Descriptive Epidemiology of Turkey Cellulitis in Minnesota and Wisconsin Turkey Flocks

Patricia D Bedford, Scott J. Wells, Morgan Hennessy, Simone Oliveira, Matheus Costa, Clint Been, and Rob Porter, College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108

Acknowledgements: The authors acknowledge funding for this study from the Minnesota Turkey Research and Promotion Council, the Midwest Poultry Council, and the Center for Animal Health and Food Safety, University of Minnesota.

Introduction

Characterizing the descriptive epidemiology of turkey cellulitis was stimulated by a 2007 national veterinary survey of current health issues facing the U.S. turkey industry, which listed “cellulitis” as a significant concern. In fact, cellulitis has been diagnosed in all geographic regions of the U.S. and is now ranked as the third most important issue facing the turkey industry.

The clinical signs attributed to cellulitis are inconsistent. Some birds present with either fluid filled blisters associated with broken feather follicles around the tail base and/or with vesicles on the skin, usually along the thighs and breast. At this time, the etiological agent associated with the development of cellulitis in turkeys is unknown. The effectiveness of antibiotic treatment suggests that bacterial agents are important components in the pathogenesis of cellulitis. Clostridia, including C. perfringens and C. septicum, are frequently isolated from field cases; however, little is known regarding the virulence factors and clonality of these isolates. Identification of the etiological agent associated with the development of turkey cellulitis, along with the characterization of potential virulence factors, is critical for the development of control strategies.

Turkey cellulitis has been reported to disproportionately affect market-age (16-18 weeks) tom turkeys, however, occurrence has been confirmed at younger ages (as early as 7 weeks) in both toms and hens and the prevalence and severity of cellulitis continues to increase. Since cellulitis affects predominantly heavy market-age tom turkeys, the economic consequences of mortality in this group are particularly hard-hitting. Affected birds are often the best physical specimens and have had few indications of disease prior to developing cellulitis. A typical case may involve the death of 100 such market-age specimens at a cost/bird of $16-18. When long-term antimicrobial therapy is initiated to control this mortality, costs quickly escalate. Overall costs of treatment and bird loss may be as high as $16,000-$18,000 per affected flock.

The objective of this study is to characterize the distribution of turkey cellulitis by time, clinical signs and bacterial isolated. Informed gained from this project will be used to
refine the current case definition for turkey cellulitis, which will improve the identification of affected herds and the communication within the turkey industry.

Materials and Methods

Retrospective Study
Turkey grower data was obtained from a large Midwest poultry company with operations in Wisconsin and Minnesota. Production data for the years 2004 – 2007 was obtained, including 4,386 flocks distributed over 91 grower farms. Production parameters (total daily mortality and daily mortality due to cellulitis) for all of the flocks on a farm were averaged for that farm. This produced a single measurement of each production parameter for each farm. Mortality of birds from 11 weeks of age to market was calculated as a proxy for cellulitis on the grower farm. The farms with the lowest 11 week to market mortality (19 farms, bottom 21%), and the highest 11 week to market mortality (22 farms, top 24%) were then selected for further analysis. The 19 farms with the lowest average 11 week to market mortality were selected and ranked based on ascending medication cost per head. The 22 farms with the highest average 11 week to market mortality were selected and ranked based on descending medication cost per head.

Ten farms from the high group and the low group were selected for final inclusion in the study. Only farms which raised toms were included in the final selection. Other reasons for excluding farms included; farms no longer in use, farms not in the study area, and farms in which flocks moved through multiple barns. The ten farms with the highest medication cost per head among those with the highest 11 week to market mortality were classified as farms with high expected incidence of cellulitis for further study. The ten farms with the lowest medication cost per head among those with the lowest 11 week to market mortality were classified as farms with low expected incidence of cellulitis for further study. Comparisons of the mean values from the high expected and low expected incidence of cellulitis farms were performed means and 95% confidence intervals.

Prospective Study
Based on the categorization of farms in the retrospective data analysis, one flock from each of the ten farms with high expected incidence and one flock from each of the ten farms with low expected incidence of cellulitis were followed from placement (6-7 weeks of age) until market age. Information was collected from each flock in a weekly farm manager report including the total daily mortality and the daily mortality due to suspected cellulitis.

