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Responses of broiler chickens orally challenged with *Clostridium perfringens* isolated from field cases of necrotic enteritis

A.A. Olkowski [a,*], C. Wojnarowicz [b], M. Chirino-Trejo [c], M.D. Drew [a]

[a] *Department of Animal and Poultry Science, University of Saskatchewan, 6D34 Agriculture Building, 51 Campus Drive, Saskatoon, Sask., Canada S7N 5A8*
[b] *Prairie Diagnostic Services, University of Saskatchewan, Saskatoon, Sask., Canada S7N 5A8*
[c] *Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, Sask., Canada S7N 5A8*

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Abstract

The present study examines the responses of broiler chickens to oral administration of *Clostridium perfringens* freshly isolated from field cases of necrotic enteritis (NE). The challenge studies included long-term exposure and short-term exposure, factored in with dietary and management variables including high levels of dietary components such as fish meal, meat meal, abrupt change of feed, and fasting. In the long-term exposure trials, the birds were orally inoculated daily, with 1 ml (1.0 or 2×10^8 CFU/ml) of an overnight culture of *C. perfringens* for 7 days. Short-term exposure trials involved challenge with 1 ml (3×10^{10} CFU/ml) administered as a single dose. The responses of broilers to orally administered *C. perfingens* under laboratory controlled conditions are presented and discussed in the context of authentic field cases of necrotic enteritis.

None of the challenge trials produced overt clinical signs of NE and there were no mortalities associated with oral exposure to high doses of *C. perfringens*. However, many of the challenged birds showed distinctly pronounced pathological changes in the intestinal tissue. On gross examination the responses in birds challenged orally with *C. perfringens* could be placed into two categories: (1) no apparent pathological changes in the intestinal tissue and (2) sub-clinical inflammatory responses with focal, multi-focal, locally extensive, or disseminated distribution throughout various sections of duodenum, jejunum, ileum, and ceca. In birds that responded with intestinal lesions, hyperemia and occasional hemorrhages were the main gross changes. In some birds, the mucosa was covered with a brownish material, but typically, the mucosa was lined by yellow or greenish, loosely adherent material. Mild gross changes were seen in some control birds, but both qualitatively and quantitatively, the lesions were distinctly more pronounced in the challenged birds.

Upon histological examination, none of the experimentally exposed birds showed overt mucosal necrosis typical of field cases of NE, but typically the lamina propria was hyperemic and infiltrated with numerous inflammatory cells. Most significant changes were seen at the interface of the basal domain of enterocytes and lamina propria. Multifocally, these areas were extensively edematous, allowing for the substantial disturbance of the structural integrity between the lamina propria and the enterocytes.

The lesions observed in the present study were consistently reproduced in all of our challenge trials, hence these responses may signify newly emerging patterns of sub-clinical enteric disorders in contemporary strains of poultry. The pathological changes observed in broilers challenged orally with *C. perfringens* in the present study, differ significantly from those reported previously, and must be clearly differentiated from those described in cases of NE or ulcerative enteritis. Although no overt necrosis of the intestinal mucosa typical of field cases of NE were observed in the present study, the birds challenged with *C. perfringens* showed strong inflammatory reaction to the introduced pathogens. The distinct features of the microscopic lesions were changes involving apparently normal enterocytes at the interface of the basal domain of villar epithelia and lamina propria. Although the pathological changes in the intestinal tissues observed in our trials appear to be rather subtle when compared to field cases of NE, the nature of these lesions suggest a significant negative effect on the digestive physiology of intestinal mucosa.

* Corresponding author. Tel.: +1 306 966 5848 (office)/8477 (lab); fax: +1 306 966 6598/4151.
E-mail address: andrew.olkowski@usask.ca (A.A. Olkowski).

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1. Introduction

Necrotic enteritis (NE) is a disease observed with variable frequency in commercial poultry. The occurrence of necrotic lesions in the intestinal tract is associated with the proliferation of the anaerobic bacterium *Clostridium perfringens*, and historically, it has been commonly accepted that this pathogen is the causative factor of NE (Prescott et al., 1978; George et al., 1982; Hamdy et al., 1983; Stutz and Lawton, 1984; Elwinger et al., 1992, 1998; Ficken and Wages, 1997). However, bacteria associated with NE can also be detected in high numbers in the intestinal tract of apparently normal birds (Ficken and Wages, 1997; Engberg et al., 2002; Pedersen et al., 2003). Thus, *C. perfringens* apparently breaks Koch's postulate that the disease-causing organism should not be present in healthy individuals. There is a lingering question as to what are the mechanisms that lead to the development of necrotic lesions.

