Campylobacter detection in broiler ceca at processing: A three-year, 211-flock survey[1]

M. E. Berrang,[*,2] R. J. Meinersmann,[*] S. R. Ladely,[†] and N. A. Cox[*]

[*]*USDA-Agricultural Research Service, U.S. National Poultry Research Center, Athens GA 30505; and* [†]*USDA-Food Safety Inspection Service, Athens, GA 30605*

Primary Audience: Poultry Researchers, Poultry Producers, Poultry Processors, Food Safety Regulators

SUMMARY

Campylobacter is associated with live broilers and chicken meat products. There is some discussion in the literature about the possibility that *Campylobacter* prevalence in broilers could be affected by season or weather conditions. The objective of this study was to measure the flock prevalence of *Campylobacter* by sampling cecal contents from multiple flocks in one commercial slaughter plant over the course of 3 years. Two-hundred-and eleven discrete cecal samples, each from a different flock, were cultured for *Campylobacter*. Weather data, collected daily at a nearby University of Georgia experiment station, was used for testing for potential relationships between environmental conditions and *Campylobacter* detection. Fifty-five percent of flocks were found to be *Campylobacter*-positive. No clear trend was uncovered for *Campylobacter* prevalence related to mo of yr or daily maximum temperature. Furthermore, no significant relationship was noted between prevalence of *Campylobacter* and rainfall on the d of slaughter ($P = 0.52$) or the total rainfall during the grow-out period ($P = 0.37$).

Key words: *Campylobacter*, broiler ceca, survey, seasonality, rainfall

2017 J. Appl. Poult. Res. 26:154–158
http://dx.doi.org/10.3382/japr/pfw052

DESCRIPTION OF PROBLEM

Campylobacter is a human pathogen that is commonly associated with poultry and poultry products. It has been shown to be present in the broiler chicken gut as well as on outer surfaces at processing [1, 2]. *Campylobacter* in the gut of broilers can result in cross-contamination and increasing carcass prevalence during transport and processing [2–4].

There is a well-defined and documented peak in human campylobacteriosis in the warmer months of the year [5–7]. The warm season peak seems to be particularly evident in cooler countries such as Finland, Sweden, and Wales; seasonality is less extreme in New Zealand [5]. Although measurable across a wide geographical area, the intensity of the peak in early summer has been reported to be dependent on region [6]. As would be expected, the seasonal peak in human campylobacteriosis is also correlated to increased environmental temperature [7]. Linkage between human campylobacteriosis and rainfall has been suggested [8].

In the past, researchers have examined broilers in attempts to uncover any seasonal trends

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[2]Corresponding author: mark.berrang@ars.usda.gov

in *Campylobacter* prevalence that may help explain human disease [9–14]. While some authors suggest an effect of season on the detection of *Campylobacter* in live poultry or carcasses, many of these studies cover only a one-year period and thus have a sample size of one season [9, 10, 12, 14]. Multi-year studies have been reported in countries or regions that are overall much cooler than Georgia in the United States [11, 13].

The objective of the current study was to measure *Campylobacter* prevalence in broiler flocks by culturing cecal contents from 211 discrete flocks at one commercial slaughter plant over the course of 3 years. A secondary objective was to determine if any relationship could be uncovered between time of yr, temperature, or rainfall and *Campylobacter* detection in broiler cecal contents.

MATERIALS AND METHODS

Experimental Overview

This study was designed to sample the highest possible number of flocks as opposed to a high number of broilers within each flock. One cecal sample was taken each from a discreet flock, over a three-year period. On each sample d between April 2013 and April 2016, one pair of ceca was collected from the evisceration line in a commercial broiler slaughter plant. Each sample was aseptically collected, individually bagged, and placed on ice for transport to the U.S. National Poultry Research Center. Cecal contents were cultured for the presence and numbers of *Campylobacter* using a filter direct plating method described below. Ratios of positive cecal samples were compared according to mo collected, temperature on d of collection, and rainfall.

Sample Collection and Preparation

On each of 211 sample d, one pair of ceca was collected from the evisceration line in a commercial broiler processing plant. Using a clean nitrile glove, ceca were removed from the viscera of eviscerated carcasses, placed into an individual clean, re-sealable plastic bag, and covered with ice for transport to the laboratory. One cecum was transferred to a sample bag, weighed, smashed with a rubber mallet to release contents, and diluted with 3 times the weight sterile phosphate buffered saline (**PBS**). Ceca with contents were then subjected to blending in a paddle blender [15] for 30 s before an aliquot was removed for dilution and direct plating as described below.

