

United States Department of Agriculture

#### Animal and Plant Health Inspection Service

Veterinary Services

National Animal Health Monitoring System

June 2012



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## Poultry 2010

Clostridial Dermatitis on U.S. Turkey-Grower Farms



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#### #645.0612

Cover photograph courtesy of Keith Weller.

#### Suggested bibliographic citation for this report:

USDA. 2012. Poultry 2010, Clostridial Dermatitis on U.S. Turkey-Grower Farms USDA–APHIS–VS–CEAH–NAHMS. Fort Collins, CO #645.0612

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#### Introduction

Clostridial dermatitis (cellulitis/gangrenous dermatitis) is a disease of turkeys and broilers and has increased in severity over the last several years. In 2010, the U.S. Animal Health Association (USAHA) ranked clostridial dermatitis among the top three disease issues in turkeys (USAHA, 2010). Dermatitis can be associated with *Staphylococcus aureus* or *Escherichia coli*; however, recently *Clostridium* spp. have been associated specifically with clostridial dermatitis. Skin lesions associated with clostridial dermatitis appear necrotic, with edema and/or gas in the underlying subcutaneous tissue. Sudden death is a frequent presentation of this illness (Clark et al., 2010).

Disease pathogenesis for clostridial dermatitis is poorly understood. The disease is believed to be caused by hematogenous transmission of clostridium from the gastrointestinal tract to muscle and skin where bacterial toxins are produced. Previous studies have suggested that bird density, litter conditions, and hygiene are potential risk factors for disease (Clark et al., 2010).

Clostridia are commonly found in the environment and intestines of healthy birds. Controlling clostridial dermatitis is difficult because managing the growth of clostridia is challenging, especially during the birds' growth phase. Uncontrolled growth of clostridia can result in toxin production, which causes disease. The basic components for controlling clostridial dermatitis include

- Reducing the number of clostridial microorganisms in birds and the environment, and
- Augmenting bird immunity (Clark et al., 2010).

The U.S. Department of Agriculture's National Animal Health Monitoring System (NAHMS) conducted the Poultry 2010 study. One objective of the study was to estimate the prevalence of and investigate the risk factors associated with clostridial dermatitis on turkey-grower farms. The clostridial dermatitis component of the NAHMS Poultry 2010 study consisted of three phases:

**1. Prevalence estimate:** This phase focused on the Nation's largest turkey companies; 15 turkey companies were selected to participate. The selected companies represented 76.8 percent of turkeys slaughtered in 2009 in the United States (Watt, 2010). Data regarding the occurrence of clostridial dermatitis on turkey-grower farms were collected from May 27 through October 16, 2010, via questionnaire. Twelve of the 15 selected companies (80 percent) participated in this phase.

**2. Case-control:** Companies that participated in the prevalence phase and had farms located in the Central and East regions were eligible to participate in the case-control phase. The West region was excluded because there were no cases of

clostridial dermatitis in the region. Participating case and control farms were chosen by the companies based on the following definitions provided by NAHMS:

- **Case farm**—farm in which at least two-thirds of flocks were affected with clostridial dermatitis during the previous 12 months.
- Affected flock—flock in which mortality due to clostridial dermatitis was greater than 0.5 birds per 1,000 birds for 2 consecutive days.
- **Control farm**—farm with little or no problem with clostridial dermatitis during the previous 12 months.

A questionnaire was completed for each selected farm. Data collection from 71 farms occurred from June 3 through December 17, 2010.

**3. Pathology:** The objective of this phase was to gain a better understanding of the role of intestinal pathology in the pathogenesis of clostridial dermatitis. Seven companies participated in this phase of the study. Nineteen case farms were visited weekly during the weeks leading up to an anticipated outbreak. Six control farms were visited weekly during the same time period. Anticipated timing of an outbreak was based on the farm's previous history. Three birds per week were euthanized and intestinal samples were collected for anaerobic culture and histopathology. Liver, spleen, muscle, litter, and beetle samples were also collected for anaerobic culture. All biologic samples were collected from May 27 through October 16, 2010.

For further details on study methodology, see Section II: Methodology (p 34).

#### **Items of Note**

The clostridial dermatitis component of the NAHMS Poultry 2010 study had three phases and was conducted to estimate the prevalence of clostridial dermatitis on turkey-grower farms in the United States, identify risk factors for clostridial dermatitis outbreaks on turkey-grower farms, and to better understand the relationship between intestinal pathology, shedding of clostridial organisms, and disease occurrence.

#### Prevalence estimate

Overall, 42.3 percent of turkey-grower farms had at least a slight problem with clostridial dermatitis during the 12 months prior to the study. No turkey-grower farms in the West region had clostridial dermatitis problems. In the Central region 48.4 percent of farms had some degree of disease, and 17.6 percent had severe disease.

#### Case-control

Companies that participated in the prevalence phase of the study and had farms located in the Central and East regions selected farms for participation in the case-control phase.

Case farms were more severely affected by clostridial dermatitis during summer and fall (8 of 10 had moderate or severe problems) than during spring or winter (6 of 10 farms had moderate or severe problems). Case farms were more likely than control farms to compost litter within 200 feet of a poultry barn (33 and 6 percent, respectively) and less likely to require employees to scrub footwear (23 and 72 percent, respectively). In addition, case farms had significantly higher humidity levels in the poultry barns than control farms and higher soil pH.

Clostridial dermatitis tends to be a disease of late finisher birds. However, disease problems appeared to be more severe in flocks in which disease onset occurred when birds were less than 13 weeks of age compared with flocks with older birds. A lower percentage of flocks on case farms were moderately or severely affected when birds' water was acidified, or when direct-fed microbials were administered.

