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Genetic diversity of *Clostridium perfringens* isolated from healthy broiler chickens at a commercial farm

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Abstract

Clostridium perfringens is an important commensal and bacterial pathogen of many animal species. It has particular significance in poultry, where it may cause necrotic enteritis. Our objective was to characterize the population diversity of *C. perfringens* colonizing healthy birds, and to observe how diversity changed over time. Isolates were obtained from broiler chicken cecal samples in two barns on a single farm, on days 7, 14, 22, 27, 30 and 34 of a single 42-day rearing cycle. Bacitracin was used as a feed additive in one of the barns and withdrawn from the second barn for the duration of the experiment. Each isolate was typed using pulsed-field gel electrophoresis (PFGE) using *Sma*I restriction endonuclease. A total of 205 cecal isolates from 49 birds were typed, as well as 93 isolates from the barn environment (bedding, drinking water and feces). Eight major PFGE types and 17 subtypes were found in the 298 total isolates. The results show that an optimal sampling strategy would involve a large number of birds, with only a few isolates sampled per bird. The diversity of *C. perfringens* in this study appears to be low within a single bird, and increases as the bird matures. There was no significant difference in genetic diversity between the two barns. In addition, isolates from fresh fecal samples appear to represent the cecal *C. perfringens* population accurately, although this was not proven statistically. Antimicrobial susceptibility testing was performed on selected isolates ($n = 41$) representing a cross-section of PFGE types. Based on minimum inhibitory concentration distributions, 95% of the isolates tested were deemed resistant to bacitracin, with a 16 μg/mL breakpoint. Three new *cpb2* (beta2 toxin gene) variants were found in the study.

Keywords: *Clostridium perfringens*; Pulsed-field gel electrophoresis; Chicken; Necrotic enteritis

1. Introduction

Clostridium perfringens is a common bacterium implicated in many enteric diseases of domestic animals (Songer, 1996). It can usually be isolated from soil and from the digestive tract of healthy animals, but

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is also known to cause disease through the production of a variety of toxins (Rood, 1998). The species is divided into mouse-lethal toxin types based on the bacteria's toxin production profile, A through E, with types A and C associated with poultry (for a genotypic summary of *C. perfringens*, see Petit et al., 1999). The bacterium causes lesion formation in the intestine of fowl, leading to a multi-factorial disease known as necrotic enteritis (Songer, 1996). The disease is usually controlled by the addition of antimicrobials in feed and has recently re-emerged in countries now banning antimicrobial growth promoters (Van Immerseel et al., 2004).

The genetic diversity of *C. perfringens* has not been studied extensively in healthy birds and there have been few publications comparing isolates from healthy and diseased broiler chickens (Engstrom et al., 2003; Nauerby et al., 2003; Gholamiandekhordi et al., 2005). Pulsed-field gel electrophoresis (PFGE) results showed that the genetic diversity of *C. perfringens* in healthy birds is generally high and much lower in cases of necrotic enteritis, where only one or two clones were recovered. These studies were carried out in Europe where there has been a 10-year ban on many antimicrobials used for growth promotion in poultry (Casewell et al., 2003; Grave et al., 2004). This is not the case in North America where antimicrobials are still commonly used for growth promotion and as preventive medication for necrotic enteritis (McEwen and Fedorka-Cray, 2002).

The objective of this study was to assess in detail the genetic diversity of *C. perfringens* isolated from broiler chickens on a single farm using a consistent sampling strategy, and also to follow the persistence of PFGE types or ''pulsotypes'' over a complete rearing cycle. The prevalence of bacitracin resistance in *C. perfringens* isolates was also assessed. Using statistical methods, implications for optimizing future sampling methods were also considered.

2. Materials and methods

2.1. Sampling strategy

Samples were obtained from male, Ross 308 breed broiler chickens on a farm in Southern Ontario during October and November of 2004; two adjacent barns of identical design (designated A and B) of the same farm were followed simultaneously. At delivery from the hatchery, 24,786 chickens were distributed randomly in each of the two barns and managed in an identical manner, with the only difference between the two barns being that chickens from Barn A were given feed supplemented with 55 ppm of bacitracin methylene disalicylate, and those from Barn B were given feed without bacitracin. Bacitracin had been used in the second barn previously however. Birds from both barns were given a coccidiostat, narasin (40 ppm) and nicarbazin (40 ppm) for the first 17 days, and salinomycin (60 ppm) for the remainder of the time. Birds were also vaccinated against infectious bronchitis virus. Feed was corn- and soybean-based, with wheat also included. Protein content for each feed type was as follows: 22% in starter feed (days 0–17), 20% in grower feed (18–31), 18% in finisher feed (32–38) and 16% in roaster finisher feed (39–42).

Each barn was physically divided into three equally sized sections during the experiment to avoid cross-over of the chickens between the three sampling areas. Two randomly selected broiler chickens were taken from each area in each barn and were sacrificed by cervical dislocation or carbon dioxide asphyxiation at each sampling date (procedure approved by the Animal Care Committee of the University of Guelph). The birds were immediately placed on ice and forwarded to a laboratory. Samples were taken six times over a rearing cycle of 42 days, at days 7, 14, 22, 27, 30 and 34. Cecal contents were removed aseptically and suspended in 5 mL Difco Brain Heart Infusion (BHI) base (Becton Dickinson Microbiology Systems, Sparks, MD) + 20% glycerol (Fisher Scientific, Fair Lawn, NJ) within 2–4 h after sacrifice and were immediately frozen at −70 °C. Barn environment samples were also obtained at the same times. These included pooled water samples aseptically taken from drinking nipples (50 mL per section in each barn), pooled bedding samples (wood shavings, 25 g per barn collected in each section of each barn) and pooled fresh fecal samples collected on plastic or paper foils spread on the ground of each barn section for approximately 30–60 min during the sampling visits.

Additionally, 20 isolates from cecal samples of a rearing cycle 2 months earlier in the same two barns were also isolated and typed using PFGE. Another 20