

Studies on Pathogenesis and Immunity to Turkey Clostridial Dermatitis

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Introduction

Over the last few years, Clostridial Dermatitis/ Cellulitis have become more prevalent in commercial turkeys. A number of agents have been isolated from Clostridial Dermatitis lesions in turkeys. Most often the bacterial agents isolated happen to belong to the Clostridial group of organisms (1, 2). It is well known that Clostridial organisms do cause subcutaneous lesions similar to Clostridial Dermatitis in mammals. There is very little information as to the exact and to what extent these Clostridial organisms play a role in Clostridial Dermatitis in turkeys. Understanding the role of these organisms in producing Clostridial Dermatitis is very important. With this information, we can develop control measures for Clostridial Dermatitis. *Clostridium septicum* seems to be more potent in causing Clostridial Dermatitis lesions and higher mortality in turkeys, although the role of *C. perfringens* in causing fatal Clostridial Dermatitis cannot be ignored (3, 4). The objectives of our study were to develop a Disease model with *Clostridium perfringens/C. septicum* for Clostridial Dermatitis in turkeys and to expand on understanding the toxic principles (protein) of *Clostridium perfringens/C. septicum* that may be responsible for Clostridial Dermatitis. This will provide the basis of pathogenicity of *Clostridial* isolates and will help us to identify an effective vaccine candidate for the control of Clostridial Dermatitis.

Materials and Methods.

I. Development of disease models for Clostridial dermatitis in turkeys

In the disease model development we had the following experiments.

We immuno-suppressed the birds either with a chemical Dexamethasone or by prior exposure to Haemorrhagic enteritis (HE) virus. Another experiment was to expose birds to a turkey specific coccidia first and then expose to Clostridial organisms to see the development of clostridial dermatitis. Later birds in all the experiments were exposed orally or by subcutaneous route to *C perfringens and/or C. septicum* to examine the development of Clostridial Dermatitis.

The Clostridium organisms isolated from field cases of turkey Clostridial Dermatitis were screened and one strain each of *Clostridium perfringens and Clostridium septicum* which produced maximum lesions in our previous studies was selected to challenge the birds. Turkey poults of 10-weeks of age were reared at RAR animal facilities at University of Minnesota. From 54 turkey poults, a group of twenty four birds were injected with 2 mg/kg of Dexamethasone (Dex) on days 1, 3 and 7. On the last day of Dex injection, two subgroups of 6 birds each from Dex injected group were infected by oral gavage with a highly potent *C. perfringens* originally isolated from Clostridial Dermatitis cases in turkeys at a dose rate of 1×10^9 cfu/ml. Another two subgroups of 6 birds each were infected with an oral gavage of *C. septicum* at a dose

rate of 10×10^9 cfu/ml.

Another group of twenty four birds with no Dex administration were also exposed to *C. perfringens* or *C. septicum* in the same way. A subgroup of 6 birds was served as non infected non-challenged controls. All the birds were monitored daily for three weeks to study the influence of chemical immunosuppression on the development of Clostridial Dermatitis and mortality. Blood was collected on days 0, 7, 14 and 28 days post administration of Clostridia for ELISA. The dead birds were necropsied and the gross lesions were recorded and the affected tissues were subjected to histopathology.

In one other experiment, birds exposed and non-exposed to *C. perfringens* and/or *C. septicum* were given turkey coccidia to investigate the development of Clostridial dermatitis. We used twelve-week old turkey poults. We exposed turkeys to high infective doses of coccidial oocysts orally.

II. Characterizing the toxic principles of Clostridium perfringens and C. septicum

The second objective of characterizing the toxic principles of Clostridium perfringens and *C. septicum* that are responsible for Clostridial Dermatitis is completed. Purified protein spots from isolates of *C. perfringens* and *C. septicum* isolated from clinical cases of Clostridial dermatitis in turkeys were used for MS analysis. Primary isolation of *C. perfringens* and *C. septicum* were made in cooked meat medium (Remel®) and grown in BHI or TGY media under anaerobic condition using AnaeroPack System for 24 h at 37C. The colonies were identified by biochemical methods using API 20A and were confirmed by PCR.

We standardized the protocols for DiGE and MALDI TOF- MS analysis with the culture supernatant of *C. perfringens* and *C. septicum* isolates obtained from Clostridial dermatitis cases to identify their secretory toxins with extremely high resolution. Briefly, One hundred microgram of protein sample were subjected to Immobilized pH gradient (IPG) Isoelectric focusing (IEF) in the 1st Dimension. This separated proteins by their charge (pI). The resulting gel was Deep-purple stained for viewing the protein spots. All gels were scanned using a GS-800 calibrated densitometer from which we obtained images (Figure 1,2). .

