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Factors associated with carcass contamination by *Campylobacter* at slaughterhouse in cecal-carrier broilers

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ABSTRACT

A study was conducted in 2009 to identify risk factors of *Campylobacter* spp. transmission from the digestive tract to the carcasses of standard broilers (slaughter age: 37 day, carcass weight: 1.3 kg on average). Counts of *Campylobacter* were performed on pools of 10 ceca and 10 neck-skins from 108 *Campylobacter* ceca-positive batches in three slaughterhouses. Technical and health data also was collected on the broilers: age, size, carcass weight (mean and standard deviation), condemnation rate, mortality rate and nature of treatment during the rearing period.

Cecal counts varied from 4.8 to 10.2 $\log_{10}$ cfu/g. In seventeen batches (15.7%), the skin count was below the detection limit. In the 91 batches with positive neck-skin test results, the counts varied from 2.0 to 5.2 $\log_{10}$ cfu/g. Standard deviation of carcass weight, condemnation rate, slaughter rate and cecal count were significantly lower and growth rate higher in the 17 batches where neck-skin results were not detected positive. Multivariate analysis showed that batches with higher standard deviation of carcass weight were 5 to 9 fold more at risk of having detectable carcass contamination. Among the 91 positive neck-skin batches, only slaughter rate and cecal counts were found to have a significant but limited effect on the level of neck-skin contamination. As far as body weight homogeneity may be affected by disease, better health control can contribute to a reduction of the contamination of the broiler carcasses in *Campylobacter* carrier batches.

1. Introduction

Campylobacter infections are the most common bacterial cause of foodborne gastroenteritis in humans in the EU (EFSA, 2010), and more generally in most developed countries (Friedman et al., 2000). Human infection is associated most commonly with *C. jejuni* followed by *C. coli*, and *C. lari* (EFSA, 2010). The clinical aspects of the human disease are most often of minor importance and self-limiting; however, in certain cases they may be more severe, especially in immune deficient, younger or older patients, with the possible further development of complications such as reactive arthritis and Guillain–Barré Syndrome (Butzler, 2004; Skirrow and Blaser, 2000). *Campylobacter* spp. is a non-pathogenic bacterium that colonizes the digestive tract of numerous domestic and wild animal species; human contamination therefore may occur under a wide variety of circumstances (Moore et al., 2005). Nevertheless, poultry meat is considered to be a major source of human infection, largely due to contamination by *Campylobacter jejuni*. The most frequent causes are the consumption of undercooked chicken meat and cross contamination from raw poultry meat to other food in the kitchen during meal preparation (knives, chopping board) (Havelaar et al., 2005). However, the exact degree to which poultry meat is responsible for human cases has not yet been determined. The latest sub-typing techniques suggest that 50 to 80% of human cases may be attributed to chickens as a reservoir, yet case–control studies estimate that 24 to 29% of human cases are related to the handling, preparation and consumption of chicken meat (EFSA Panel on Biological Hazards, 2010). If the question is considered from another angle, there was a 40% drop in human cases in Belgium when the consumption of chicken meat was temporarily banned following the dioxin crisis (Vellinga and Van Loock, 2002).

The contamination of broiler meat occurs on the slaughter and processing chain, either at slaughter, when carcasses of colonized birds may become contaminated by fecal matter, or while passing down the line due to cross-contamination (Johannessen et al., 2007). The count of *Campylobacter* found on the carcass varies along the processing chain, with a peak at defeathering followed by a reduction along the rest of the chain, although an increase sometimes has

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been observed at evisceration (Guerin et al., 2010). Thereafter, the level of contamination generally decreases along the processing line, and is reduced even further when freezing is included (Georgsson et al., 2006). As the infective dose of *Campylobacter* is typically low (EFSA, 2010), even batches contaminated at a low level may be considered to be threats to public health. However, risk analyses indicate that highly contaminated carcasses contribute most to the risk of human illness, and that a reduction of fecal leakage would be the intervention with the best cost-utility ratio (Havelaar et al., 2005; Lindqvist and Lindblad, 2008).