#### Pathology

Seven companies participated in this phase of the study. Nineteen case farms were visited weekly during the weeks leading up to an anticipated outbreak. Six control farms were visited weekly during the same period. Samples were collected for culture and histopathology. Sixteen case farms had clostridial dermatitis outbreaks, while 3 case farms and all 6 control farms had no outbreaks during the study.

Prior to an outbreak, birds on outbreak farms and nonoutbreak farms had similar prevalence of *C. septicum* (1 percent of birds). About half the birds (42 percent) were positive for *C. septicum* during an outbreak. Approximately 20 percent of birds were positive for *C. perfringens*, regardless of outbreak status.

*C. septicum* was found in liver and spleen samples, which is consistent with the theory of hematogenous spread to muscle. *C. perfringens* was found less commonly in internal organs.

Over half the birds sampled during an outbreak had gross lesions consistent with clostridial dermatitis in the skin/subcutaneous tissue/muscle, but lesions were rarely seen before an outbreak. Intestinal pathology was frequently found, especially in the lamina propria and muscle of the ileum and Meckel's diverticulum, on both outbreak and nonoutbreak farms. Intestinal pathology did not appear to be related to outbreak status.

Two-thirds of *C. septicum*-positive birds (67 percent) had gross lesions consistent with clostridial dermatitis compared with less than one-tenth of *C. septicum*-negative birds. There was no substantial difference in the percentage of *C. perfringens*-positive birds and *C. perfringens*-negative birds with lesions. *C. septicum*- and *C. perfringens*-positive birds had no more intestinal pathology than *C. septicum*- and *C. perfringens*-negative birds.

*C. septicum* was found on 22 percent of nonoutbreak farms and on 87 percent of outbreak farms. *C. perfringens* was found on more than 80 percent of farms, regardless of outbreak status.

#### **Terms Used in This Report**

**Affected turkey:** Lesions present consistent with clostridial dermatitis (skin/dermal vesicles or accumulation of gelatinous fluid or gas under the skin, particularly along the thighs and breast).

**Direct-fed microbial/probiotic:** A source of live (viable) naturally occurring microorganisms administered to give a health benefit.

Farm: A premises with one or more poultry barns under common management.

**Case farm:** Farm in which at least two-thirds of flocks were affected with clostridial dermatitis within the previous 12 months.

**Control farm:** Farm with little or no clostridial dermatitis problem during the previous 12 months.

Flock: A group of birds kept in one barn and managed as a unit.

**Affected flock:** A flock with affected turkeys and mortality due to clostridial dermatitis greater than 0.5 per 1,000 for 2 consecutive days.

**Ionophore:** An organic compound that transports specific ions across cell membranes. Ionophores are used for the prevention and control of coccidiosis in poultry.

**Odds ratio:** The likelihood or odds of a farm with a certain characteristic being a case farm compared with farms lacking that characteristic. The 95-percent confidence interval for odds ratio is the range within which one can be 95 percent certain that the true odds ratio falls.



**Population estimates:** Population estimates in this report are provided with a measure of precision called the standard error. A 95-percent confidence interval can be created with bounds equal to the estimate plus or minus two standard errors. If the only error is sampling error, the confidence intervals created in this manner will contain the true population mean 95 out of 100 times. In the example to the left, an estimate of 7.5 with a standard error of 1.0 results in limits of 5.5 to 9.5 (two times the standard error above and below the estimate). The second estimate of 3.4 shows a standard error of 0.3 and results in limits of 2.8 and 4.0. Alternatively, the 90-percent confidence interval would be created by multiplying the standard error

by 1.65 instead of 2. Most estimates in this report are rounded to the nearest tenth. If rounded to 0, the standard error was reported (0.0). If there were no reports of the event, no standard error was reported (—). References to estimates being higher or lower than other estimates are based on the 95-percent confidence intervals not overlapping.

Outbreak: See Affected flock.

**p-value:** The probability that an association between a variable and an outcome is due to chance alone. A probability lies between 0.0 (never) and 1.0 (absolute).

#### Region:

Farms were reported by region, not by individual State.

**Central:** Arkansas, Illinois, Iowa, Kansas, Louisiana, Minnesota, Missouri, Nebraska, North Dakota, Oklahoma, South Dakota, Texas

**East:** Alabama, Connecticut, Delaware, Florida, Georgia, Indiana, Kentucky, Maine, Maryland, Massachusetts, Michigan, Mississippi, New Hampshire, New Jersey, New York, North Carolina, Ohio, Pennsylvania, Rhode Island, South Carolina, Tennessee, Vermont, Virginia, West Virginia, Wisconsin.

**West:** Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, Wyoming.

#### Severity of clostridial dermatitis—farm level:

Slight: Few flocks, low dermatitis-related mortality Moderate: Between slight and severe Severe: Nearly every flock, high dermatitis-related mortality

#### Severity of clostridial dermatitis—flock level:

Mild: Not treated Minimal: Treated once and responded Moderate: Multiple or continuous treatment, responded Severe: No or poor response to treatment

#### NAHMS Poultry 2010 study regions



Companies reported number of farms affected by region, not by individual State.

#### Section I: Results

#### A. Prevalence

Respondents reported the number of farms that had none, slight, moderate, or severe problems with clostridial dermatitis. Criteria for farm-level severity were based on number of flocks affected and mortality. Overall, 42.3 percent of turkey-grower farms had at least a slight problem with clostridial dermatitis during the 12 months prior to the study.

Overall, 42.3 percent of turkey-grower farms had at least a slight problem with clostridial dermatitis during the 12 months prior to the study. No turkey-grower farms in the West region had clostridial dermatitis problems. In the Central region 48.4 percent of farms had some degree of disease, and 17.6 percent had severe disease.

