Inter-laboratory and inter-assay comparison on two real-time PCR techniques for quantification of PCV2 nucleic acid extracted from field samples

Several real-time PCR assays for quantification of PCV2 DNA (qPCR) have been described in the literature, and different in-house assays are being used by laboratories around the world. A general threshold of 7 copies of PCV2 per millilitre serum for postweaning multisystemic wasting syndrome (PMWS) diagnosis has been suggested. However, neither inter-laboratory nor inter-assay comparisons have been published so far. In the present study two different qPCR probe assays used routinely in two laboratories were compared on DNA extracted from serum, nasal, and rectal swabs. Results showed a significant linear association between the assays (p < 0.0001) and a systematic difference of 1.4 log(10) copies of PCV2 per millilitre of sample (p < 0.0001). This difference indicated that the assay from laboratory 1 yielded a higher output than the one from laboratory 2. Results also showed that there was no linear association between the amount of PCV2 DNA and the amount of total DNA, neither in nasal (p = 0.86) nor in rectal (p = 0.78) swabs, suggesting that normalizing of PCV2 DNA load in swab samples to total DNA concentration is not suitable. The present exploratory study highlights the need for the performance of ring trials on qPCV2 protocols between laboratories. Meanwhile, the proposed thresholds for PMWS diagnosis should only be considered reliable for each particular laboratory and each particular assay.

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