During the dressing process at slaughter, fecal contamination of the carcass may be increased by the accidental rupture of the digestive tract. This event may be caused at evisceration if the machine is not correctly adjusted to the size of the carcass (Rosenquist et al., 2006). A large range of carcass weights within a batch therefore may impair the efficiency of the slaughter process as the machine is calibrated for a mean carcass weight (Northcutt, 2001). Heterogeneous weights can have various origins, including disease. Certain diseases furthermore may damage the digestive tract directly (coccidiosis, necrotic enteritis) or cause adhesions between viscera, such as aerosacculitis (Russell, 2003), thereby increasing the occurrence of rupture of the digestive tract at evisceration. Given these considerations, poultry health may be related to food safety as stated by Singer et al. (2007) to support the implementation of a model relating food animal health and human foodborne illness. Therefore it may be useful to assess the relationship between the contamination of the carcasses and some flock health indicators, such as mortality rate, occurrence of treatment, condemnation rate, to determine how the improvement of poultry health could positively influence food safety.

The objective of this study was to identify the factors influencing the transmission of *Campylobacter* from colonized digestive tracts to carcass skin in batches of standard broilers (37 days of age and 1.3 kg of carcass weight on average, fast growing strains, exclusive indoor rearing, high density), with special reference to health indicators of the slaughtered batches.

Table 1
Number of broiler batches tested for *Campylobacter* contamination, separated by risk-factors.

Variable	All lots (n = 108)	Lots with skin counts below detection limit (n = 17)
Campylobacter count in ceca (log_{10} cfu/g)		
<7.5	31	8
7.5–<8.5	45	5
≥8.5	32	4
Age (d)		
<37	38	10
37–38	34	4
>38	36	3
Average daily gain (g of carcass)		
<33.5	28	1
33.5–<37	48	10
≥37	32	6
Standard deviation of carcass weight at slaughter (g)[a]		
<165	32	12
165–<200	37	2
≥200	39	3
Condemnation rate at slaughter (%)		
<0.2	27	9
0.2–<1	52	6
≥1	29	2
Batch size		
<14,000	31	8
14,000–<22,000	45	6
>22,000	32	3
Slaughter rate (n/h)		
<7200	30	7
7200–<9000	40	9
≥9000	38	1
Slaughterhouse		
A	40	2
B	42	12
C	26	3

[a] Threshold values divided by the mean carcass weight give thresholds of 12.6% and 15.2% respectively for coefficient of variation.

2. Materials and methods

2.1. Data collection

The study was conducted from May to August 2009 in three different slaughterhouses that were each owned and operated by three different standard broiler companies. The three slaughterhouses used the same scalding procedure (2 mn 30 s at 51.5 °C–53 °C) followed by mechanical evisceration.

Technical and health data were obtained for each batch from the slaughter and production companies. A batch consisted of broilers from the same flock, i.e. a group of broiler chickens raised together in the same broiler house during the same period of time.

The following data were gathered (Table 1): age at slaughter, number of slaughtered broilers, average weight and distribution of individual carcass weights before chilling (i.e. without condemned carcasses), condemnation rate, mortality rate, and treatments. Treatments were documented by the regulatory information sheet sent by the farmer to the slaughter company specifying the treatments given to the broilers during the last 30 days before slaughtering: health reason, name of the drug, date and number of days of treatment, withdrawal period before slaughter and registration number of the veterinarian's prescription. Although an anticoccidial-additive is given systematically in poultry feed until about 8 days before slaughter, coccidiosis outbreaks nevertheless may occur; the treatment for coccidiosis was recorded specifically. To calculate the average slaughter rate, the duration of the slaughter process was recorded for each batch, from first stunning to the entrance of the last carcass in the chilling equipment.