A. Percentage of turkey-grower farms by **farm-level** severity of clostridial dermatitis problems during the previous 12 months, and by region:

			Percer	nt Turkey	/-growei	<sup>-</sup> Farms		
				Reg	gion			
	W	est	Cei	ntral	E	ast	A	
Farm-level severity*	Pct.	Std. error	Pct.	Std. error	Pct.	Std. error	Pct.	Std. error
None	100.0	(—)	51.6	(2.2)	61.0	(1.4)	57.7	(1.2)
Slight (few flocks, low mortality)	0.0	(—)	15.7	(1.4)	25.0	(1.3)	20.1	(0.9)
Moderate	0.0	(—)	15.1	(1.6)	12.1	(1.1)	13.2	(0.9)
Severe (nearly every flock, high mortality)	0.0	(—)	17.6	(1.7)	1.9	(0.4)	9.0	(0.8)
Total	100.0		100.0		100.0		100.0	

\*Criteria for farm-level severity were based on number of flocks affected and mortality.



Percentage of turkey-grower farms by farm-level severity\* of clostridial dermatitis problems during the previous 12 months, and by region

Percent

\*Criteria for farm-level severity were based on number of flocks affected and mortality.

## B. Case-control 1. Background Study

Companies were asked to select case farms and control farms and complete a questionnaire for each selected farm. A case farm was defined as a farm in which at least two-thirds of flocks were affected with clostridial dermatitis during the previous 12 months. An affected flock was one in which mortality due to clostridial dermatitis was greater than 0.5 per 1,000 birds for 2 consecutive days.

A control farm was a farm with little or no problem with clostridial dermatitis during the previous 12 months.

#### 2. Severity of problem

Criteria for severity at the flock level were based on treatment response. Over 80 percent of flocks on case farms had moderate or severe problems that either required multiple treatments for response or did not respond to treatment.

B.2.a. Of **flocks** placed during the previous 12 months, percentage of flocks on case farms and percentage of flocks on control farms by **flock-level** severity of clostridial dermatitis:

	Percent Flocks			
	Case farms	Control farms		
Flock-level severity*	Percent	Percent		
None	7	95		
Mild (not treated)	8	5		
Minimal (treated once and responded)	4	0		
Moderate (multiple or continuous treatment, responded)	68	0		
Severe (no or poor response to treatment)	13	0		
Total	100	100		

\*Criteria for severity at the flock level were based on treatment response.

Case farms were more severely affected by clostridial dermatitis during summer and fall (8 of 10 farms had moderate or severe problems) than during spring or winter (6 of 10 farms had moderate or severe problems).

B.2.b. Percentage of case and control farms by **flock-level** severity of clostridial dermatitis and by season:

	Percent Farms							
		Ca	se			Con	trol	
	Spring	Summer	Fall	Winter	Spring	Summer	Fall	Winter
Flock-level severity*	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
None	14	6	8	0	91	94	94	97
Mild (not treated)	8	6	6	9	9	6	6	3
Minimal (treated once and responded)	14	6	3	31	0	0	0	0
Moderate (multiple or continuous treatment, responded)	53	51	60	54	0	0	0	0
Severe (no or poor response to treatment)	11	31	23	6	0	0	0	0
Total	100	100	100	100	100	100	100	100

\*Criteria for severity at the flock level were based on treatment response.



Percentage of case farms by flock-level severity of clostridial dermatitis problems, and by season

Percent

The severity of a clostridial dermatitis problem was fairly consistent from one flock to the next, which may be a reflection of aggressive treatment on problem farms.

B.2.c. Percentage of case and control farms by **flock-level** severity of clostridial dermatitis in the last three flocks:

		Percent Farms							
		Case			Control				
	Most recent flock	Previous flock	Two flocks prior	Most recent flock	Previous flock	Two flocks prior			
Flock-level severity*	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.			
None	6	6	6	91	91	87			
Mild (not treated)	6	6	3	9	9	13			
Minimal (treated once and responded)	11	11	23	0	0	0			
Moderate (multiple or continuous treatment, responded)	69	69	60	0	0	0			
Severe (no or poor response to treatment)	8	8	8	0	0	0			
Total	100	100	100	100	100	100			

\*Criteria for severity at the flock level were based on treatment response.

#### 3. Risk factor characteristics for evaluation

The risk factor evaluation follows an analytic process that compares affected (case) farms to unaffected (control) farms. The questionnaire contained one or more questions relating to the following potential risk-factor characteristics:

- **Barn characteristics:** water source, litter management, carcass disposal, culling practices, cleaning practices
- Animal characteristics: bird sources, types of animals on farm, pest problems and control methods
- **Biosecurity requirements:** producer/employee requirements, catch and vaccination crews, sharing equipment, vehicle requirements

• Characteristics of last completed flock: bird types, litter management, water treatment, coccidiosis control, clostridial vaccinations, feed ingredients, concurrent disease problems

#### 4. Selected variables for the model

Each variable was modeled individually by logistic regression, with region included as a covariate and the outcome being farm status (case/control). The season that flocks were placed was also included as a covariate for those variables related to the last completed flock. Variables with a p-value of 0.10 or less were selected for multivariable model building. The following variables met the selection criteria:

- · Municipal water system for birds' drinking water
- · Carcass (daily mortality) disposal method via composting
- · Carcass (daily mortality) disposal method via landfill
- Designated cull pen in the turkey barns
- Cull throughout growing period
- C&D ≥4 days after birds removed
- · Barns washed down and disinfected after every flock
- Water lines flushed with chlorine bleach
- Feeders disinfected with iodophor or bleach
- · Litter composted within 200 feet of poultry barn
- Litter tilled during life of the flock
- · Wood shavings litter
- · Livestock on farm
- · Employees required to shower
- Employees required to scrub footwear
- Catch crews that catch for other companies
- Share equipment with other farms
- Turkey strain

Additionally, a separate model was constructed for a subset of farms that measured humidity in the poultry barn and soil pH just outside the barn. Region and season were included as covariates in this model.