A draft case definition for turkey cellulitis was developed by our team in collaboration with experts in the turkey industry (Oct 30, 2007 Conference call of the National Turkey Federation Turkey Health and Welfare Committee’s Turkey Cellulitis Working Group). This case definition was refined for use in this study.
The manager’s report also noted when medication was started and the daily dose administered. If mortality reached a point of ≥ 0.5 dead/1,000-birds fitting the individual bird definition for two consecutive 24-hour periods, the flock veterinarian examined up to a maximum of 10 dead birds with clinical signs of cellulitis and signs were recorded. If mortality did not reach this cut point, up to a maximum of 10 random healthy birds were sacrificed at 8, 16, and 20 weeks of age and their clinical signs recorded. The prospective monitoring of study flocks included the training of farm managers to collect weekly information, as well as production veterinarians to monitor disease, collect biologic samples, and to handle and ship samples for laboratory testing. As part of the characterization, starting at 6 weeks of age, up to 20 dead turkeys were examined for signs of cellulitis once every two weeks. Specific clinical signs noted included subcutaneous emphysema; “feel crepitus under the skin”, moist, dark, wrinkled skin, especially breast/inguinal area, vesicles on the skin, especially on the breast/inguinal area, vesicles on the skin, on the tail area and moist, dark, wrinkled skin, on the tail area.

If signs of cellulitis were observed at the time of weekly examination or any time signs were noticed, clinically affected or recently found dead birds along with an age-matched healthy bird (up to a maximum of 10 affected birds and one healthy bird/site/week) were sacrificed and submitted to the Minnesota Veterinary Diagnostic Laboratory for diagnostic testing and histopathology. Samples from cellulitis lesions and internal organs (liver and spleen) were collected. Eligible samples included the following: (1) Whole birds submitted for necropsy to the MVDL; (2) Anaerobic swabs collected aseptically from cellulitis lesions (3) Fresh muscle and liver samples (2 square inches each), placed in separate plastic bags; (4) Fixed muscle, liver, and spleen samples (1 square inch each, thin cut) placed in a single container/bird with 10% buffered formalin. In addition, three randomly selected healthy birds were submitted from each study flock at 6, 8, 16, and 20 weeks of age, if no dead birds were submitted for diagnostic testing. This allowed for testing in each flock regardless of clinical signs, in order to characterize the presence or absence of specific pathogens (specifically including *Clostridium* sp.) on farms.

*Clostridium* sp. isolates obtained from clinical samples were further identified to the species level by 16S rRNA sequencing. *Clostridium perfringens* and *C. septicum* isolates were tested for the presence of toxin genes by PCR and were genotyped by multilocus sequence typing (MLST). Presence and quantity of *C. perfringens* and *C. septicum* in fecal and litter samples collected throughout the study were evaluated using real-time PCR.

The incidences of bird mortality and mortality of birds with cellulitis per flock were calculated by age of the bird. Clinical signs, using the case definition, were noted by the production veterinarians when birds with cellulitis and those that were healthy were examined.

**Results**
Retrospective study
On the ten high-expected farms, there were a total of 1074 flocks, with an average of 107 flocks per farm. The 10 farms in the low expected group had a total of 160 flocks with an average of 16 flocks per farm. This difference in mean number of flocks per farm was significantly different (95% CI: 50-132). Flocks from the high expected incidence group had an average live weight of 1.83 pounds (95% CI=2.26, 1.41) less than farms from the low expected group. Flocks from the high expected farms had an average 4% higher 11 week to market mortality, and a final livability 3% lower (95% CI=2%,4%). The difference between medication costs per head of the two groups was also found to be statistically significant (P < 0.01) with the low expected group having a medication cost per head, which was 54% of the all flocks average.