2.2. Sampling

The *Campylobacter* status of the batches sampled was not known before slaughter. As we aimed to obtain the maximum amount of contaminated poultry batches, the study was undertaken during spring and summer because previous research found that these were the peak periods of infection in France (for example, Huneau-Salaun et al., 2007 and Refregier-Petton et al., 2001). All of the batches slaughtered during one day were sampled over several days in each plant.

Samples were taken from a total of 140 batches. Ten ceca and 10 neck-skin samples were taken from each of batch and pooled together (for a total of 140 pools of ceca samples and 140 pools of neck-skin samples). Detection is based on a quantitative estimation above a detection limit. When 10 samples are pooled, the minimal contamination count theoretically is the highest concentration of the ten samples in the pool divided by ten. While low prevalences of ceca contamination in a batch have been described (Reich et al., 2008; Hansson et al., 2010), levels of contamination generally are high (e.g. >4 log_{10} cfu/g in Rosenquist et al., 2006; 6.9 ± 2.2 log_{10} cfu/g in Reich et al., 2008). Therefore, one contaminated ceca among ten would give a positive result, detecting a prevalence of 30% with 95% sensitivity, which according to Allen et al. (2007) is the threshold between low and high prevalence.

Due to contamination on the slaughter line, the prevalence of carcass contamination is most often either high (>85%) or absent (Hansson et al., 2010) but the level of contamination on the skin usually is lower than in the ceca, and may fall under the detection limit. A pool of ten was then assumed to include, according to the

within batch variability of carcass contamination, some higher levels which would contribute to a positive test result as it was observed, for example, in Reich et al. (2008) and Hansson et al. (2010).

The sampling of ceca was conducted at the sanitary inspection point between defeathering and evisceration following this procedure: a carcass was chosen at random, unhooked from the chain, put on its back on a table, vent oriented toward the operator. One incision was made in the abdominal wall above the vent with a lancet, in order to introduce a finger to hook the ceca and pull them out of the carcass. One of the ceca (if they were of different sizes, the fullest one) was chosen and gently detached to be stored in a sterile plastic bag. The sampling of the neck-skin (4 cm × 4 cm) was conducted after the carcass had been washed inside and out before chilling. A piece of neck skin was cut from a carcass chosen at random on the line with a lancet, without unhooking the carcass. The piece of skin then was cut out with scissors to obtain a 4 cm × 4 cm square and put in a sterile plastic bag.

Sampling started about 20 min after the beginning of the slaughter process in each batch, taking first a cecum, then a neck skin, and was repeated every 3 min until 10 samples of each were gathered. New, sterile instruments were used for each batch (e.g. we used a new sterile lancet for incision at cecum sampling, a separate new sterile lancet for skin removal, and a new sterile pair of scissors for skin sampling). Gloves were changed before each new skin sampling (e.g. new gloves were used for each carcass).

Air was pressed out manually from each plastic bag before it was hermetically closed and stored in a refrigerator before transportation. Batch sampling was scheduled in such as way that pooled samples could be kept chilled (between 2 and 10 °C) and transported to the laboratory within 48 h. Analysis started directly upon arrival.

2.3. Microbiological analysis

Campylobacter was recovered from ceca and neck skins using direct plating and enumeration. The entire contents of all 10 ceca were mixed together and 1 g of the content was then analyzed. Neck skins were weighed, put into a sterile bag and diluted at 1:10 (w/v) in a buffered peptone water broth. The mix then was homogenized for 2 min in a peristaltic homogenizer. Ten-fold serial dilutions of samples were made in tryptone salt broth, ranging from 10^1 to 10^7 for cecal pool samples and 10^1 to 10^5 for skin pool samples. An aliquot from each of the serial dilutions was plated onto a selective modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA), followed by incubation for 44 h ± 4 h at 41.5 °C ± 1 °C under microaerobic conditions. Each plate was examined to find characteristic *Campylobacter*-like colonies. Colony identification was confirmed by gram staining. If the typical spiral morphology was displayed, one colony was subcultured onto blood agar plates and incubated as above for *Campylobacter* genus identification. The enumeration limit of the method was 100 cfu/g.