#### 5. Results

Four variables were significantly associated with clostridial dermatitis status. Case farms were more likely than control farms to compost litter within 200 ft of a poultry barn (OR=8.3) and were less likely to require employees to scrub footwear (OR=0.09). Case farms were also more likely than control farms to have a soil pH  $\geq$ 6.0 and humidity level in the barn  $\geq$ 60 percent. The other variables evaluated were not significantly associated with clostridial dermatitis. It is a commonly held belief that higher frequency of removing carcasses decreases clostridial dermatitis risk. Surprisingly, frequency of mortality removal was not associated with case-control status in this study. This finding may be due to the overwhelming contamination level already present on severely affected farms, making the impact of frequent carcass removal in this short time period hard to evaluate.

B.5.a. Percentage of case farms and control farms with the following characteristics, and results of logistic regression:

	Case	Control		
Characteristic	Percent	Percent	Odds ratio	p-value
Litter composted within 200 ft of poultry barn <sup>1</sup>	33	6	8.3	0.01
Employees required to scrub footwear <sup>1</sup>	23	72	0.09	0.0004
Soil pH ≥6.0²	70	24	17.5	0.02
Humidity ≥60 percent <sup>2</sup>	55	14	11.7	0.02

<sup>1</sup>Adjusted for region.

<sup>2</sup>Adjusted for region and season.

Case farms had significantly higher soil pH and humidity levels than control farms.

B.5.b. Average soil pH and humidity for case and control farms:

Average						
	Case farms	Control farms	p-value*			
Soil pH	6.4	5.3	0.04			
Humidity	60.5	49.3	0.02			

\*Adjusted for region and season.

The severity of clostridial dermatitis in the last completed flock was evaluated for case farms in relation to the characteristics of the flock. Due to the small sample size, and because three-fourths of flocks had a moderate to severe problem, statistical inferences could not be generated. However, a few variables were interesting numerically, and their relationship with clostridial dermatitis should be considered for future studies.

Clostridial dermatitis tends to be a disease of late finisher birds. However, disease problems tended to be more severe in flocks in which disease onset occurred when birds were less than 13 weeks of age compared with flocks with older birds. A lower percentage of flocks were moderately or severely affected when birds' water was acidified, or when birds were administred ionophores or direct-fed microbials. The association with ionophore use was unexpected. The questionnaire did not collect information on timing or duration of ionophore administration, which may affect the association with clostridial dermatitis. These intervention strategies deserve further investigation in future studies, as the small sample size in this report limited the ability to determine whether there was a true association between clostridial dermatitis severity and use of these interventions.

Characteristic of last flock	n	Percent farms
Age of onset for clostridial dermatitis		
13 weeks or more	23	78
Less than 13 weeks	11	91
Acidify the birds' drinking water		
Yes	18	67
No	17	88
Flocks fed ionophores*		
Yes	23	65
No	13	100
Direct-fed microbials/probiotics		
Yes	12	67
No	10	80

B.5.c. For case farms, percentage of farms with moderate or severe clostridial dermatitis problems, by characteristic of last flock:

\*Includes Coban and Avatec coccidiostats.

Data on litter management, water treatments, vaccinations, coccidiostat use, feed ingredients, and concurrent diseases were collected for the last three flocks. Ionophore use increased in the most recent flock compared with previous flocks. Other than ionophore use, practices remained fairly stable over time.

B.5.d. Percentage of case farms and control farms by management practices used for the last three flocks:

		Percent Farms						
		C	ase			Co	ntrol	
		Most recent flock	Previous flock	Two flocks prior		Most recent flock	Previous flock	Two flocks prior
Practice	n	Percent	Percent	Percent	n	Percent	Percent	Percent
Acidify the birds' drinking water	35	51	49	41	32	19	19	19
Flocks fed ionophores*	36	64	33	36	35	66	54	43
Flocks fed direct-fed microbials/ probiotics	22	55	50	43	24	29	21	21

\*Includes Coban and Avatec coccidiostats.

Overall mortality, mortality due to clostridial dermatitis, and age of onset stayed relatively stable over the last three flocks.

B.5.e. Average overall mortality, mortality due to clostridial dermatitis, and age of birds at clostridial dermatitis onset for the last three flocks:

	Case farms Control				ontrol farm	arms	
		Flock			Flock		
Mortality/onset	Most recent flock	Previous flock	Two flocks prior	Most recent flock	Previous flock	Two flocks prior	
Average overall mortality (percent)	17.0	14.2	14.5	10.1	10.7	10.1	
Mortality due to clostridial dermatitis (percent)	4.0	4.0	4.2	<0.1	<0.1	<0.1	
Age of birds at clostridial dermatitis onset (weeks)	13.3	13.7	14.1	NA	NA	NA	

# **C. Pathology** Seven companies participated in this phase of the study. Nineteen case farms were visited weekly during the weeks leading up to an anticipated outbreak. Six control farms were visited weekly during the same period. Sixteen case farms had clostridial dermatitis outbreaks and 3 case farms and all 6 control farms had no outbreaks.

Samples were collected for culture and histopathology. The types of biologic samples collected varied based on farm type and collection week. Appendix I shows which tissues were collected for culture and histopathology.

