Evaluation of PCR and DNA Sequencing for Direct Detection of Clostridium perfringens in the Intestinal Tract of Broilers

The aim of this investigation was to determine the presence of the opportunistic pathogen Clostridium perfringens by PCR and DNA sequencing, without previous cultivation. This methodology was then used to investigate how C. perfringens was affected by different preventive measures, such as ionophores and feed additives, for necrotic enteritis in broilers chickens. DNA was extracted from the intestinal content or intestinal tissue by DNA extraction kits. Detection limits for 16S rRNA, alpha-toxin, and cpb2 PCR gene targets were approximately $1 \times 10^3$, $5 \times 10^4$, and $1 \times 10^6$ cells per g of intestinal content or tissue, respectively, as determined with samples spiked with C. perfringens. The method was evaluated with samples from single conventional broilers or from pools of six birds of experimentally reared broilers. Conventional chickens, raised with salinomycin in their feed, showed reduced numbers of C. perfringens-positive samples ($P < 0.05$) for all three PCR tests. With respect to cpb2, a tendency to detect more samples as positive for C. perfringens was observed with increasing age. The addition of sodium butyrate and lactic acid in the feed for experimental birds had a minor effect ($P < 0.10$) on positive samples, as detected with the 16S rRNA PCR. For experimental birds fed whole wheat, only three out of six pools of six birds allowed detection of C. perfringens by the 16S rRNA PCR, compared to five for the untreated controls or the Avilamycin- or prebiotic-treated birds. All 16S rRNA partial gene sequences obtained were identical and were 99.5% similar to the rrnB gene of the type strain of C. perfringens. Two types of the partial cpb2 gene sequence were detected with a similarity of 93%. One type was translated into protein, whereas a stop codon was found in the other type. Both types were located in the "atypical" phylogenetic group of the cpb2 gene sequences. The PCR test, based on extraction of DNA from intestinal content, provided rapid screening of poultry for C. perfringens without the need to have access to facilities in order to immediately cultivate and identify bacteria at the location of sampling. Further work is suggested to determine the relationship between the degree of necrotic enteritis, the actual level of C. perfringens in the animal, and the detection achieved by PCR.

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