Presence of *Mycobacterium avium* subs. *paratuberculosis* DNA in milk used to feed calves in Portugal

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Short title: *Map* DNA in milk used to feed calves

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This Technical Research communication describes results of a study aimed at detecting the presence of *Map* in milk fed to calves, and identifying possible risk factors for that presence. A questionnaire was performed on 37 dairy farms and waste milk samples were collected on 3 occasions separated by a minimum of one week. For farms not feeding waste milk, bulk tank milk samples were collected instead. A real time PCR for the detection of the *IS900* sequence was performed for the detection of *Map*. A majority of farms (89·2%) fed waste milk, with only one pasteurizing the milk before feeding it to calves. Results of the PCR showed that 51·5% of the farms that were feeding waste milk had a positive result for *Map* on that milk. None of the studied risk factors were significantly associated with the presence of *Map* in milk samples, possibly due to the small number of farms entering the study. However, the prevalence of positive samples for *Map* on PCR was 3·5 times higher for farms that bought in animals from a single origin and 1·9 times higher for farms that bought from multiple farms, when compared with closed farms. Having a calving area for multiple cows also increased the risk of a positive *Map* result by 1·5 when compared with single pens. The risk of having a positive *Map* result on waste milk was 1·6 times higher for farms feeding that milk to male calves and 1·4 for farms feeding to both male and female calves, when compared with farms not feeding waste milk. This study highlights paratuberculosis as one of the potential risks of feeding waste milk to calves, and the need for mitigations strategies to be in place to avoid unnecessary disease transmission.