Culture Methods

Aliquots of serially diluted cecal contents were placed on solid plating media for isolation and identification of *Campylobacter*. Samples were plated on the surface of Campy-cefex agar (**CCA**) [16], Campy-Line agar (**CLA**) [17] or RF *Campylobacter jejuni*/*coli* agar (**RFA**) [18]. The diluted sample (0.5 mL) was placed dropwise onto the surface of a nitrocellulose filter [19] previously laid on the agar surface and allowed to soak in at room temperature (approximately 25 °C) and ambient atmosphere until dry (up to 60 min.). Similar filter methods have been shown to allow *Campylobacter* to make its way through the filter while excluding most non-*Campylobacter* background bacteria [20, 21]. Filters were aseptically removed and plates were incubated at 42 °C for 48 h in a sealable bag flushed with a micro-aerobic atmosphere (10% CO_2, 5% O_2, and 85% N_2). Colonies characteristic of *Campylobacter* spp. were selected for confirmation. All suspect colony types on all media were confirmed as *Campylobacter* by observation of cellular morphology and motility under phase contrast microscopy and further confirmed as thermophilic *Campylobacter* by a positive reaction on a latex agglutination kit [22].

Weather Data

Temperature and rainfall were recorded by a University of Georgia weather data collection station at the J. Phil Campbell Sr. Natural Resources Conservation Center in Watkinsville, GA, within the area from which broilers are grown for the slaughter plant used in this study [23].

Table 1. Mean ratio, ± 95% confidence interval, of broiler ceca collected in commercial processing plant positive for *Campylobacter* by mo of the year (n = 3 yr).

Month	n[1]	Ratio positive
JAN	14	0.36 ± 0.29[2]
FEB	19	0.42 ± 0.24
MAR	23	0.52 ± 0.22
APR	21	0.52 ± 0.23
MAY	18	0.89 ± 0.16
JUN	17	0.47 ± 0.26
JUL	24	0.54 ± 0.21
AUG	18	0.50 ± 0.26
SEP	16	0.62 ± 0.27
OCT	15	0.47 ± 0.29
NOV	13	0.69 ± 0.29
DEC	13	0.61 ± 0.31
Total	211	0.55 ± 0.25[3]

[1]Sample size, total number of samples drawn in mo across 3 yr of sampling.
[2]95% confidence interval.
[3]General linear model analysis uncovered no effect of mo on detection of *Campylobacter* ($P = 0.22$).

Table 2. Mean ratio, ±95% confidence interval, of broiler ceca collected in commercial processing plant positive for *Campylobacter* sorted by maximum temperature on d of sampling (n = 3 yr).

Max temp (C)	n[1]	Ratio positive
≤ 2.8	3	0.33 ± 1.44[2]
2.9 to 6.7	4	0.25 ± 0.80
6.8 to 10.5	9	0.44 ± 0.40
10.6 to 14.4	20	0.55 ± 0.24
14.5 to 18.3	19	0.42 ± 0.24
18.4 to 22.2	31	0.64 ± 0.18
22.3 to 26.1	34	0.62 ± 0.17
26.2 to 30.0	38	0.42 ± 0.16
30.1 to 33.9	43	0.65 ± 0.15
40.0 to 37.8	10	0.60 ± 0.37
Total	211	0.55 ± 0.25[3]

[1]Sample size, total number of samples drawn on d with maximum temperatures in this range across 3 yr of sampling.
[2]95% confidence interval.
[3]General linear model analysis uncovered no effect of maximum temperature on detection of *Campylobacter* ($P = 0.39$).

Statistical Analysis

Campylobacter detection was converted to a ratio (number of positive samples/total number of samples per subset) for mo of the yr, ranges of maximum daily temperature values, d of collection rainfall values or 35-day total rainfall values. Mean ratio of positive samples was compared using General Linear Model (**GLM**) [24].

RESULTS AND DISCUSSION

Overall, 55% of cecal content samples were positive for *Campylobacter*. The ratio of positive samples by mo of the yr is presented in Table 1. The mo of May had numerically higher prevalence of *Campylobacter* than any other month. However, when analyzed by GLM, there was no significant effect ($P = 0.22$) of mo on *Campylobacter* detection in ceca of broilers in the slaughter plant. Similar results were found when the detection of *Campylobacter* was examined relative to maximum daily temperature on d of collection (Table 2). These data are different from many published studies that claim an increase in *Campylobacter* prevalence or numbers during warmer mo [9–11, 13, 14] or cooler months [12]. Some of the earlier studies may not be comparable because they were conducted in Northern Europe, which in general has a much cooler climate than Georgia, United States [11, 13, 14] or sampled in only one yr and therefore having a sample size of just one for season [9, 11, 12].