#### 1. Bird-level results

Other than 12 hens from 1 case farm and 1 hen from 1 control farm, nearly all birds sampled were toms. The average age of bird sampled (13 weeks) was the same for case and control farms. Samples were collected from 56 birds that were showing lesions, of which 19 were live and 37 had recently died.

	Ν	umber of Sampled Bi	rds
	Case farms	Control farms	All farms
Description	Number	Number	Number
Total birds	307	90	397
Gender			
Tom	295	89	384
Hen	12	1	13
Status			
Live, no lesions	251	90	341
Live, with lesions	19	0	19
Dead, with lesions	37	0	37
Age (weeks)			
Average	13	13	13
Minimum	5.5	9	5.5
Maximum	19	17	19

C.1.a. Description and number of birds sampled on case and control farms:

Prior to an outbreak, birds on outbreak farms and nonoutbreak farms had similar levels of *C. septicum* (1 percent of birds). About half the birds were positive for *C. septicum* during an outbreak. Approximately 20 percent of birds were positive for *C. perfringens,* regardless of outbreak status.

C.1.b. Percentage of birds positive for *C. septicum* and *C. perfringens,* by farm outbreak status:

		Percent Birds Positive							
		Outbreak Status							
	Nonoutb	reak farm	Outbrea not outbr	k farm— eak week	Outbrea outbrea	k farm— Ik week			
Species	n	Percent	n	Percent	n	Percent			
C. septicum	2/141	1	2/171	1	36/85	42			
C. perfringens	30/141	21	29/171	17	19/85	22			

## Percentage of birds positive for *C. septicum* and *C. perfringens*, by farm outbreak status



#### Percent

*C. septicum* was found in liver and spleen samples, which is consistent with the theory of hematogenous spread to muscle. *C. perfringens* was found less commonly in internal tissues.

C.1.c. Percentage of birds positive for *C. septicum* and percentage of birds positive for *C. perfringens*, by farm outbreak status and by tissue tested:

	Percent Birds Positive								
	Outbreak Status								
	Nonoutb	reak farm	Outbrea not outbr	k farm— eak week	Outbrea outbrea	k farm— ak week			
Tissue tested	n	Percent	n	Percent	n	Percent			
C. septicum									
GI	1/140	1	1/162	1	0/48	0			
Liver	0/138	0	1/169	1	17/82	21			
Spleen	0/21	0	0/3	0	13/74	18			
Muscle	1/21	5	0	NA	31/64	48			
Any	2/141	1	2/171	1	36/85	42			
C. perfringens									
GI	30/140	21	28/162	17	18/48	37			
Liver	0/138	0	1/169	1	1/82	1			
Spleen	1/21	5	0/3	0	0/74	0			
Muscle	0/21	0	0	NA	0/64	0			
Any	30/141	21	29/171	17	19/85	22			

About half the birds were positive for *C. septicum* during an outbreak, but levels were similar to nonoutbreak farms in the weeks prior to the outbreak. The percentage of birds with *C. perfringens* increased with birds' age on nonoutbreak farms. On outbreak farms, *C. pefringens* did not increase during the outbreak week, although *C. septicum* increased dramatically. Based on these results, it appears that *C. septicum* has a major role in clostridial dermatitis outbreaks, but the role, if any, of *C. perfringens* is unclear.

C.1.d. Percentage of birds with *C. septicum* and *C. perfringens* on outbreak and nonoutbreak farms, by number of weeks before outbreak and by bird age:

		Percent Birds			
		C. septicum	C. perfringens		
Outbreak farm/ number weeks	n	Percent	Percent		
3+ weeks prior to outbreak	96	1	9		
2 weeks prior to outbreak	33	3	27		
1 week prior to outbreak	42	0	26		
Outbreak week	85	42	22		
Nonoutbreak farm/bird age	n	Percent	Percent		
Less than 11 weeks old	24	0	4		
11 to less than 14 weeks old	66	2	20		
14+ weeks old	51	2	31		

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Percentage of birds with C. septicum

and C. perfringens on outbreak farms,

Percentage of birds with *C. septicu* and *C. perfringens* on nonoutbreak farms, by bird age



Intestinal pathology was assessed microscopically by assigning lesion scores to four areas of the duodenum, ileum, and cecum. A single lesion score was also assigned to Meckel's diverticulum. The presence of luminal bacteria, coccidia, and "other parasites" was also noted. Further characterization of these organisms was not done. Intestinal pathology was frequently found on both outbreak and nonoutbreak farms, especially in the lamina propria and muscle of the ileum and Meckel's diverticulum. Intestinal pathology did not appear to be related to farm outbreak status.

C.1.e. Percentage of birds that had pathology/pathogens in the following areas of the intestines, by farm outbreak status:

		Percent Birds				
	Outbreak farm					
	Nonoutbreak farm n=140	Not outbreak week n=159	Outbreak week n=48 Percent			
Pathology/pathogens in the	Percent	Percent				
Duodenum						
Villi	0	0	0			
Crypts	2	1	2			
Lamina propria	4	6	0			
Muscle	0	1	0			
Any duodenum	6	7	2			
Bacteria	37	28	25			
Coccidia	3	1	4			
Other parasites	0	0	0			
lleum						
Villi	4	7	0			
Crypts	1	3	0			
Lamina propria	55	55	42			
Muscle	52	53	35			
Any ileum	69	71	54			
Bacteria	46	37	40			
Coccidia	9	11	8			
Other parasites	11	18	8			
Meckels diverticulum	46	42	35			
Cecum						
Crypts	8	8	4			
Lamina propria	10	20	13			
Mucosa	1	1	2			
Muscle	0	0	0			
Any cecum	17	27	19			



## Percentage of birds that had pathology in the following areas of the intestines, by farm outbreak status

Changes in pathology of the tissues collected were not apparent in the weeks leading up to an outbreak.

