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

B.B. Kunkel, BS[1]; **I.R. Grant**, BSc, PhD[2]; **A.C.G. Foddai**, DVM, MSc, PhD[2]; **J.C. Tarrant**, DVM[1]; **S.M. McGuirk**, DVM, PhD, DACVIM[3]; **M.T. Collins**, DVM, PhD, DACVM[1]
[1]Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, 2015 Linden Dr., Madison, WI 53706
[2]Institute for Global Food Security, Queen's University Belfast, 97 Lisburn Rd., Belfast, NI BT9 7BL
[3]Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, 2015 Linden Dr., Madison, WI 53706

Introduction

When advising farmers on how to control Johne's disease, the number 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 of 8 (12.5%) samples tested positive for live MAP organisms by the peptide-mediated magnetic separation-phage assay (PMS-PA). Previously, 30 of 68 (44%) 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 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. IS*900* 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 US. 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 to assess their overall hygienic quality.

Results

Sixteen of 83 (19.2%) CMR samples tested positive for live MAP based on PMS-PA with IS*900* PCR confirmation of MAP DNA in plaques obtained. All conventional microbiology results were within the US 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.