Because of the occurrence of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in the food supply, the question of whether or not MAP is a causal agent of Crohn’s disease is of paramount interest. Seemingly well-designed research studies have often produced contradictory results; therefore, the association of MAP with Crohn’s disease remains quite contentious.

Although the conclusions of the meta-analysis by Martin Feller and colleagues appear to indicate an association between MAP and Crohn’s disease, I have reservations concerning the decision to exclude 13 studies in which MAP was not detected in any patients with Crohn’s disease or in controls. Because MAP occurrence was the primary outcome under analysis, this decision undoubtedly skewed the conclusions of the meta-analysis. The authors’ post-hoc rationale for exclusion was that the DNA extraction protocols used in these studies might not have been sufficiently robust to release MAP DNA. Was a similar exclusion criterion applied to the studies in which MAP DNA was detected?

Failure to detect MAP in any sample in a study could indicate either poor assay sensitivity or a true finding that MAP is not associated with Crohn’s disease. Because no standards exist for PCR assays of MAP in human specimens, it is not possible to assess which possibility holds for any given study. Thus, the exclusion of studies based solely on failure to detect MAP is capricious and inappropriate. If assay sensitivity were a valid criterion for study inclusion/exclusion, then I would argue that overly sensitive PCR assays (ie, nested PCR followed by hybridisation with labelled probes), which likely result in overestimates of the prevalence of MAP in samples, must also be treated as suspect. That many of the studies included in this meta-analysis were not blinded also suggests a substantial possibility of systematic error in interpreting borderline results; unblinded control samples are likely to be judged negative and unblinded Crohn’s disease samples judged positive.

Finally, an additional source of bias that was not addressed by the authors is the fact that negative results generally are not published, and so would not enter into this meta-analysis. Indeed, in parallel with a recently published culture-independent study of microbial communities associated with inflammatory bowel disease, my colleagues and I undertook quantitative PCR surveys of MAP using both IS900 and 16S ribosomal DNA primer sets; results were uniformly negative and so were discussed only in passing. Thus by default, the meta-analysis of Feller and colleagues would appear to be biased towards studies that support the hypothesis that MAP is associated with Crohn’s disease. Whether or not an association truly exists remains just as contentious as before.

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I declare that I have no conflicts of interest.


Authors’ reply

We thank Daniel Frank for his interest in our meta-analysis, and would like to respond to his reservations regarding our decision to exclude 13 studies in which *Mycobacterium avium* subspecies *paratuberculosis* (MAP) could not be detected in any of the samples from patients with Crohn’s disease or controls. We made this decision a priori in our study protocol, based on the fact that in this situation no estimate of an association between MAP and Crohn’s disease can be calculated. In the discussion section of our paper, we speculated that the laboratory techniques used in these studies could have led to negative findings because only three of the 13 studies used an enzymatic step in combination with a mechanical step or sonication for DNA extraction.