C.1.f. Percentage of birds that had pathology/pathogens in the following areas of the intestines, by outbreak/weeks category:

	Percent Birds						
	Outbreak farm				Nor	noutbreak f	arm
	Outbreak week n=48	1 week prior n=42	2 weeks prior n=24	3+ weeks prior n=93	Less than 11 weeks old n=24	11 to less than 14 weeks old n=66	14+ weeks old n=50
Pathology/ pathogens in the	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Duodenum							
Villi	0	0	0	0	0	0	0
Crypts	2	0	0	1	4	0	4
Lamina propria	0	5	8	5	0	5	6
Muscle	0	2	0	1	0	0	0
Any duodenum	2	5	8	7	4	5	10
Bacteria	25	38	8	28	21	39	42
Coccidia	4	0	0	2	0	2	6
Other parasites	0	0	0	0	0	0	0
lleum					-		
Villi	0	10	8	5	8	6	0
Crypts	0	2	0	4	0	2	0
Lamina propria	42	52	54	57	58	58	49
Muscle	35	45	58	55	63	52	48
Any ileum	54	60	79	74	71	74	61
Bacteria	40	31	37	39	46	47	45
Coccidia	8	14	17	9	8	11	8
Other parasites	8	10	25	20	13	8	16
Meckels diverticulum	35	35	48	44	54	43	47
Cecum							
Crypts	4	10	0	10	4	11	6
Lamina propria	13	24	35	14	4	12	10
Mucosa	2	0	4	0	0	0	2
Muscle	0	0	0	0	0	0	0
Any cecum	19	33	35	22	8	22	16

Over half of birds sampled during an outbreak had gross lesions consistent with clostridial dermatitis in the skin/subcutaneous tissue/muscle, but lesions were rarely seen before an outbreak.

C.1.g. Percentage of birds that had gross lesions consistent with clostridial dermatitis, by outbreak/weeks category:

Percent Birds						
Outbreak farm				No	noutbreak fa	arm
Outbreak week n=85	Outbreak 1 week 2 weeks 3+ weeks week prior prior prior n=85 n=42 n=33 n=96			Less than 11 weeks old n=24	11 to less than 14 weeks old n=66	14+ weeks old n=51
Percent	Percent	Percent	Percent	Percent	Percent	Percent
59	5	9	1	0	0	0

*C. septicum-* and *C. perfringens-*positive birds had no more intestinal pathology than *C. septicum-* and *C. perfringens-*negative birds.

C.1.h. Percentage of birds that had pathology/pathogens in the following areas of the intestines, by *C. septicum* and *C. perfringens* status of birds:

		Percent Birds				
	<b>C</b> . s	C. septicum and C. perfringens Status				
	<i>C. septicum</i> negative n=331	<i>C. septicum</i> positive n=16	C. perfringens negative n=272	<i>C.</i> perfringens positive n=75		
Pathology/ pathogens in the	Percent	Percent	Percent	Percent		
Duodenum						
Villi	0	0	0	0		
Crypts	1	6	1	1		
Lamina propria	4	6	5	3		
Muscle	1	0	1	0		
Any duodenum	6	12	7	4		
Bacteria	32	19	31	31		
Coccidia	2	13	2	4		
Other parasites	0	0	0	0		
lleum						
Villi	5	0	6	3		
Crypts	2	0	2	0		
Lamina propria	53	44	56	41		
Muscle	51	25	53	41		
Any ileum	68	56	71	55		
Bacteria	41	44	43	35		
Coccidia	10	13	10	11		
Other parasites	13	25	14	13		
Meckels diverticulum	44	29	43	44		
Cecum						
Crypts	8	6	7	10		
Lamina propria	15	13	16	11		
Mucosa	1	0	0	3		
Muscle	0	0	0	0		
Any cecum	22	13	22	21		



## Percentage of birds that had pathology in the following areas of the intestines, by *C. septicum* and *C. perfringens* status of birds

About two-thirds of birds positive for *C. septicum* (67 percent) had gross lesions consistent with clostridial dermatitis compared with less than one-tenth of birds negative for *C. septicum*. There was no substantial difference in the percentage of *C. perfringens*-positive birds and *C. perfringens*-negative birds with lesions.

C.1.i. Percentage of birds that had gross lesions consistent with clostridial dermatitis, by *C. septicum* and *C. perfringens* status of birds:

	Percent Birds						
	C. septicum and C. perfringens Status						
C. septicum negative n=357	C. septicumC. septicumC. perfringensC. perfringensnegativepositivenegativepositiven=357n=40n=319n=78						
Percent	Percent	Percent	Percent				
8	67	16	8				

#### 2. Farm-level results

A farm was considered positive for *C. septicum* or *C. perfringens* if one or more sampled birds tested positive or if a sample from litter or beetles tested positive during any week.

*C. septicum* was found on 22 percent of nonoutbreak farms and on 87 percent of outbreak farms. *C. perfringens* was found on more than 80 percent of farms, regardless of outbreak status.

C.2.a. Percentage of farms positive for *C. septicum* and *C. perfringens,* by farm outbreak status:

	Percent Farms Positive				
	Outbreak Status				
	Nonoutbreak farm n=9 Outbreak farm n=16				
	Percent	Percent			
C. septicum	22	87			
C. perfringens	89	81			

The percentage of farms in which birds had pathology/pathogens in the intestines in the weeks prior to an outbreak was generally similar for outbreak farms and nonoutbreak farms. *C. perfringens* was found in birds on outbreak and nonoutbreak farms prior to the final week of sampling, while *C. septicum* was only found prior to the final week in birds or litter on outbreak farms.

