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Research Note—

Comparison of *Campylobacter* Levels in Crops and Ceca of Broilers at Slaughter

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SUMMARY. A considerable fraction of the poultry carcasses becomes contaminated with *Campylobacter* by cross-contamination from the digestive tract of colonized broilers at slaughter. *Campylobacter* in the crop may serve as a possible source of cross-contamination, because the crop may contain high numbers of *Campylobacter* and is more likely to rupture during the slaughtering process than intestines. In this study, the correlation between *Campylobacter* colonization levels in crop and cecum was assessed in 48 broilers of 31 days of age. In addition, the effect of drinking water supplemented with 0.2% volatile fatty acid (VFA) on these *Campylobacter* colonization levels was studied. No correlation between crop and cecal colonization levels was found ($\rho = 0.09$; $P = 0.71$), indicating that future studies on cross-contamination should include an examination of not only cecal colonization levels but also crop colonization levels. Supplementation of drinking water with VFA did not result in a significant reduction of colonization levels in either the crop ($P = 0.50$) or the ceca ($P = 0.92$), indicating that this is not an effective measure to reduce cross-contamination at slaughter.

RESUMEN. *Nota de Investigación*—Comparación de los niveles de *Campylobacter* en buche y ciegos de pollos de engorde durante el procesamiento.

Una fracción considerable de las canales de las aves comerciales se contamina con *Campylobacter* por la contaminación cruzada a partir del tracto digestivo de los pollos de engorde. La bacteria *Campylobacter* que se encuentra en el buche puede servir como una posible fuente de contaminación cruzada, porque el buche puede contener grandes cantidades de *Campylobacter*, además, de que es más probable la ruptura de este órgano durante el procesamiento en comparación con los intestinos. En este estudio, se evaluó la correlación entre los niveles de la colonización de *Campylobacter* en el buche y en el ciego en 48 pollos de 31 días de edad. Además, se estudió el efecto del agua potable suplementada con 0.2% de ácidos grasos volátiles sobre los niveles de colonización por *Campylobacter*. No se observó correlación entre la colonización en el buche y en el ciego ($\rho = 0.09$; $P = 0.71$), lo que indica que estudios futuros sobre la contaminación cruzada deben incluir un examen no sólo de los niveles de la colonización del ciego, sino también de los niveles de colonización en el buche. La suplementación de agua potable con ácidos grasos volátiles no resultó en una reducción significativa de los niveles de colonización en el buche ($P = 0.50$) ni en el ciego ($P = 0.92$), lo que indica que esto no es una medida eficaz para reducir la contaminación cruzada durante el procesamiento.

Key words: *Campylobacter* control, food safety, broiler, cross-contamination, colonization level

Abbreviations: BWG = body weight gain; cfu = colony-forming units; PBS = phosphate-buffered saline; VFA = volatile fatty acid

Campylobacter spp. are a common cause of diarrhea in humans, and such infections are often associated with the handling and consumption of contaminated poultry meat (7). In broilers, highest colonization levels are observed 5 days after onset of colonization (21). Although a slight decrease in colonization level might occur afterward, the majority of broilers shed *Campylobacter* in concentrations above 6 $\log_{10}$ colony-forming units (cfu)/g feces for the remaining rearing period (9). Risk assessment studies have indicated that reduction of *Campylobacter* colonization levels at slaughter could result in lower contamination levels of poultry carcasses and meat, which in turn could reduce the risk of human infection (11,14). To predict the efficacy of an intervention that reduces colonization levels, knowledge of the relationship between *Campylobacter* levels in broilers and carcass contamination levels is required. To date, it is not clear to what extent the different parts of the digestive tract of colonized birds contribute to carcasses contamination. Although *Campylobacter* levels in the ceca are considered a good predictor for the colonization levels in the broiler, cecal levels seemed to predict levels of carcass contamination poorly (1,12,17). The crop is also a possible source of cross-contamination because it can contain 4.8–5.0 $\log_{10}$ cfu *Campylobacter* (16), and it is more likely to rupture during evisceration than intestines (8) after which the content may easily spread out over the carcass, because it is rather fluid (4).

It is hard to prevent colonization of broiler flocks (13); consequently, there is a need for effective intervention measures to reduce colonization levels of broilers at slaughter. Interventions using medium-chain fatty acids, bacteriocins, or phage therapy applied shortly before slaughter have only been shown to decrease colonization levels in ceca (6,18,22). Oral application of volatile fatty acids (VFAs, e.g., formic and acetic acids) might reduce *Campylobacter* levels in ceca and crops because VFAs are effective

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Table 1. Effect of 0.2% volatile fatty acid drinking water treatment during 3 days on colonization levels in crop and ceca of 31-day-old broilers (combined data of two replicate experiments).