The current data are interesting in comparison to reported studies on human campylobacteriosis. There is very good published evidence of a peak in human *Campylobacter* infection and disease during warm months [5–7]. A further link has been demonstrated between density of poultry growing operations and processing plants and warm weather human campylobacteriosis in people between the ages of 16 and 34, an age range that may well include many of those employed by the poultry industry [8]. An interesting observation is that in some studies in which a warm weather peak in *Campylobacter* prevalence in chicken has been suggested, the human warm weather disease peak actually precedes it [25, 26]. This suggests that chicken may not be the primary source for warm weather human campylobacteriosis but, rather, there may be some other common environmental source of *Campylobacter* to humans and poultry.

Campylobacter detection relative to rainfall on the d of collection is presented in Table 3. In an earlier Canadian study, low rainfall was found to be significantly associated with detection of *Campylobacter* on processed carcass rinses [27].

Table 3. Mean ratio, ±95% confidence interval, of broiler ceca collected in commercial processing plant positive for *Campylobacter* sorted by rainfall on d of sampling (n = 3 yr).

1-d rainfall (cm)	n[1]	Ratio positive
≤ 0.76	181	0.55 ± 0.07[2]
0.77 to 2.5	22	0.59 ± 0.22
2.6 to 5.1	4	0.75 ± 0.80
5.2 to 7.6	4	0.25 ± 0.80
Total	211	0.55 ± 0.25[3]

[1]Sample size, total number of samples drawn on d with rainfall in this range across 3 yr of sampling.

[2]95% confidence interval.

[3]General linear model analysis uncovered no effect of sample d rainfall on detection of *Campylobacter* ($P = 0.52$).

However, *Campylobacter* is sensitive to drying and can be killed in transport coops when contaminated fecal matter is allowed to dry out [28, 29]. We therefore hypothesized that rain on the d of sample collection (and presumably high humidity during transport and holding) would lead to an increase in *Campylobacter* detection. The current data did not support this hypothesis; GLM revealed no effect of sample d rainfall on detection of *Campylobacter* in ceca from eviscerated broiler carcasses ($P = 0.52$).

Jorgensen et al. [13] reported that weather, including total rain in the mo of chick placement, could be somewhat predictive of *Campylobacter*, explaining 46% of the prevalence. We examined detection of *Campylobacter* according to total rainfall in a 35-day period prior to slaughter (data presented in Table 4). No significant relationship was noted between total rainfall and detection of *Campylobacter* in ceca ($P = 0.37$).

CONCLUSIONS AND APPLICATIONS

1. The three-year mean *Campylobacter* prevalence by culture of cecal contents was 55% of flocks in one Georgia, United States, processing plant.
2. Detection of *Campylobacter* was not significantly related to mo of the yr or maximum daily temperature at slaughter.
3. Detection of *Campylobacter* was not significantly related to rainfall on d of slaughter or for 35 d prior to slaughter.

Table 4. Mean ratio, ±95% confidence interval, of broiler ceca collected in commercial processing plant positive for *Campylobacter* sorted by total rainfall for 35 d prior to the d of sampling (n = 3 yr).

35-d rainfall (cm)	n[1]	Ratio positive
0 to 2.5	3	0.67 ± 1.43[2]
2.6 to 5.1	5	0.60 ± 0.68
5.2 to 7.6	26	0.58 ± 0.20
7.7 to 10.2	32	0.44 ± 0.18
10.3 to 12.7	44	0.57 ± 0.15
12.8 to 15.2	35	0.54 ± 0.17
15.3 to 17.8	16	0.62 ± 0.27
17.9 to 20.3	11	0.64 ± 0.34
20.4 to 22.3	12	0.83 ± 0.25
22.4 to 25.4	6	0.83 ± 0.43
25.5 to 27.9	3	0.33 ± 0.1.43
28.0 to 30.5	4	0.25 ± 0.80
33.1 to 35.6	9	0.33 ± 0.39
35.7 to 38.1	2	0.0.0 ± 0.00
38.2 to 40.6	3	0.33 ± 1.43
Total	211	0.55 ± 0.25[3]

[1]Sample size, total number of samples drawn on d with 35-day rainfall total in this range across 3 yr of sampling.

[2]95% confidence interval.

[3]General linear model analysis uncovered no effect of 35-day rainfall total on detection of *Campylobacter* ($P = 0.37$).

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Acknowledgments

The authors wish to acknowledge expert technical assistance by Steven W. Knapp.