Improved Culture Medium (TiKa) for Mycobacterium avium Subspecies Paratuberculosis (MAP) Matches qPCR Sensitivity and Reveals Significant Proportions of Non-viable MAP in Lymphoid Tissue of Vaccinated MAP Challenged Animals

The quantitative detection of viable pathogen load is an important tool in determining the degree of infection in animals and contamination of foodstuffs. Current conventional culture methods are limited in their ability to determine these levels in Mycobacterium avium subspecies paratuberculosis (MAP) due to slow growth, clumping and low recoverability issues. The principle goal of this study was to evaluate a novel culturing process (TiKa) with unique ability to stimulate MAP growth from low sample loads and dilutions. We demonstrate it was able to stimulate a mean 29-fold increase in recoverability and an improved sensitivity of up to three logs when compared with conventional culture. Using TiKa culture, MAP clumping was minimal and produced visible colonies in half the time required by standard culture methods. Parallel quantitative evaluation of the TiKa culture approach and qPCR on MAP loads in tissue and gut mucosal samples from a MAP vaccine-challenge study, showed good correlations between colony counts (cfu) and qPCR derived genome equivalents (Geq) over a large range of loads with a 30% greater sensitivity for TiKa culture approach at low loads (two logs). Furthermore, the relative fold changes in Geq and cfu from the TiKa culture approach suggests that non-mucosal tissue loads from MAP infected animals contained a reduced proportion of non-viable MAP (mean 19-fold) which was reduced significantly further (mean 190-fold) in vaccinated "reactor" calves. This study shows TiKa culture equates well with qPCR and provides important evidence that accuracy in estimating viable MAP load using DNA tests alone may vary significantly between samples of mucosal and lymphatic origin.

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