	Percent Farms			
	Outbreak	< Status		
	Nonoutbreak farm n=9	Outbreak farm n=16		
Pathology/pathogens in the	Percent	Percent		
Duodenum				
Villi	0	0		
Crypts	11	6		
Lamina propria	33	37		
Muscle	0	13		
Any duodenum	44	44		
Bacteria	89	81		
Coccidia	44	13		
Other parasites	0	0		
lleum				
Villi	44	31		
Crypts	11	19		
Lamina propria	100	87		
Muscle	100	87		
Any ileum	100	87		
Bacteria	100	87		
Coccidia	89	63		
Other parasites	56	63		
Meckels diverticulum	100	87		
Cecum				
Crypts	67	37		
Lamina propria	89	81		
Mucosa	11	6		
Muscle	0	0		
Any cecum	89	81		
C. septicum in litter	0	6		
<i>C. septicum</i> in beetles (n=10 farms)	0	0		
C. septicum in bird	0	13		
C. perfringens in bird	89	69		

C.2.b. Percentage of farms that had birds with pathology/pathogens in the following areas of the intestines **prior to the final week** of sampling, by farm outbreak status:

No nonoutbreak farms and about one of five outbreak farms identified gross lesions in sampled birds prior to an outbreak.

C.2.c. Percentage of farms that had birds with gross lesions consistent with clostridial dermatitis **prior to the final week** of sampling, by farm outbreak status:

Percent Farms					
eak Status					
Outbreak farm n=16					
Percent					
19					

*C. septicum*-positive farms and *C. septicum*-negative farms did not differ substantially by the presence of intestinal pathology in birds.

C.2.d. Percentage of farms that had birds with pathology/pathogens in the following areas of the intestines **prior to the final week** of sampling, by farm *C. septicum* status:

	Percent Farms			
	C. septic	um Status		
	Negative n=9	Positive n=16		
Pathology/pathogens in the	Percent	Percent		
Duodenum				
Villi	0	0		
Crypts	0	13		
Lamina propria	33	37		
Muscle	0	13		
Any duodenum	33	50		
Bacteria	89	81		
Coccidia	33	19		
Other parasites	0	0		
lleum				
Villi	33	37		
Crypts	11	19		
Lamina propria	100	87		
Muscle	100	87		
Any ileum	100	87		
Bacteria	89	94		
Coccidia	67	75		
Other parasites	56	63		
Meckels diverticulum	89	94		
Cecum				
Crypts	67	37		
Lamina propria	78	87		
Mucosa	11	6		
Muscle	0	0		
Any cecum	78	87		
C. septicum in litter	0	6		
<i>C. septicum</i> in beetles (n=10 farms)	0	0		
C. septicum in bird	0	13		
C. perfringens in bird	67	81		

About 10 percent of *C. septicum*-positive and *C. septicum*-negative farms had birds with gross skin lesions prior to the final week of sampling.

C.2.e. Percentage of farms that had birds with gross lesions consistent with clostridial dermatitis **prior to the final week** of sampling, by farm *C. septicum* status:

Percent Farms					
C. septicum Status					
Negative n=9	Positive n=16				
Percent	Percent				
11	13				

#### Section II: Methodology

#### NAHMS develops study objectives by exploring existing literature and contacting industry A. Needs members and other stakeholders about their informational needs and priorities during Assessment a needs assessment phase. For the NAHMS Poultry 2010 study, the following activities were conducted: A focus group consisting of industry, State, Federal, and university representatives met at the World Poultry Exposition in Atlanta, GA, in January 2008. A needs assessment questionnaire was distributed to poultry veterinarians via the presidents of the egg layer, broiler, turkey, and primary breeder veterinary groups. This questionnaire was also distributed to State and Federal veterinarians and laboratory and research personnel. Discussions were held with each of the poultry veterinary groups at the American Association of Avian Pathologists meeting in New Orleans, LA, in July 2008, and in Seattle, WA, in July 2009. Additional discussions occurred at the USAHA Transmissible Diseases of Poultry Committee. B. Sampling and 1. Prevalence estimate (company survey) Data Collection A total of 15 turkey companies were selected to participate in Phase I of the Poultry 2010 study. Turkey breeder companies were not included. Subparts of some large turkey companies were considered to be separate companies. Large turkey co-ops were considered to be companies. Data collection was conducted from May 27 through October 16, 2010. Company veterinarians or representatives completed one company questionnaire per company.

Inferences cover the population of the selected poultry companies. The selected companies represent 76.8 percent of turkeys slaughtered in 2009 in the United States (WATT, 2010). All respondent data were statistically weighted to reflect the populations from which they were selected. Because companies were selected with certainty, the initial selection weight was equal to one for all selected companies. This selection weight was adjusted for nonresponse.

#### 2. Case-control study

Companies that participated in Phase I (prevalence estimate: company survey) and that had grower farms located in the Central and East regions were eligible to participate in the clostridial dermatitis case-control study. The West region was excluded because there were no cases of clostridial dermatitis in the West region. Companies selected case and control farms for participation in the case-control study. A case farm was defined as a farm with at least two-thirds of flocks affected with clostridial dermatitis during the previous 12 months. An affected flock was one in which mortality due to clostridial dermatitis was greater than 0.5 per 1,000 birds for 2 consecutive days. A control farm was a farm with little or no problem with clostridial dermatitis during the previous 12 months. A questionnaire was completed for each selected farm. Data collection occurred from June 3 through December 17, 2010.

