Reviewer's report

Title: Development of a multiplex PCR to detect and discriminate porcine circoviruses in clinical specimens

Version: 1 Date: 29 Apr 2019

Reviewer: Karen Harmon

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The authors describe a multiplexed PCR for the detection of Porcine Circoviruses types 1,2, and 3. Various assays for these targets are available singly or in combinations of two of the three but nothing for all three. As more PCV subtypes are identified, this is a useful tool, but in this reviewer's opinion, case must be taken in interpretation of results, especially when clinical significance of the various agents may not be well understood.

Recommended edits:

Line 10: change "special primers" to "primer pairs"
Line 11: change "detected" to "tested"
Line 15: change "and PCV3" to OR PCV3"
Line 78: How many GenBank sequences of each were used in primer design?
Line 151: change to "was added to make a total volume of 50 ul per reaction" or similar.
Line 163- 164: I assume the PCV1, PCV2 and PCV3 target genes were amplified in the multiplexed reaction. The way it is currently worded is ambiguous in that it may be interpreted that these were run with the individual primer pairs. Please clarify.
Line 172: Change "and" to "or"
Line 177: change "detected" to "tested"
Line 205: change "known" to "believed" or similar.
Lines 228 - 229: change to "the sizes of the amplified PCR products..."
Lines 237 and 240: change Practiced" to "run"
Line 240:1: change to "conventional PCR instrumentation" (or equipment)
Line 261: change "consumption" to "cost"

Were any of the specimens tested negative for all 3 of the viruses? From Table 2 it would appear so. A column in this table with number of samples negative for all 3 agents would be helpful. Also, were any samples from "non-clinical" pigs tested? This would also be useful information to evaluate the presence of these agents in the absence of clinical signs to perhaps understand how to best use this assay.

Given the very scarce evidence of the involvement of PCV1 in pathogenicity (only one paper referenced here suggests a link, and the final statement in that paper is "More research is needed to confirm the pathogenic character of PCV1 for porcine foetuses.") I think this paper needs to be
very careful about stating that this multiplex PCR could be used as a diagnostic tool for monitoring PCV1. Perhaps it would be more appropriate to say this multiplex PCR could be useful in determining the presence of the various PCVs, but at this point I think it is premature to say it would be a useful diagnostic tool. There should also be a statement that the significance of detection by PCR must be coupled with the clinical status of the animals being tested, and in the case of PCV1 in particular, additional research should be performed to better explore its role in pathogenesis.

Are the methods appropriate and well described?  
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?  
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?  
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?  
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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Needs some language corrections before being published
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