Among the 140 batches studied, samples from four batches slaughtered on the same day where not assayed because that particular day was very hot and the conservation conditions were deemed unsatisfactory. In two other batches, the carcass weight distribution was not available; and for another batch, samples were subject to an error of manipulation in the assay. Microbiological analyses therefore were performed on 133 batches.

The period between the moment the sample was taken and the time it was delivered to the laboratory ranged between 19 and 49 h, with a seven hour gap, between 32 and 39 h, during which no samples were delivered. Significant negative correlation coefficients were found between delivery time and (i) count of *Campylobacter* in the ceca ($p<0.03$), (ii) count in the neck skin ($p<0.01$). No significant correlation was found for samples whose delivery time was under 32 h, therefore only batches having a delivery time <32 h (n = 118) were taken into account.

2.4. Statistical analysis

The statistical analysis was conducted in two steps. The first step considered the factors associated with the presence or the absence of detectable skin contamination in the batches using the mean of a *T*-test for quantitative variables and a Khi2 test for categorical variables (presence/absence of treatment). A multiple logistic regression model for a multivariate analysis then was computed. Relations between quantitative variables were assessed by Spearman's correlation rate. Relations between standard deviation of carcass weight on one hand, and condemnation rate, slaughter rate and batch size on the other were found to be low or moderate (0.2 to 0.6). Therefore, all significant variables in Table 2 were introduced in the logistic regression model. The logit linearity was checked with regard to the independent variables (4 classes of equal size); none showed any linearity. Three classes consequently were defined for each variable based on each variable distribution (Table 1). Variables were introduced in the model if $p<0.20$ in the univariate analysis. A backward selection, PROC LOGISTIC procedure in SAS® was used and variables were excluded if $p>0.05$. Slaughterhouse effect was tested by a mixed logistic model with slaughterhouse as a random effect.

In the second step of the statistical analysis, the factors associated with the contamination level within the skin positive/ceca positive batches were considered. These factors were assessed by Spearman's correlation rate followed by a multiple linear regression model. The variable selection procedure was similar to that used for the logistic regression model. PROC GLM procedure in SAS® was used and variables were excluded if $p>0.05$.

The effect of treatment on cecal counts was investigated using Student's test. To analyse the effect of the treatments among the positive skin batches, the variable "skin count" was divided into three classes: low (2–<3.15 $\log_{10}$ cfu, n = 23), intermediate (3.15–3.85 $\log_{10}$ cfu, n = 45), high level (>3.85 $\log_{10}$ cfu, n = 23) and the effect was investigated by a Khi2 test. All of the statistical analyses were conducted with SAS statistical software (version 9.1; SAS Institute Inc., Cary, North Carolina).

3. Results

Out of 118 batches, 108 batches carried a detectable number of *Campylobacter* in the cecal contents (Fig. 1), resulting in a prevalence of 91.5%. The counts varied between 4.8 and 10.2 $\log_{10}$ cfu (mean = 7.97 $\log_{10}$ cfu, s.d. = 0.95 $\log_{10}$ cfu). Seventeen of the positive batches (15.7%) were below the detection limit of 100 cfu/g while in the remaining 91 batches, the count of *Campylobacter* in the

Table 2
Comparison of variables in batches according to the status of skin contamination.