#### a. Measurement of humidity and pH

The questionnaire contained one question which asked the humidity level in the barn when turkeys in the last completed flock were 10 weeks old. Farms that routinely monitored poultry barn humidity answered this question. Respondents who were willing to measure pH were provided with pH paper in order to measure pH of water, litter, and soil. This was done at the time the questionnaire was completed.

#### 3. Biologic sampling

The objective of this phase of the study was to gain a better understanding of the role of intestinal pathology in the pathogenesis of clostridial dermatitis. Seven companies participated in this phase of the study. Nineteen case farms were visited weekly during the weeks leading up to an anticipated outbreak. Six control farms were visited weekly during the same time period. Anticipated timing of an outbreak was based on the farm's previous history. Three birds per week were euthanized and intestinal samples collected for culture and histopathology. Liver samples, litter, and beetles were also collected weekly for culture. On the final visit, additional samples for culture included spleen and muscle from three euthanized birds and case farms also collected liver, spleen, and muscle for culture from three dead birds. Biologic samples were collected from May 27 through October 16, 2010. See Appendix I for details regarding biologic sampling.

#### C. Data Analysis 1. Validation and estimation

Data were entered into a SAS data set. Validation checks were performed to identify numeric extremes, improper categorical responses, skip patterns not followed, and relational checks. Analyses were performed using SUDAAN software, which accounts for complex study design. Weights were created for prevalence estimates only. Because companies were selected with certainty, the initial selection weight was equal to 1 for all selected companies. This selection weight was adjusted for nonresponse.

#### 2. Risk factor analysis

The risk factor analysis follows an analytic process of comparing affected (case) farms with unaffected (control) farms. Each variable was modeled individually by logistic regression, with region included as a covariate. Season of flock placement was also

included as a covariate for those variables related to the last completed flock. Variables with a p-value of 0.10 or less were selected for multivariable model building in a forward selection procedure. Additionally, a separate model was constructed for a subset of farms that measured soil pH and humidity in the poultry barn. Region and season were included as covariates in this model.

#### D. Response Rate

	Companies	Case farms	Control farms	Total farms
Selected for Phase I* (company-level survey)	15			
Participated in Phase I	12 (80%)			
Eligible for Phase II (case-control study)	11			
Participated in Phase II	10 (91%)	36	35	71
Biologic sampling	7	19	6	25

\*For the purpose of this study, subparts of some large turkey companies were considered to be separate (unique) companies. Also, large turkey co-ops were considered to be companies.

	CASE farm			CONTROL farm	
	No outbreak	Outb	reak or final	No outbreak	Outbreak or final
	Weeks without outbreak sacrifice three live birds	At outbreak— Sacrifice three live birds and, if available, sample three dead birds with symptoms.		Sacrifice three live birds (If an outbreak occurs, take at outbreak samples described on left.)	
Sample	Weeks without an outbreak	Live birds	Dead birds with symptoms, dead less than 8 hr	Weeks 1 –4	Week 5
For histopathology					
1/4-inch duodenal bend; include a small piece of the pancreas	×	х		х	х
1/4-inch jejunum including the diverticulum	х	x		Х	Х
1/4-inch cecum at its midpoint	Х	х		Х	х
For culture					
3–4-inch jejunum	Х	Х		Х	Х
Whole cecal pouch	Х	Х		Х	Х
Whole liver	Х	Х	Х	Х	Х
Whole spleen		Х	Х		Х
1-inch cube of skin, subcutaneous tissue, and muscle from the inguinal region or breast		X (lesion if seen)	X lesion		Х
1/4-inch cube of skin, subcutaneous tissue, and muscle from the inguinal region or breast		X (lesion if seen)	X lesion		Х
One pooled litter from sampled barn	Х		х	х	х
One set of beetles from sampled barn	х		х	х	Х

## Appendix I: Biologic Sampling Protocol

## Appendix II: Number of Farms and Number of Turkeys on Farms

Region/State	Turkey farms (number)	Turkeys (inventory)	
Central			
Arkansas	530	9,437,181	
Illinois	362	845,971	
Iowa	417	4,002,111	
Kansas	220	561,484	
Louisiana	127	801	
Minnesota	601	18,298,316	
Missouri	868	8,604,222	
Nebraska	209	761,232	
North Dakota	60	444,274	
Oklahoma	418	(D)	
South Dakota	148	2,232,577	
Texas	1,210	1,986,815	
Wisconsin	780	3,685,648	
East			
Alabama	279	8,788	
Connecticut	114	3,564	
Delaware	21	701	
Florida	478	13,780	
Georgia	350	1,986	
Indiana	498	5,971,548	
Kentucky	434	30,785	
Maine	181	3,097	
Maryland	142	223,233	
Massachusetts	198	17,501	
Michigan	731	1,978,869	
Mississippi	203	1,396	
New Hampshire	145	2,534	
New Jersey	151	18,405	
New York	529	99,333	
North Carolina	846	17,865,896	
Ohio	627	2,074,750	
Pennsylvania	835	3,537,517	
Rhode Island	32	1,912	
South Carolina	337	5,484,201	
Tennessee	526	3,479	
Vermont	106	5,748	
Virginia	572	6,330,958	
West Virginia	214	1,641,120	
United States	17,226	107,173,804	

Source: NASS 2007 Census of Agriculture.

### **Appendix III: References**

Clark S, Porter R, McComb B, Lippert R, Olson S, Nohner S, Shivaprasad HL. 2010. Clostridial dermatitis and cellulitis: an emerging disease of turkeys. *Avian Dis* 54(2):788–794.

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