acknowledgments

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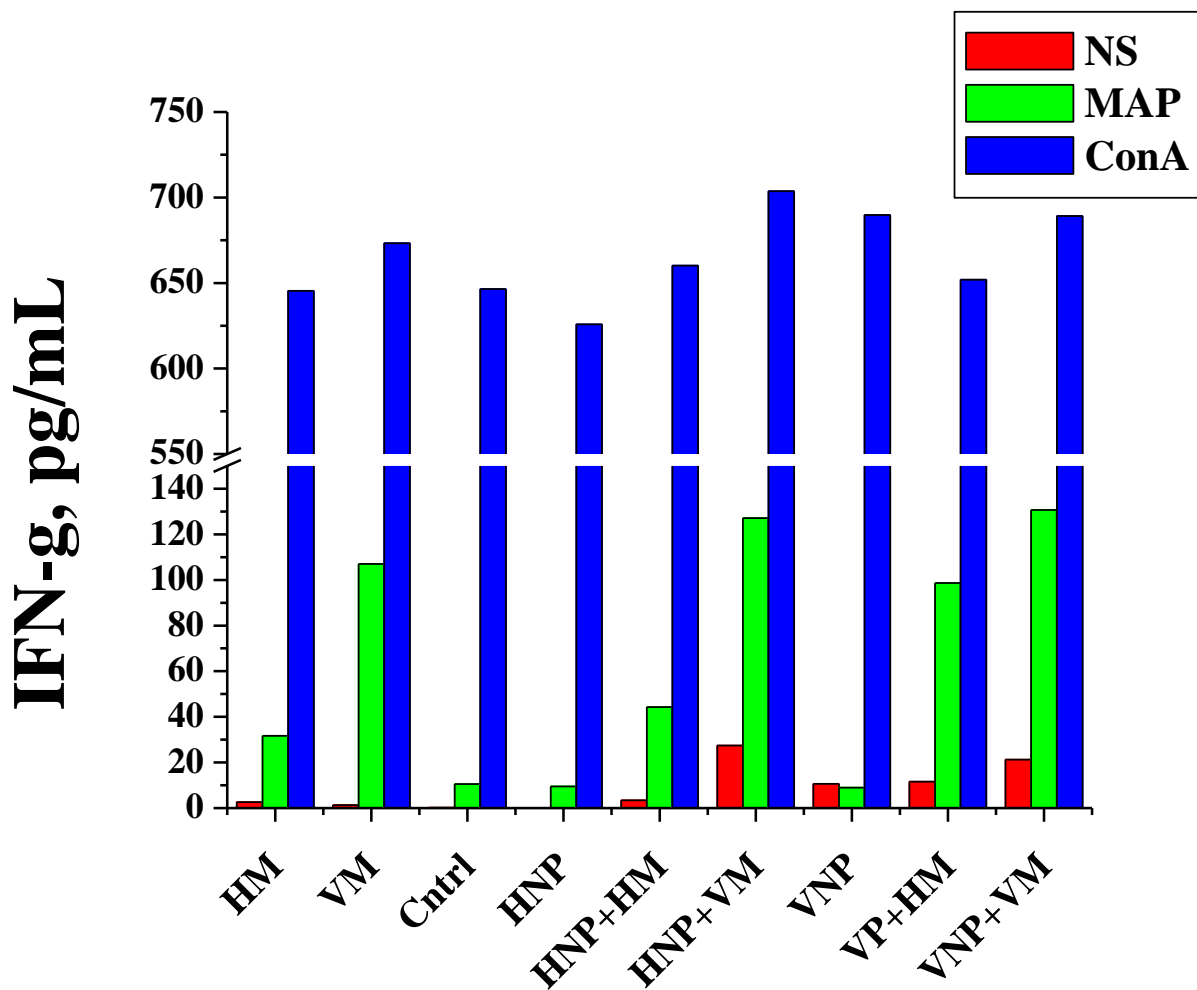


Figure 1. Concentrations of interferon gamma (IFN- $\gamma$ ) on d 45 from in vitro culture of splenocytes of mice fed chow plus maltodextrin (control), chow plus maltodextrin and challenged with heat-killed MAP (HM), chow plus maltodextrin and challenged with viable-MAP (VM), chow plus heat-killed NP51 (HNP), chow plus HNP and challenged with heat-killed MAP (HNP+HM), chow plus HNP and challenged with VNP (HNP+VM), chow plus viable-NP51 (VNP), chow plus VNP and challenged with the HM (VNP + HM) or chow plus VNP and challenged with the VM (VNP + VM). Splenocytes were either nonstimulated (NS) or stimulated with concanavalin A (ConA) or MAP antigen (MAP).