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Necrotic enteritis in chickens: A paradigm of enteric infection by *Clostridium perfringens* type A

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ABSTRACT

Withdrawal of antimicrobial growth promoters and ionophore coccidiostats has been accompanied by a resurgence in incidence of necrotic enteritis (NE), a severe *Clostridium perfringens*-induced disease which some consider the most clinically dramatic bacterial enteric disease of poultry. Lesions, in jejunum and ileum, are focal-to-confluent, often with a tightly adhered pseudomembrane, and hemorrhage is uncommon. The key risk factor for development of NE is an intestinal environment that favors growth of the organism. Birds on high energy, protein-rich, wheat- or barley-based diets experience NE at a rate up to ten times greater than do birds on maize-based diets. Specific strains of type A cause NE, although only a few specific virulence attributes are known. The role of alpha toxin (CPA) has been called into question by the finding that an engineered CPA mutant retained full virulence *in vivo*, although the counterpoint to this is the finding that immunization with CPA toxoids provides substantial protection against NE. A recently described toxin, NetB, seems likely to be involved in pathogenesis of infection by most NE strains. Immunization with CPA, NetB, or other proteins, delivered by conventional means or vectored by recombinant attenuated *Salmonella* vectors may help the industry deal with NE. Future progress may be based in large part on genomic and proteomic analyses.

1. Introduction

The poultry industry has grown dramatically over the past 40 years, transforming itself into a highly specialized field requiring substantial economic investment [1]. Improvements in housing and equipment have led to dramatic increases in stocking density, and genetic selection has resulted in more rapid growth, improved feed conversion, higher meat yields, and lower mortality rates. Advances in growth rates and feed efficiency partially offset the 65–70% of producers' total costs that are invested in feed. However, the difference between profit and loss is often determined by the incidence of the many infectious diseases that can affect poultry. One of these is necrotic enteritis (NE), which costs the worldwide poultry industry $2 billion annually [2]. Growth retardation and increased mortality significantly affect profitability of poultry production [3], and the peracute and acute forms of NE may be the most clinically dramatic bacterial infections of poultry [3].

2. Etiology and risk factors

Clostridium perfringens is the most important clostridial pathogen of poultry, causing a panoply of diseases which include avian malignant disease, gizzard erosions, and gangrenous dermatitis [4–7]. NE is a common and severe *C. perfringens*-induced disease, and some consider it the most clinically dramatic bacterial enteric disease of poultry [8]. Bennetts isolated *C. perfringens* (*Bacillus welchii*; *Clostridium welchii*) from intestinal lesions in a Black Orpington pullet in 1930, and attributed the bird's death to this infection [9–11]. The designation "six-day disease" appeared later, and was characterized by *C. perfringens* invasion of chick intestinal mucosa [12–14]; this condition was reproduced in day-old chicks by *per os* administration of *C. perfringens* [12,15–17]. "Necrotic enteritis" was first used in description of an intestinal disorder in young cockerels [8,9,18–20], and the disease was reproduced by administration of *C. perfringens per os*, with opium to abrogate gut motility [18,21,22].

C. perfringens is an anaerobic, spore-forming, large Gram-positive rod [3,23], which is motile by way of type IV pili [24]. It is a commensal in the intestinal tract of vertebrates, and is, thus, commonly isolated from environments contaminated by feces

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[3,23]. It is responsible for many diseases of humans and domestic and wild animals, and may be the most widely occurring pathogenic bacterium [23,25,26].

Genomic analysis has revealed that *C. perfringens* lacks the genetic machinery to produce 13 essential amino acids [27,28], and it obtains these *in vivo* via the action of exotoxins, some of which are enzymes [29]. It produces at least 17 toxic or potentially toxic exoproteins [23,29], and is classified into toxigenic types A–E based on production of four so-called major toxins (alpha, beta, epsilon, and iota) [23,25,26] (Table 1). NE is commonly caused by *C. perfringens* types A and C, although the latter is apparently uncommon [3,4,11,30–32].

C. perfringens enteric infection is sometimes exacerbated by co-infection with coccidia. Early NE outbreaks were associated specifically *Eimeria brunetti* and *Eimeria maxima* infection [9,33]. Intestinal mucosal damage by sporozoites and merozoites [12], together with reductions in intestinal pH [34,35] and increased transit time [33], allow establishment and proliferation of *C. perfringens* [34,36–38]. Numbers of *C. perfringens* in birds with acute coccidiosis are several logs higher than normal in small intestine and ceca. *C. perfringens* adheres at a higher rate to cecal mucosa of germ-free chicks infected with *Eimeria* than in non-infected birds [37]. The mortality rate in conventional birds infected with *C. perfringens* and *Eimeria* spp. is typically at least 25% higher than in *Eimeria*-free birds [39].