Historically, necrotic enteritis has always been perceived as a major threat to the poultry industry, and various aspects of this condition have been intensively studied for several decades (Prescott et al., 1978; George et al., 1982; Hamdy et al., 1983; Stutz and Lawton, 1984; Branton et al., 1987, 1997; Elwinger et al., 1992, 1998; Ficken and Wages, 1997; Kaldhusdal and Hofshagen, 1992; Riddell and Kong, 1992; Kaldhusdal and Skjerve, 1996; Ficken and Wages, 1997; Kaldhusdal et al., 1999; Engberg et al., 2002; Pedersen et al., 2003; Williams et al., 2003). Despite considerable research efforts, however, the pathogenesis of this condition is still poorly understood. A good deal of research effort has been devoted to characterizing factors that may promote proliferation of *C. perfringens*. Dietary components such as fish meal, wheat, and barley have been widely accepted as predisposing factors (Kaldhusdal and Hofshagen, 1992; Riddell and Kong, 1992; Kaldhusdal and Skjerve, 1996; Ficken and Wages, 1997). Engberg et al. (2002) observed that birds fed pelleted diets harbored fewer *C. perfringens* organisms in the caeca than those fed otherwise identical mash diet. Hence, even the physical form of feed has been implicated as a possible risk factor.

It is commonly accepted that natural outbreaks of NE are associated with proliferation of pathogens in the environment. One can argue that, a reasonable model of the disease should closely resemble the probable route of natural pathogen exposure, i.e., oral. However, consistent reproduction of the disease by oral inoculation with *C. perfringens* has not been achieved. Interestingly, numerous attempts to experimentally reproduce NE by oral inoculation in different labs has resulted in extremely variable results including substantial mortality, severe clinical signs observed in the majority of treated birds, sub-clinical NE seen in small number of exposed birds, and no lesions at all (Al-Sheikhly and Truscott, 1977a,b; Truscott and Al-Sheikhly, 1977; Kaldhusdal et al., 1999; Brennan et al., 2001, 2002, 2003; Pedersen et al., 2003). Such a significant lack of consistency in scientific data generated in different labs is puzzling indeed, particularly in view of a large number of claims identifying predisposing factors. However, many factors previously identified as predisposing poultry to NE are poorly defined, and results have been contradictory. There is insufficient information to enable a proper evaluation of the impact of putative risk factors under experimental conditions. Accordingly, the objectives of this work were to comprehensively evaluate the responses of broiler chickens to oral administration of *C. perfringens* freshly isolated from fulminant field cases of NE. However, rather than using lesion scoring system, we evaluated the lesions in the context of pathological changes observed in naturally occurring outbreaks of NE. Our experimental approach also included all variables that have been identified and could reasonably be presumed to be risk factors in commercial situations, including high levels of dietary components such as fish meal, meat meal, diet form (pelleted vs. mash), abrupt change of feed, and dietary restriction (fasting).

2. Materials and methods

2.1. General

The methodological approach taken in this work is based on a collection of materials obtained from routine diagnostic work submitted to Prairie Diagnostic Services, University of Saskatchewan, observations of commercial flocks, and experiments trials. The experimental work was focused on the laboratory reproduction of necrotic enteritis via oral administration of *C. perfingens* isolated from field cases of fulminant necrotic enteritis. All experimental protocols were approved by the Animal Care Committee and the procedures were performed in accordance with the requirements of the Guide to the Care and Use of Experimental Animals (Canadian Council On Animal Care, 1993).

2.2. Field studies

Field data collection included screening of commercial flocks for necrotic enteritis, and collection of necropsy material from birds showing signs of pathological changes in the gastrointestinal (GI) tract. The field cases diagnosed with fulminant necrotic enteritis were used for microbiological evaluation and pathogen isolation.