	Campylobacter status of skin				
	Below detection limit (n = 17)		Positive (n = 91)		p-value (*T* test)
	Mean	*s.d.*	Mean	*s.d.*	
Mean slaughter rate (broilers/h)	7530.7	1099.7	8478.5	1556.8	$p<0.01$
Delivery time of samples (h)	25	3.3	25.6	3.6	n.s.
Age at slaughter (d)	35.8	2.7	37.6	2.4	$p<0.05$
Average carcass weight (g)	1312.4	119.4	1312.9	117.1	n.s.
Average daily gain (g of carcass)	36.7	2.4	35.1	3.5	$p<0.05$
Carcass weight standard deviation (g)	169.3	40.6	192.2	35.0	$p<0.05$
Cecal count of *Campylobacter* ($\log_{10}$ cfu)	7.4	1.1	8.1	0.9	$p<0.05$
Mortality rate in rearing period (%)	3.5	1.2	3.4	1.7	n.s.
Condemnation rate (%)	0.4	0.5	1.1	1.3	$p<0.001$
Batch size (broilers)	15,152	6435	18,625	6545	$p<0.05$

neck-skin varied between 2.0 and 5.2 $\log_{10}$ cfu (mean = 3.48 $\log_{10}$ cfu, s.d. = 0.62 $\log_{10}$ cfu, n = 91).

The last 10 batches were found to have not detected cecal contents. Five nevertheless were found to be positive for neck skin, with a count varying between 2.0 and 3.3 $\log_{10}$ cfu.

The following results regard the 108 positive ceca batches. Their distribution according to the status of skin contamination and the classes used in the different variables for logistic regression are displayed in Table 1.

A comparison of variables according to skin contamination status is displayed in Table 2. There are significant differences between the two skin status groups: on average, the poultry in the batches that were below the detection limit grew more rapidly, were slaughtered at a lower rate and at a younger age, had a smaller standard deviation of carcass weight, a lower condemnation rate and a lower level of cecal contamination than the poultry in the batches with skin counts above the detection limit.

Various treatments were administrated for colibacillosis (sulfadiazin–trimethoprim, colistin, enrofloxacin, amoxicillin), coccidiosis (amprolium, toltrazuril), and enteritis (tylosin, ampicillin, lincomycin, amoxycillin). No treatment had an identifiable effect on cecal counts. Various conditions were tested: treated/not treated against a disease (3 diseases), treated or not treated against one or both of 2 diseases (3 combinations of disease), treated or not treated whatever the disease(s). No significant difference was found in the proportion of modality among the various conditions between the two batch groups (with detected contamination and without detected contamination) (Table 3).

The computing of the logistic regression models by backward selection showed that the only variable that had a significant effect was "standard deviation of carcass weight" (Table 4; p = 0.01); for values between 165 g and 200 g and for values >200 g, batches were respectively 9.2 and 5.7 times more likely to be positive than batches <165 g. The introduction of the variable, "slaughterhouse", as a random effect had no effect in the model. To take into account confounding effect, different variables that had been found previously to be nonsignificant were introduced successively in the model. Only introduction of "age at slaughter" changed the estimated OR (variation of 21%), this variable therefore was kept for adjustment in the final model.

In the analysis of the 91 batches with a positive neck-skin sample, weak but significant correlations were found between the contamination level of neck-skin and, respectively, the level of ceca contamination (r = 0.28, p = 0.007) and the slaughter rate (r = 0.22, p = 0.049). Distribution of these batches according to their respective *Campylobacter* counts in the ceca and on the skin is displayed in Fig. 2. A positive tendency was found with the condemnation rate (r = 0.19, p = 0.067). The proportion of therapeutic treatments did not differ between classes of contamination. A significant multiple linear regression model was performed (p<0.004) which accounted for 12% of the total variability and showed that the variables "cecal count" and "slaughter rate" were positively associated with the variable "skin count".