Treatment	No. crops	Median *Campylobacter* count and range (log_{10} cfu /g)	No. ceca	Mean *Campylobacter* count ± SD (log_{10} cfu /g)
No	23	4.8 (n.d.[A]–6.5)	24	8.5 ± 0.7
Yes	21	4.0 (n.d.–6.6)	23	8.5 ± 0.7
Test result		$P = 0.50$ (Mann-Whitney *U*-test)		$P = 0.92$ (linear regression)

[A]n.d. = *Campylobacter* not detected, with a detection limit of approximately 0.8 log_{10}/g crop content.

against many bacteria (19), including *Campylobacter* spp. (5). However, Bird *et al.* (18) showed that application of drinking water supplemented with 0.5% formic acid or 0.5% acetic acid results in reduced water consumption in broilers (3), which may result in reduced body weight at slaughter. Lowering the VFA dose may overcome this problem and might still be effective with respect to reducing *Campylobacter* levels in the intestinal tract.

The aims of this study were to determine 1) the correlation of *Campylobacter* colonization levels in ceca and crop and 2) the effect of 0.2% VFA in drinking water on *Campylobacter* colonization levels in both ceca and crops and on body weight gain (BWG).

MATERIALS AND METHODS

Experimental design. Two replicate experiments were carried out. In total, we used 48 *Campylobacter*-colonized commercial broilers (Ross 308), originating from the (nontreated) control group of a *Campylobacter* dose-response intervention study (20). Broilers had been orally inoculated with a dose of *Campylobacter jejuni* C356P varying between 1.19 and 5.47 log_{10} cfu at 14 (Experiment 1) or 18 days (Experiment 2) of age. To ensure independence of observations, birds were housed individually on litter in 40- × 40-cm cages from 14 days of age. Because previous pilot studies did not show any effect of inoculation dose on cecal colonization levels at 4 days postinoculation (data not shown) and birds included in the current study were detected *Campylobacter*-colonized 6 to 11 days before the current study was started (17–22 days of age), a dose effect was assumed to be absent. An effect of possible stress due to individual housing on *Campylobacter* colonization levels was assumed to be absent as well, because previous experiments with individually housed broilers (data not shown) showed cecal colonization levels similar to those observed in groups (e.g., 15). Birds were fed a mash diet, free from antibiotics and anticoccidia drug, *ad libitum* until 6 to 8 hr before the end of the experiments, as described below.

At 28 days of age, birds were weighed and randomly allocated to two groups (treatment or control), each consisting of 12 broilers within each experiment. From 28 to 31 days of age, the treatment group was provided drinking water supplemented with 0.2% (g/g) of a VFA blend, consisting of ammoniated formic acid (90%) and acetic acid (10%). The control group received nonsupplemented drinking water. Water was provided in drinking cups and refreshed twice daily. Control broilers were used to assess the correlation between cecal and crop colonization levels and served as control group to study the effect of VFA supplemented drinking water on these levels. The experimental setup provided 90% statistical power to detect 1.5 log_{10} cfu change in colonization levels in each of the replicate experiments, with expected SD of 1.25 log_{10} cfu (10,16) and α error of 5% (Win Episcope 2, www.clive.ed.ac.uk/winepiscope). Experiments were approved by the animal ethical committee of Utrecht University (2008.II.03.034), and animal care was provided in accordance with the Dutch law on animal experiments.

Sampling and testing. At 31 days of age, feed was withdrawn but water remained available. After 6 to 8 hr, euthanasia was performed by injection of T 61® (Intervet B.V., Boxmeer, the Netherlands) into the jugular vein. After euthanasia, birds were weighed and sexed. Ceca were removed from the carcass aseptically. Ligatures were placed on the esophagus proximal and distal of the crop, and the crop surface was dissected. Subsequently, crop surface was sprayed with 70% ethanol and because the crop contained less than 1 ml of fluid content, 2 ml of saline was injected into the crop. Crops and ceca were transported to the laboratory and processed within 1 hr.

Enumeration of cecal and crop content was performed by adding 1 g of diluted crop content or cecal content to 9 ml of phosphate-buffered saline (PBS) and plating 10-fold dilutions of content (PBS) on modified charcoal cefoperazone deoxycholate agar plates (Biotrading Benelux B.V., Mijdrecht, the Netherlands). All plates were incubated microaerobically at 37 C and examined for the presence of *Campylobacter*-suspect colonies after 48 hr. Microscopic examination of morphology and motility was used as confirmation. The *Campylobacter* levels are expressed in log_{10} cfu/g (diluted) content.