Results:

I. Development of disease models for Clostridial dermatitis in turkeys

In studies with birds infected with *C. perfringens* or *C. septicum* orally and immunosuppressed with Dexamethasone, all but one bird from the immuno-suppressed group which were challenged S/C with a low dose of *C. perfringens* and *C. septicum* developed Clostridial Dermatitis lesions and died within 12 hours. The birds which were not given Dexamethasone but challenged with a low dose of *C. perfringens* and *C. septicum* showed no signs of Clostridial Dermatitis or mortality in both *C. perfringens* and *C. septicum* inoculated group. The birds either immuno-suppressed with dexamethasone or not and orally challenged with a low dose of *C. perfringens* or *C. septicum* did not develop any Clostridial Dermatitis lesions.

With higher dose of inoculum, only one bird died in CS oral gavaged group. But no Clostridial Dermatitis lesions were noticed. On necropsy, liver showed extensive

necrotic foci, intestines showed hemorrhages and had necrotic areas. Spleen was not found enlarged. However, with the higher dose all the birds died of Clostridial Dermatitis in both CP and CS subcutaneous inoculated groups.

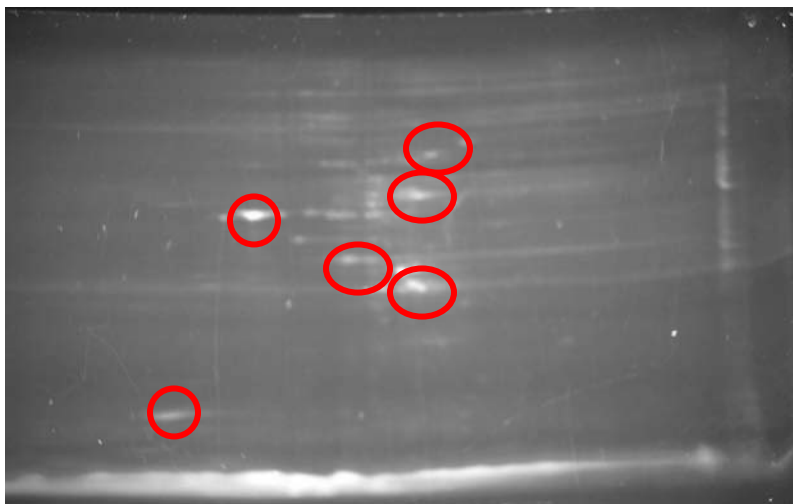
In summary, immuno-suppressed birds were found to be more susceptible to Clostridial Dermatitis through subcutaneous challenge only. Where as oral inoculation was found to be ineffective in causing Clostridial Dermatitis.

The following was the observation in the experiment, where birds exposed and non-exposed to *C. perfringens* and/or *C. septicum* were later given turkey coccidia. Birds exposed to *C. perfringens* orally alone did not show any clinical signs whereas birds exposed to *C. perfringens* and coccidia showed signs of diarrhea, necrotic enteritis and Clostridial Dermatitis. However, birds exposed to *C. septicum* orally with or without coccidia showed no signs of enteritis or Clostridial Dermatitis. In addition, birds exposed to Clostridium perfringens and Clostridium septicum through subcutaneous route showed highest incidence of Clostridial Dermatitis. Our results support more of a subcutaneous route of infection than oral route of infection for Clostridial Dermatitis in turkeys.

II. Characterizing the toxic principles of Clostridium perfringens and C. septicum

The second objective of characterizing the toxic principles of *Clostridium perfringens* and *C. septicum* that is responsible for Clostridial Dermatitis. Purified protein spots from isolates of *C. perfringens* and *C. septicum* isolated from clinical cases of Clostridial dermatitis in turkeys were used for MS analysis.

Figure 1: showing unique secretory proteins of Clostridia subjected for mass spectrometry analysis.