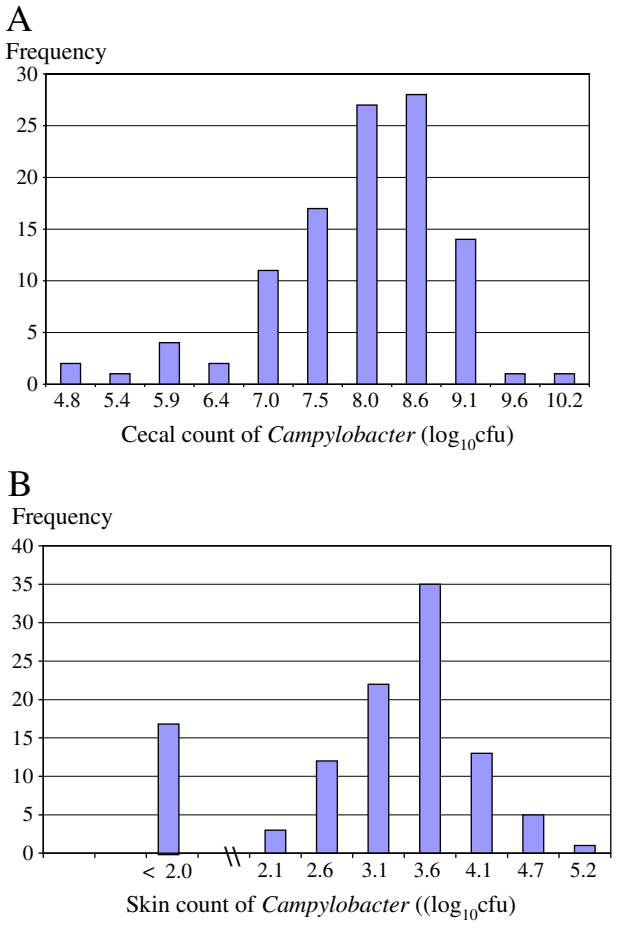

Fig. 1. Distribution of (A) cecal and (B) skin counts in pools of ten individual samples in *Campylobacter* colonized batches (n = 108).

4. Discussion

The present study successfully included in the survey a sufficient number of broiler batches colonized by *Campylobacter spp*. before slaughter thanks to the high prevalence rate (91.5%), which had been expected in the summer based on the findings of previous studies in France (Huneau-Salaun et al., 2007; Refregier-Petton et al., 2001). The sampling method was conducted at the batch level and involved pools of samples to qualify presence/absence and to evaluate the level of contamination for each batch. The rational for this procedure, which is the same as that followed by Hansson et al. (2007), was presented in the preceding Materials and methods section. This approach differs from those used in other studies which focus on within batch percentages of contaminated carcasses (Arsenault et al., 2007) or the percentage of positive samples (Figueroa et al., 2009).

The manner by which *Campylobacter* rapidly colonizes a broiler batch as soon as it enters a poultry facility has been described extensively (see, for example, Berndtson et al., 1996). Nevertheless, as shown by Hansson et al. (2010), Allen et al. (2007) and Rosenquist et al. (2006), it can be demonstrated that, although 0 and 100% are the values most frequently found, the percentage of positive individual cecal counts in a batch may vary between 0 and 100%. Therefore, a pooled sample of 10 ceca would allow the detection of one positive sample with counts >3 log10 cfu. This appears to be a sufficiently sensitive and reliable indicator that a batch is actively shedding

Table 3
Distribution of treatments among the batches with neck-skin status for *Campylobacter* below detection limit or positive.

Reason for treatment	Number of batches treated at least once with *Campylobacter* skin status		P value (Khi²)
	Below detection limit (n = 17)	Positive (n = 91)	
Colibacillosis	3	25	0.39
Coccidiosis	2	17	0.49
Enteritis[a]	11	38	0.08
Coccidiosis and/or Enteritis[a]	11	43	0.18
Enteritis[a] and/or colibacillosis	12	50	0.23
Colibacillosis and/or Coccidiosis	5	37	0.38
Colibacillosis and/or Coccidiosis and/or Enteritis[a]	12	54	0.38

[a] Other than Coccidiosis.