Statistical analyses. The correlation between crop and cecal colonization levels in control birds was assessed by calculating Pearson's correlation coefficient (ρ) and by testing whether ρ was significantly larger than zero.

Colonization levels in crops of treated birds and controls were compared for pooled experiments using the Mann-Whitney *U*-test. Parametric statistics could not be applied, because data was not expected to be normally distributed as crop levels under the detection limit were to be expected (8). Cecal colonization levels in treated birds and controls were compared by linear regression, with treatment and experiment as predictive variables, according to the following equation:

$$Qcecal_i = b_0 + b_1 exp_{i1} + b_2 treatment_{i2} + e_i.$$

Broilers in Experiment 2 were heavier at the start of the VFA treatment, which is likely to affect BWG. Sex also affects BWG. Therefore, the effect of VFA on BWG was assessed by linear regression, with experiment, sex, and treatment as predictive variables, according to the following equation:

$$BWG_i = b_0 + b_1 exp_{i1} + b_2 Sex_{i2} + b_3 treatment_{i3} + e_i.$$

RESULTS

Cecal samples from 47 of the 48 broilers were included in the analyses; one broiler died before the end of the trial. Crop samples were obtained from 44 birds, because three crops ruptured at sampling or could not be analyzed due to overgrowth with concurrent bacterial flora. Cecal contents of all birds were *Campylobacter*-positive and contained 8.5 log_{10} cfu/g on average (Table 1). In control birds, *Campylobacter* was detected in eight of 12 crops (Expt. 1) and 12 of 12 crops (Expt. 2). The median crop colonization level was 4.8 log_{10} cfu/g (Table 1). Cecal and crop colonization levels were not correlated for these birds ($\rho = 0.09$; $n = 20$; $P = 0.71$). In samples from broilers that had received VFA-supplemented drinking water, *Campylobacter* was detected in six of 10 crops (Expt. 1) and 10 of 11 crops (Expt. 2). Because the data did not differ between experiments, pooling of data was considered legitimate. There was no difference in crop colonization levels between VFA-treated birds and control birds ($P = 0.50$; Table 1). Cecal colonization levels also did not differ between control and treated broilers ($P = 0.92$) nor between replicate experiments ($P = 0.11$; Table 1). Moreover, VFA-supplemented water did not have an effect on BWG ($P = 0.87$), corrected for experiment ($P < 0.001$) and sex ($P = 0.023$).

DISCUSSION

The aims of this study were to quantify a possible correlation between crop and cecal *Campylobacter* colonization levels and to quantify the effect of drinking water supplemented with VFA on crop and cecal colonization levels and BWG. No correlation between cecal and crop colonization levels was found, indicating that the lack of correlation between cecal and carcass *Campylobacter* levels might be due to contamination from the crop. Although the total amount of *Campylobacter* was lower in crops than in ceca, the observed crop colonization levels (4.8 $\log_{10}$ cfu/g) are considered to be sufficiently high to cause previously observed carcass contamination levels (17) and were similar to previously reported levels (16). Smith and Berrang (16) compared data from two different studies and suggested that the contribution of crop content to carcass contamination is limited. A study specifically designed to determine to what extent crop content contributes to carcasses contamination would however be required to conclude on this point. Once the mechanism of cross-contamination is understood and the relation between colonization levels in various parts of the intestinal tract and carcass contamination are quantified, interventions that may reduce carcass contamination could be developed.

Water supplemented with 0.2% VFA throughout the 3 days before euthanasia did not result in a significant reduction of *Campylobacter* colonization levels either in the crop or in ceca. This suggests that this treatment will not contribute to a reduction of carcass contamination during slaughter. The lack of effect is possibly caused by the low concentrations that were applied with the aim of preventing reduced BWG. Although BWG was not impaired, water intake during the feed withdrawal period might have been too low to affect *Campylobacter* levels in the crop (2). To date, it is unclear whether crop *Campylobacter* levels are a consequence of ingestion of *Campylobacter* present in the bird environment or a result of true colonization at that site. More insight on its biology might be useful for the development of interventions aimed at reducing crop *Campylobacter* levels. Whether other measures applied shortly before slaughtering, such as phage therapy (14), may reduce crop *Campylobacter* levels needs to be determined.

This study shows that crop content should be considered as one of the additional sources causing carcass contamination. We recommend including quantification of crop colonization levels in future studies on cross-contamination during slaughter to get a more comprehensive quantitative picture of broiler carcass contamination sources.

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