

**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 to feed calves

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Summary

Dairy farmers often feed calves with waste milk, a mixture of excess colostrum and non-saleable milk. *Mycobacterium avium* subsp. *paratuberculosis* (*Map*), the causal agent of paratuberculosis is transmitted more frequently through the fecal-oral route, with evidence that calves under 6 months of age are the most susceptible to infection. This study aimed at detecting the presence of *Map* on milk fed to calves, and to address 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. Out of these, only one pasteurized 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, however the risk of having a sample positive 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.

Keywords: *Mycobacterium avium* subsp. *paratuberculosis*; Johne's disease; calf feeding; waste milk; milk direct detection