Table 4
Final multivariable logistic regression model for risk-factors for *Campylobacter* neck skin contamination in batches of broilers carrying *Campylobacter* in the ceca (n = 108).

Variable	Odds- ratio		95% CI
	Estimate	Global P value	
Standard deviation of carcass weight at slaughter (g)			
<165			
165–<200	9.2	0.01	1.8, 47.7
≥200	5.7		1.2, 28.1

Campylobacter when it enters the slaughterhouse, representing, therefore, a source of contamination for carcasses. Allen et al. (2007) detected three batches with a low-prevalence of contaminated ceca and very low counts in ceca (mainly detected after enrichment). In these batches, a large proportion of the carcasses were contaminated at a low level, but these batches also were exposed to a cross contamination from fully colonized batches slaughtered previously. In our study, it is noteworthy that no intermediate count was found between the detection limit and the count of 4.8 $\log_{10}$cfu, suggesting a clear cut difference in status between positive batches and batches under the detection limit.

The cecal counts ranged from 4.2 to 10.6 $\log_{10}$ cfu which appear similar to those observed in a contemporary survey conducted on a larger scale in France (Hue et al., 2010) following a similar protocol.

In the study of Hansson et al. (2010), the percentage of positive carcasses (rinsed carcasses after chilling) was either 0% or between 85 and 100%. This distribution is fairly different from the results of Arsenault et al. (2007), who found that the prevalence of *Campylobacter* positive carcasses varied between 0 and 100% with a mean of 35.8% and a 95% confidence interval of 27.1–44.5%. In the latter study, the authors observed that over 25% of the carcasses were contaminated in *Campylobacter* colonized batches (i.e. 8 out of 30 samples per batch) which is close to the 30% threshold rate used in our study. Unfortunately, the detection limit and *Campylobacter* counts were not given in the paper so it is not possible to make further comparisons. However, as other studies have reported higher incidence rates and sufficiently high levels of contamination, it seemed reasonable to consider that a pooled sample of 10 neck-skins was a reliable indicator that could be used to study the link between contamination of skin and ceca.

With the limitation on the comparison that only one carcass per batch was sampled in their study, Hue et al. (2010) found a range of count values in skin that were lower (1.0–4.4 $\log_{10}$ cfu) than our results (2.0 to 5.2 $\log_{10}$ cfu). This discrepancy may be explained by the likelihood that the lowest value is linked to the lower threshold of the detection method (1.0 vs 2.0 $\log_{10}$ cfu) and that the highest values are linked to a sampling done after chilling rather than before as in our study. Chilling is known to significantly lower the level of contamination of the carcasses by about 1 $\log_{10}$ cfu (Guerin et al., 2010).

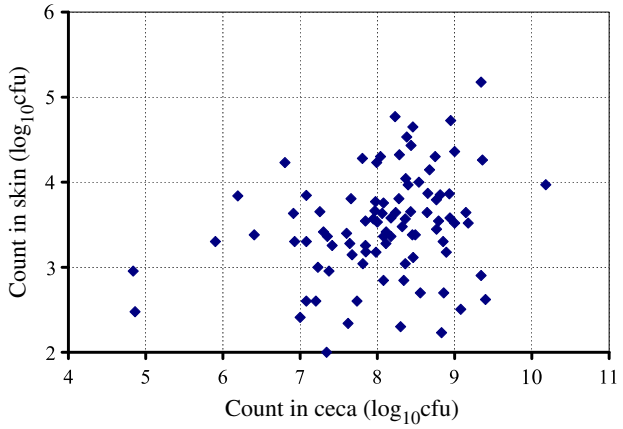

Fig. 2. Distribution of carcass positive batches according to their respective counts of *Campylobacter* in ceca and on skin (n = 91). Each batch is represented by a plot.

