Reviewer's report

Title: Potential role of real-time PCR for detection of Mycobacterium avium subsp. paratuberculosis (MAP) in chronically diseased milking cows: a case control study

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Reviewer number: 1

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The manuscript is quite interesting, but it requires, in my opinion, some changes and corrections in order to make it suitable for publication.

Here my comments and suggestions according to the scheme of BMC Vet Res

Major Compulsory Revisions

Rows 86-94: The authors stated the kits used was not officially certified in Germany, why they used these kits? In other words, have the kits been validated according to any procedures? Which is the limit of detection of the kits for both faeces and milk? Did the authors carry out a preliminary set of experiments in order to have any ideas about the performances of the kits? Please, give more details about this issue.

Rows 130-135: please, the authors should discuss their results based on the current literature.

Rows 160-169: Did the criteria for eligibility in control or case groups have been specified elsewhere? The idea was to ascertain if the MAP exposure can increase the cases status, maybe more information about how and why these criteria have been selected should be added and explained. Please, can the authors add further explanations about this important point? Based on what I have read in the table 1, both cases and control have the same percentage of MAP positive herds, so the idea was to estimate if MAP DNA positive animals can be more prone to fall into the cases group? In case of positive answer, the authors should state it in the objectives of the study, as well as in the abstract section, because in the current format, the aims of the paper are misleading for the readers.

Minor Essential Revisions

Rows 74-83: Since the manuscript is focused on testing dairy herds, why report here data about the seroprevalence in small ruminants? I would suggest to remove this part.

Row 91: Please, could the authors report some citations in literature to justify the statement relative to the higher sensitivity of this kit to respect to ELISA and cultural assays?

Rows 112-114: Please, could you better explain this point?
Row 106: .. and 5 out of 350 (1.43%) milk samples....., suppose here the authors are referring to foremilk? Please check because in the row before they have stated “all bulk tank milk samples tested as MAP-negative”.

Rows 145-150: this statement is true, but more recently news primers and probes for IS900 target have been designed and validated. Also the specificity of these primers and probes has been evaluated in a panel of mycobacteria very close to MAP. On the other hand, since IS900 is present in a variable number of copies, from 14 to 20, according to some authors, it remains the target which permits to achieve the higher sensitivity. Please discuss.

Rows 172-174: I did not understand this statement. Is the paper a preliminary report of a bigger study?

Rows 191-193: According to the procedures of the kit, this phase follows the centrifugation at 1500 x g for 10 min. Please check it.

Row 243 and other part of the manuscript: I feel it should be better report the result as Cp>40 instead of Cp= 0.

Row 252. Should be “MAP DNA-negative sample” instead of “MAP DNA-positive sample”?

Rows 254-259: this part it’s not clear and I have the impression some typing mistakes occurred; moreover, icr samples were tested again with the Real time PCR without a new DNA extraction? I suggest to rewrite it in a clear and concise form because it’s just difficult to follow the text.

Row 260: which is the utility of this classification in the manuscript? The authors did not stratify the samples based on their Cp values.

Discretionary Revisions

Regarding the performances of the kits used for the study, do the authors feel it should be possible give any idea about the positive and negative predictive values of these kits for both faeces and milk?

According to the concept of discretionary revisions, I have here a curiosity: the authors used the OR for the assessment of MAP exposure as potential risk factor for the case status (rows 269-279); in this regard, do the authors believe it would be possible use another statistical approach/model? Something like a logistic regression perhaps? Or it’s not possible use here this kind of model)?

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.