



***Clostridium perfringens* population dynamics in commercial broiler chicken flocks submitted to two different farm rearing protocols, including one without antimicrobial agents**

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Abstract

Introduction: The growing pressure arising from both consumers and governmental authorities to reduce and even to ban the use of antimicrobials in food-producing animal represents a major challenge for the poultry industry. The ban on the use of these compounds in broiler chicken production has led to various problems in some European Union countries, necrotic enteritis being the most significant issue. In an effort to identify new alternatives in replacement of antimicrobials, it becomes relevant to better understand *Clostridium perfringens* population dynamics in commercial broiler chickens flocks.

Objective: We studied how a conventional rearing protocol, including the use of antimicrobials, and an alternative protocol without antimicrobials, rather including different alternatives, influence the resident *Clostridium perfringens* population dynamic over a 14-month period.

Methods: Pulse Field Gel Electrophoresis (*Sma*I) was used to assess the genetic relatedness of the strains while PCR was used for toxinotyping. Two modified multiplex PCR protocols were used for the detection of the toxin genes *cpa* (alpha), *cpb* (beta), *cpb2* (β2), *tpcL* (TpeL), and *cpe* (enterotoxin), *netB* (NetB), *iA* (iota), *etx* (epsilon), respectively. Computed rarefaction curves with 95% confidence intervals were used to compare the genetic diversity of isolated *Clostridium perfringens* strains. Rarefaction curves are plots of the cumulative species richness as a function of the numbers of individuals sampled. A Simpson's diversity index which considers the number of genotypes (n) function of the total number of strains (N) was also calculated to compare genotypic diversity among treatments.

Results: We found higher numbers of isolates in barns submitted to the alternative rearing protocol. However, rarefaction curves showed that *Clostridium perfringens* species richness was not different

among farm protocols. Moreover, a balanced increasing diversity was noted for both rearing protocols between initial and final time points of the study. We also observed an increased prevalence of strains harboring more virulence genes at the end of the experiment, this situation being correlated with a decreased frequency in strains carrying a single virulence gene.

Conclusion: We thus conclude that *Clostridium perfringens* populations found in poultry barns are dynamics and that feed additives do not seem to play a major role in the temporal genotypic evolution of this bacterial species.