

# Are we feeding *Mycobacterium paratuberculosis* in calf milk replacer?

**B.B. Kunkel**, BS<sup>1</sup>; **I.R. Grant**, BSc, PhD<sup>2</sup>; **A.C.G. Foddai**, DVM, MSc, PhD<sup>2</sup>; **J.C. Tarrant**, DVM<sup>1</sup>; **S.M. McGuirk**, DVM, PhD, DACVIM<sup>3</sup>; **M.T. Collins**, DVM, PhD, DACVIM<sup>1</sup>;

<sup>1</sup>Dept. Pathobiological Sci., School of Veterinary Medicine, Univ. Wisconsin-Madison, 2015 Linden Dr., Madison, WI 53706

<sup>2</sup>Institute for Global Food Security, Queen's University Belfast, 97 Lisburn Rd., Belfast, NI BT9 7BL

<sup>3</sup>Dept. Medical Sci., School of Veterinary Medicine, Univ. Wisconsin-Madison, 2015 Linden Dr., Madison, WI 53706

## Introduction:

When advising farmers on how to control Johne's disease, the #1 recommendation is to avoid feeding waste milk to calves and instead to feed them calf milk replacer (CMR). Obviously, this advice is based on the assumption that milk replacer is free of live *Mycobacterium avium* subsp. *paratuberculosis* (MAP) organisms capable of causing infection. No one has ever challenged this assumption. Preliminary work on CMR sourced in Wisconsin found that 1 (12.5%) of 8 samples tested positive for live MAP organisms by the peptide-mediated magnetic separation-phage assay (PMS-PA). Previously, 30 (44%) of 68 powdered milk products intended for human consumption were positive for live MAP by the same assay. The study objective was to expand the survey of CMR and to test for MAP as well as standard measures of microbiological quality.

## Materials and Methods:

The PMS-PA permits sensitive and rapid detection of live MAP. MAP cells are separated from the sample using magnetic beads coated with MAP-specific peptides and incubated with a mycobacteriophage, before treatment with viricide (to kill extracellular phages) and then plating with fast-growing *M. smegmatis*. Plaques in the resulting *M. smegmatis* lawn indicate the presence of live MAP organisms that have burst as a result of phage amplification. IS900 PCR is applied to plaques to confirm that the plaque is derived from MAP cell(s). CMR products (n=83) were obtained from dairy farms across the USA. In addition to the PMS-PA, conventional microbiological methods were used to quantify total mesophilic bacterial counts, coliforms, *Salmonella*, staphylococci, and streptococci in the CMR samples, in order to assess their overall hygienic quality.

## Results:

Sixteen (19.2%) of 83 CMR samples tested positive for live MAP based on PMS-PA with IS900 PCR confirmation of MAP DNA in plaques obtained. All conventional microbiology results were within the USA regulatory guidelines for bulk tank milk. No

correlation was found between conventional microbiology and presence of MAP indicated by the PMS-PA. Corroboration of PMS-PA findings was attempted using sample processing with and without PMS, and solid and liquid medium-based MAP culture; some culture results are pending.

## **Significance:**

Live MAP in CMR was confirmed by a novel, highly sensitive assay. However, these results could not be confirmed by conventional culture methods for MAP (as of the time of abstract submission). Thus, the findings must be considered as unverified, but concerning. Detection of MAP in CMR was not correlated with any conventional measures of microbiological quality of milk making it impossible to use alternative methods to predict presence of MAP.