Prospective study
Of the 20 farms selected for study, complete data was received from 15 farms including 18 flocks. Of these 18 flocks, nine were from high-expected incidence farms, and nine were from low expected incidence farms. Twelve of these flocks had cases of cellulitis reported by farm managers, with cumulative incidences ranging from 0.02% to 8.7% of placed birds. Eight of the nine expected high incidence flocks had cases of cellulitis, with cumulative incidences ranging from 0.4% to 8.7% of placed birds. Four of the nine low expected incidence flocks had cases of cellulitis, with cumulative incidences ranging from 0.02% to 1.8% of placed birds. Among the flocks experiencing cellulitis, cases started appearing in the flocks during the 9th week of age with a peak in mean mortality occurring during the 16th week of age (Figure 1). Cases continued to occur after treatment in the flocks, with mean incidence continuing to increase up until time of marketing of birds.

Subcutaneous emphysema was found to be the most common clinical sign in cellulitis birds (68% of birds with cellulitis, Table 1). Other signs associated with cellulitis were serosanguineous subcutaneous fluid and moist, dark wrinkled skin especially in the breast and inguinal area.

Clostridium septicum was frequently isolated (84.1%) from cellulitis birds, followed by C. perfringens type A (15 %), and C. limosum (0.9 %). Toxin testing revealed that all C. septicum and C. perfringens isolates recovered from cellulitis birds carried the alpha toxin gene. Clostridium perfringens and C. septicum were more likely to be detected in high numbers in fecal samples from cellulitis birds compared to healthy ones. Clostridium perfringens isolates characterized by MLST were highly diverse with 16 sequence types being identified among 18 isolates typed. Clostridium septicum isolates recovered from this study were highly clonal with only 13 sequence types identified among 87 isolates typed by MLST. Two of the C. septicum sequence types identified in this study accounted for 65% of the cellulitis isolates typed. One sequence type (one single strain) was exclusively isolated from flocks classified as ‘high risk’ for the development of cellulitis.

Discussion
While turkey cellulitis has been reported to affect birds as young as 7 weeks old,\textsuperscript{2} this study monitored turkey flocks beginning at 6 weeks of age and no birds under 9 weeks of age were detected with cellulitis. A peak prevalence of the disease occurred around 16 weeks of age, followed by a brief decline perhaps due to treatment with antibiotics, which was followed by an increase in cellulitis incidence until time of marketing.

In summary, study results indicated that turkey cellulitis is commonly observed in turkey flocks with up to nearly 9\% of placed birds affected. In affected flocks, turkey cellulitis is the most frequent cause of mortality in older birds. Clinical signs most frequently observed in turkeys with cellulitis were subcutaneous emphysema, serum or serosanguineous subcutaneous fluid, and moist dark wrinkled skin especially in the breast or inguinal area. \textit{Clostridium septicum} was identified as the main agent associated with the development of turkey cellulitis. This pathogen was found to be highly clonal and carry the alpha-toxin gene. One single strain was associated with high risk for development of cellulitis. The features that differentiate this specific strain from strains isolated from low risk flocks will be the subject of future studies in our laboratory.

References

4. Olkowski AA, Kumor, L et al Cellulitis in commercial turkeys identified during processing. The Veterinary Record. 1999 August 21; 228-229.
Table 1: Percentage of suspected cellulitis turkeys on farms with specific clinical signs observed on necropsy by production veterinarians

<table>
<thead>
<tr>
<th>Clinical signs</th>
<th>% of birds affected by clinical signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcutaneous emphysema</td>
<td>68%</td>
</tr>
<tr>
<td>Serum/serosanguineous subcutaneous fluid</td>
<td>65%</td>
</tr>
<tr>
<td>Vesicles on the breast/inguinal area skin</td>
<td>20%</td>
</tr>
<tr>
<td>Moist, dark, wrinkled skin, especially breast/inguinal area</td>
<td>53%</td>
</tr>
<tr>
<td>Vesicles on tail skin</td>
<td>16%</td>
</tr>
<tr>
<td>Moist, dark, wrinkled tail skin</td>
<td>3%</td>
</tr>
<tr>
<td>Organ involvement (spleen/liver)</td>
<td>32%</td>
</tr>
</tbody>
</table>

Fig. 1: Mean incidence of cellulitis and total mortality among turkey flocks with cellulitis