Key factors in experimental reproduction of NE, and major risk factors for development of natural disease, are inoculation with a virulent strain of *C. perfringens* and creation of an intestinal environment that favors anarchic growth of the organism [30,40–44]. Strains vary in virulence (Table 2), and NE has been reproduced with isolates from field cases of NE and cholangiohepatitis [20,42]. However, many isolates putatively from NE cases are, in fact, avirulent for chickens (KK Cooper and JG Songer, unpublished).

Preparation of the intestinal environment often includes creating minor enterocyte damage by such methods as challenge with *Eimeria* spp. [34,35] or simply immunizing with the commercial coccidial vaccine. Mortality rates and numbers of *C. perfringens* in the intestinal tract are higher in co-infected birds [35]. Another approach is manipulation of the diet. Feeds that are high in fishmeal [usually ~50% [45]] and deploy rapidly fermentable carbohydrates and/or proteins into the small intestine (usually based upon wheat or barley) are most effective [41,46]. Supplemental dietary zinc increases the attack rate in birds challenged with *Eimeria* and *C. perfringens* [40]. Exposure to *C. perfringens* can be by gavage [32,47,48], by offering feed mixed with broth cultures of *C. perfringens* [7,20,35,41,42,45], or by exposure to litter from infected flocks [7,40,49]. Results from various studies should be compared with severity of challenge methods and lesion scoring system in mind. The latter are extraordinarily variable (Table 3), but System 1 is the standard.

3. Disease and pathogenesis

Many signs of NE are common in poultry disease. Birds are depressed [3,32,50], reluctant to move [32,33], and have ruffled feathers [32,50]. They may be somnolent [35], diarrheic [50], inappetant, and anorexic [6,32], and dehydrated [8]. The course is often peracute, with death in 1–2 h [6,51]. Mortality rates range from 0 to 50% [18,48,52].

Gross lesions are usually restricted to jejunum and ileum [3,8,18,32,50,53], but can occur in duodenum and ceca [50]. Small intestine is usually distended with gas [3,32,50] and the wall is thin and friable [50,54]. The mucosa is characterized by a focal-to-confluent, often tightly adhered pseudomembrane, and hemorrhage is uncommon [32]. Microscopic examination reveals extensive villous necrosis [8,55], and cellular degeneration may reach the submucosa [32] or muscularis mucosa [9]. Coagulation necrosis is common at villous apices [32], with a line of demarcation between necrotic and viable tissue and an accumulation of mononuclear cells at the junction [8,18,35]. Necrotic villi, degenerated epithelial cells, and inflammatory cells trapped in fibrin comprise the diphtheritic membrane seen grossly [32,33,35]. Large, Gram-positive rods compatible with *C. perfringens* are associated with areas of necrosis [32], but do not invade viable cells [3].

C. perfringens gains access to portal circulation and biliary ducts, resulting in cholangiohepatitis [56], with pale, focal liver lesions [11,32,50]. Microscopically, there is extensive multifocal coagulative necrosis in liver [9] and bile ducts [57]. Gall bladder and extra-hepatic ducts are distended with inspissated material [58], and Gram-positive bacilli are found in necrotic areas [57]. Hepatitis, without bile duct and gall bladder involvement [11], is common [5].

Chickens without *C. perfringens* among the normal flora are uncommon [48,50,59], although published estimates of numbers are extraordinarily variable [50,60–62]. Intestines of NE-affected birds contain large numbers of *C. perfringens* [e.g., 10^6–10^8 CFU per g of mucosa [8,34]], although high numbers of *C. perfringens* in the intestinal tract are not sufficient to produce NE.

Table 1
Toxinotypes of *C. perfringens*.

Type	Major toxins			
	Alpha (CPA)	Beta (CPB)	Epsilon (ETX)	Iota (ITX)
A	X			
B	X	X	X	
C	X	X		
D	X		X	
E	X			X

Table 2
Virulence of selected *C. perfringens* strains in a necrotic enteritis model.

Strain number	Toxinotype	Source	Virulence for chickens
JGS4143	A	Poultry necrotic enteritis	+++
JGS5252	A	Poultry necrotic enteritis	++++
JGS1235	A	Poultry ulcerative enteritis, hepatitis	+
JGS1882	A	Porcine neonatal enteritis	–
JGS	C	Porcine neonatal hemorrhagic enteritis	–
JGS4142	A	Bovine neonatal hemorrhagic enteritis	–
JGS1473	A	Chicken normal flora	–

Table 3
Lesion scoring systems for necrotic enteritis.

Score	System 1[a]	System 2[b]	System 3[c]
0		No gross lesions	
1+	Thin-walled, friable intestine	Small lesions totaling ≤ 2.5 cm^2	<10 Lesions
2+	Focal necrosis, ulceration	Lesions covering > 2.5 cm^2	≥10 Focal lesions
3+	Large patches of necrosis	Severe lesions throughout intestine	≥ 1 lesion extending > mucosal circumference
4+	Severe/extensive necrosis – field cases	NA[d]	NA
5+	Birds dying with 4+ lesions	NA	NA

[a] [6,45,51,97].
[b] [111].
[c] [7].
[d] Not applicable.