The present study demonstrates that the contamination of carcasses by *Campylobacter* is not systematic within a batch containing chickens harboring the bacteria in their digestive tract. This was recorded in all three slaughterhouses, all of which followed the same scalding and evisceration procedures up to the stage of neck-skin sampling. This observation was made in a context where, for food safety reasons, the main concern is to avoid cross contamination between batches, i.e. that a carrier batch contaminates the carcasses of non-carrier batches following after it on the slaughter line (Johannessen et al., 2007; Reich et al., 2008). To our knowledge, no study on the rate of intra-batch contamination by *Campylobacter* has been conducted to date. Data could nevertheless be calculated based on the findings of Hansson et al. (2007): from 2002 to 2005, 10.4% of the 2638 batches with positive cloacal swabs (with a range of annual occurrence of 8.6% to 12.5%) were not detected positive on neck-skin before chilling, compared with 15.7% in this study. The lower mean value in Hansson et al. may be explained by the lower threshold of detection on skin (1 vs 2 $\log_{10}$ cfu), which may increase the number of batches detected positive, combined with the use of a transport medium and a shorter delivery time of the samples to the laboratory, providing better survival conditions to *Campylobacter*.

The present study also demonstrates that heterogeneity in carcass weight within a batch can be a risk factor for contamination of carcasses by *Campylobacter* spp. in batches of broilers carrying *Campylobacter* in their digestive tract. Little information is available on this parameter in the literature. In our sample, 70% of the batches had a standard deviation of carcass weight >165 g, which represents an average coefficient of variation of 12.6%. In the study of Arsenault et al. (2007), only 23% of the batches, which were reared in single-sex flocks, showed a coefficient of variation>12%. However, in contrast with our results, no cecal carrier batch was found to be free of carcass contamination. It would have been interesting to consider whether the slaughter process involved in the Arsenault et al. (2007) study structurally enabled a larger contamination than that found in our study and that of Hansson et al. (2007). In our study, the slaughterhouse was taken into account in the mixed model but did not have a significant effect. Only a low positive correlation rate was found between the slaughter rate and the count of *Campylobacter* on the skin.

When a carcass was contaminated by *Campylobacter*, the level of skin contamination appeared to be poorly correlated (r = 0.28) to the level of cecal contamination in the batches where the skin test was positive. Various studies conducted in the past have assessed the colonization of ceca in relation to different variables (mean of individual counts, pooled samples, individual counts), with conflicting results. Allen et al. (2007) found no correlation between numbers of *Campylobacter* detected in the ceca and on carcasses, yet Chemaly et al. (2010) and Reich et al. (2008) found higher correlation rates (0.59 and 0.81 respectively). In contrast to our study, batches, the ceca of which were not detected positive, were taken into account in these two studies for the correlation rate calculation, resulting in a larger range of cecal counts.

The role of weight heterogeneity in the contamination of broiler carcasses by *Campylobacter*, which has been suggested in the past but previously never evaluated on a sample, seems to be validated in our study. When there are a variety of weights within a batch, some of the broilers will not fit the carcass format of the slaughtering machine, with one consequence being fecal leakage on the carcasses. Weight heterogeneity furthermore may be an indicator of a lower health status, as illustrated by the lower growth rate and the higher condemnation rate in the batches where neck skin was found to be contaminated. Other health indicators, i.e. therapeutic treatments, were studied but were found related neither to the status nor to the level of contamination of the skin. This may be explained by the

hypothesis that, in case of disease, some slightly diseased and/or well treated flocks succeed in maintaining homogeneous weights while other, highly diseased and/or unsatisfactorily treated flocks do not.

5. Conclusion

Alongside efforts to reduce *Campylobacter* prevalence in chicken batches and cross contamination of carcasses on the slaughter line, measures should be taken to homogenize the weights of birds in a batch. This homogenization, which can be achieved through an improvement in the health status of broilers, can contribute to lowering the risk of contamination of broiler carcasses by *Campylobacter* when batches entering slaughterhouses are carriers of the bacteria in their intestinal tracts.

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