The secreted and whole-cell protein profiles of the strains of *C. perfringens* and *C. septicum* from Clostridial dermatitis turkeys were screened for unique proteins. To identify the role of these toxins in the immunity to Clostridial dermatitis, we conducted Western blot with convalescent serum. The protein profiles of *C. septicum* isolates were transferred to a PVDF membrane from SDS gels. Protein spots were spot-picked; trypsin digested and then identified using mass spectrometry (MALDITOF MS) analysis at the University of Minnesota, Center for Mass Spectrometry and Proteomics. Some of the unique proteins we identified were lecithinases, hyaluronidase, DNase K, chaperone proteins and hypothetical proteins. Knowing this helps us to refine the composition with the right vaccine candidate for a better protection than what we have demonstrated hitherto.

The major secretory toxins in *C. septicum* isolates we identified were alpha toxin, septicolysin, sialidase, Dnase, flagellin and Gelson precursor (actin depolymerizing factor). The Gelson precursor (actin depolymerizing factor) might have some role in the pathogenesis of Clostridial Dermatitis, but more studies are warranted to confirm this assertion. Since *C. septicum* genome is not completely sequenced, not much information is available for the development of a recombinant vaccine at this point. Unlike *C. perfringens* we observed only one type of proteomic profile for *C. septicum* isolates. These results support our findings in the MLST analysis as well as previous reports (Neumann et al., 2010) that genomes of *C. septicum* poultry isolates are highly conserved.

Figure 2. Two distinct proteomic profiles of *C. perfringens* UMNCP01 and UMNCP 06 isolates after 2-DiGE.

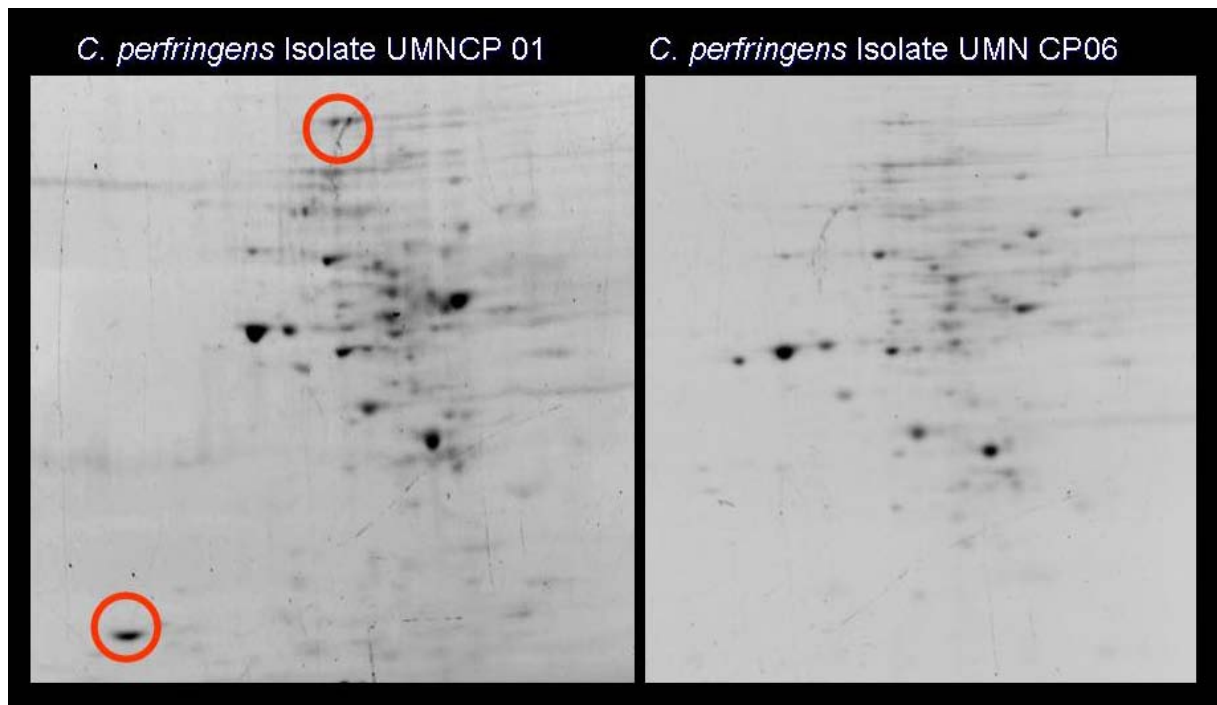
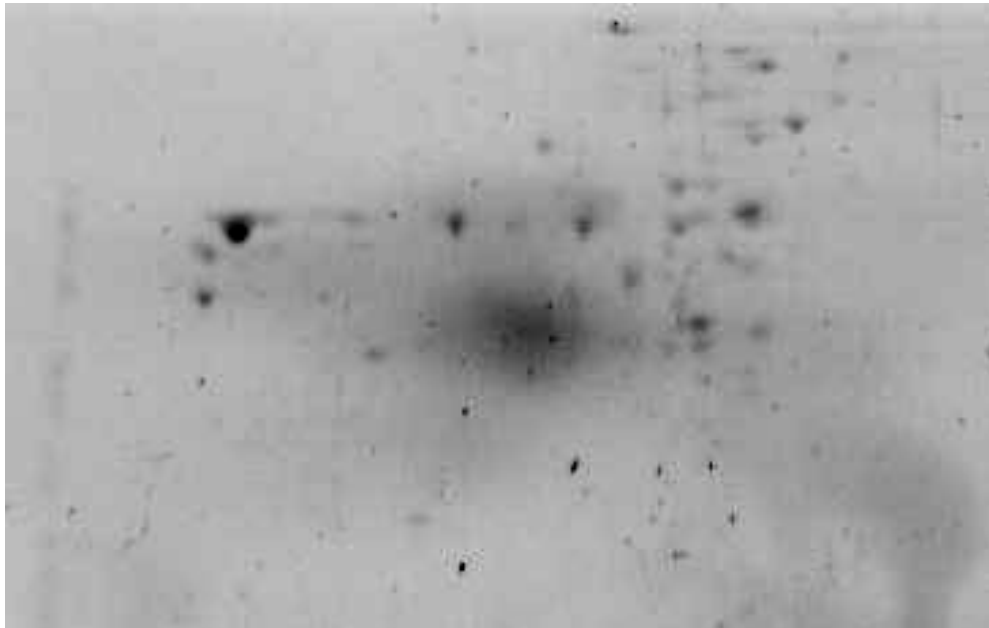


