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Letter to the Editor

Application of PCR-based detection of *Clostridium perfringens cpb2* in fecal samples

We have evaluated three strategies for the PCR-based detection of *cpb2* carrying *Clostridium perfringens* in feces (van Asten et al., 2008). Thirty-three fecal samples were anaerobically cultured on sheep blood agar for *C. perfringens* and of each sample, approximately 10 *C. perfringens*-like colonies were tested for *cpb2* by PCR. Twenty-four samples appeared to contain *cpb2* positive colonies. In 11 of these samples also *cpb2* negative *C. perfringens* strains were demonstrated. Two of these samples also harbored strains with different *cpb2* variants as determined by MboI digestion (van Asten et al., 2008). As a first alternative 10 colonies of each sample were pooled and 1 μl of this mixture was used as template in the PCR. Five micrograms of bovine serum albumin (BSA, Sigma) and a double amount of primers were added to improve the sensitivity of the PCR. All pooled samples that harbored *cpb2* carrying *C. perfringens* colonies gave a positive PCR result for *cpb2* and the subsequent MboI digestion of the PCR product enabled detection of the *cpb2* variants.

As a second alternative the total DNA of 18 fecal samples was directly isolated using QIAmp DNA Stool Mini Kit (Qiagen). Five microlitres of the isolated DNA was used in a PCR for *cpb2*: only 9 out of the 15 samples positive for *cpb2* as demonstrated by the simultaneously performed colony method tested positive for *cpb2*. The use of a more sensitive polymerase (FastStart High Fidelity PCR System Roche) did not improve this result.

These experiments demonstrated:

(a) the limitations involved with testing a single or only few *C. perfringens* like colonies of a fecal sample for the presence of *cpb2* harboring *C. perfringens* in e.g. prevalence studies;
(b) that the pooling of colonies leads to similar PCR results as testing separated single colonies;
(c) that testing feces directly for *cpb2* results in fewer positive samples.

We conclude that out of the three methods evaluated, the ''pooled sample method'' is the most sensitive and efficient one.

Reference

van Asten, A.J.A.M., Allaart, J.G., Meeles, A.D., Gloudemans, P.W.J.M., Houwers, D.J., Gröne, A., 2008. A new PCR followed by MboI digestion for the detection of all variants of the *Clostridium perfringens* cpb2 gene. Vet. Microbiol. 127, 412–416.

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