Figure 3. Proteomic profile of *C. septicum* after 2-DiGE



Discussion

Dexamethasone (Dex) is a gluco-corticoid known to induce cell mediated immunosuppression and lower resistance to infection in various animal species including turkeys (5, 6). Dex at a dose rate of 2mg/kg was found to cause osteomyelitis in turkeys when challenged with *E. coli* (5). The results from our study showed that turkeys become susceptible to Clostridial Dermatitis when immuno-suppressed. However, development of Clostridial Dermatitis lesions was minimal.

Coccidial infection is very common in turkeys. A breach in the integrity of the intestinal tract is attributed as the reason for the increased colonization, severe infection and mortality caused by *C. perfringens* in these birds. In our studies, *C. perfringens* and *C. septicum* does not appear to cross the intestinal barrier in turkeys following a breach caused by coccidial infection to cause Clostridial Dermatitis in turkeys. Our findings were contrary to earlier findings where, a breach in the integrity of the intestinal tract is attributed as the reason for the increased colonization, severe infection and mortality caused by *C. perfringens* in chickens (7).

Clostridium perfringens isolate UMNCP01 that appeared most potent differed in their secretory protein profile from less potent isolate like UMNCP06. The role of hypothetical protein 1232 needs to be further investigated. The functional annotation of this protein is suggestive of a protease. It has been reported that *C. perfringens* produces extracellular toxins including beta2 toxin, enterotoxin, perfringolysin, collagenase, lambda toxin, hyaluronidase, dnase, neuraminidase and urease. Our results also suggest involvement of different toxins of *C. perfringens* in pathogenesis of

Clostridial Dermatitis in turkeys.

The proteomic profiles of all *C. septicum* isolates appeared identical. The major secretory toxins in *C. septicum* isolates we identified were alpha toxin, septicolysin, sialidase, Dnase, flagellin and Gelson precursor (actin depolymerizing factor). The Gelson precursor (actin depolymerizing factor) might have some role in the pathogenesis of Clostridial Dermatitis, but more studies are warranted to confirm this assertion. Since *C. septicum* genome is not completely sequenced, not much information is available for the development of a recombinant vaccine at this point. Unlike *C. perfringens* we observed only one type of proteomic profile for *C. septicum* isolates. These results support our findings in the MLST analysis as well as previous reports (8) that genomes of *C. septicum* poultry isolates are highly conserved.

The results of the study enabled us to understand the pathogenesis of Clostridial Dermatitis in depth. Our Clostridial Dermatitis disease model offer promise to use it as a challenge model in the development of vaccines against Clostridial Dermatitis in turkeys. The findings also helped us in refining the composition of the inactivated vaccine for better protection against Clostridial Dermatitis in turkeys.

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