Food Microbiology 29 (2012) 105–112

Contents lists available at SciVerse ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Campylobacter contamination in broiler carcasses and correlation with slaughterhouses operational hygiene inspection

Ihab Habib[a,h,*], Dirk Berkvens[b], Lieven De Zutter[c], Katelijne Dierick[d], Xavier Van Huffel[e], Niko Speybroeck[f], Annemie H. Geeraerd[g], Mieke Uyttendaele[a]

[a] Laboratory of Food Microbiology and Food Preservation, Faculty of Bioscience Engineering, Ghent University, Coupure links 653, B-9000 Ghent, Belgium
[b] Department of Animal Health, Unit of Epidemiology and Biostatistics, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium
[c] Department of Veterinary Public Health and Food Safety, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium
[d] Scientific Service Food-borne pathogens, Operational Directorate for Communicable and Infectious Diseases, Institute of Public Health, Brussels, Belgium
[e] Staff Direction Risk Assessment, Federal Agency for the Safety of the Food Chain (FASFC), Kruidtuinlaan 55, B-1000 Brussels, Belgium
[f] Institute of Health and Society, Université Catholique de Louvain, Clos Chapelle-aux-Champs 30, bte 30.05, 1200 Brussels, Belgium
[g] Division of Mechatronics, Biostatistics and Sensors (MeBioS), Department of Biosystems (BIOSYST), Katholieke Universiteit Leuven, W. de Croylaan 42, B-3001 Leuven, Belgium
[h] Division of Food Hygiene and Control, Department of Nutrition, High Institute of Public Health (HIPH), Alexandria University, 165 El-Horrya Avenue, Alexandria, Egypt

ARTICLE INFO

Article history:
Received 17 June 2011
Received in revised form
7 September 2011
Accepted 8 September 2011
Available online 17 September 2011

Keywords:
Broiler carcasses
Campylobacter
Risk factors
Slaughterhouse hygiene
Survey

ABSTRACT

This study investigates factors associated with *Campylobacter* contamination of broiler carcasses, using survey data collected from nine Belgian slaughterhouses in 2008 in accordance with a European Union baseline study. *Campylobacter* were detected in 51.9% (202/389) (95% confidence interval, 46.8%–56.9%) of broiler carcasses. *Campylobacter* concentration was <10 CFU/g in 49.6% of carcasses, while 20.6% were contaminated with ≥1000 CFU/g. The mean *Campylobacter* concentration, as calculated by maximum likelihood estimation for left-censored data, was 1.8 $\log_{10}$ CFU/g, with a standard deviation of 1.9 $\log_{10}$ CFU/g. There was statistically significant variation among slaughterhouses in prevalence and concentrations of *Campylobacter* in their sampled carcasses. *Campylobacter* prevalence (but not concentrations) was positively associated with increase in broilers age. Both *Campylobacter* prevalence and concentration were significantly higher in carcasses sampled during June and September (but not in July and August) than carcasses sampled in January. We also investigated the correlation (Spearman's rank correlation test) between the scores of official control inspections and *Campylobacter* prevalence for eight out of the nine slaughterhouses. The control inspections were routinely performed by the Belgian Federal Agency for the Safety of the Food Chain, and the concluded inspection scores were used as a general numerical indicator for the status of operational hygiene and quality of management in the slaughterhouses. Ranking of slaughterhouses based on their inspection scores was statistically correlated (Spearman's correlation coefficient = 0.857) with their ranking based on prevalence of *Campylobacter*. In the present study we demonstrate how the outcomes from a routine baseline survey could be coupled with other readily available data from national control authorities in order to enable a better insight over *Campylobacter* contamination status in broiler slaughterhouses. Findings from this work call for subsequent in-depth investigations on technical and hygiene management factors that could impact *Campylobacter* contamination across broiler slaughterhouses.

1. Introduction

Campylobacter jejuni and *Campylobacter coli* are among the most common bacterial causes of human gastroenteritis worldwide. Infected humans exhibit a range of clinical symptoms varying from mild, watery to severe inflammatory diarrhoea (Humphrey et al., 2007). In addition, *C. jejuni* has been identified as an important infectious trigger for Guillain-Barré syndrome, the most common cause of acute flaccid paralysis in polio-free regions (Godschalk et al., 2004). In Belgium, 47.9 cases of human campylobacteriosis per 100,000 people were reported in 2008 by the Network of National Reference and Sentinel Laboratories (Anonymous, 2009). The majority of human campylobacteriosis cases are sporadic, and

* Corresponding author. Laboratory of Food Microbiology and Food Preservation, Faculty of Bioscience Engineering, Ghent University, Coupure links 653, B-9000 Ghent, Belgium. Tel.: +32 09 264 60 85; fax: +32 09 225 55 10.
E-mail address: ihab.habib@ugent.be (I. Habib).

0740-0020/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fm.2011.09.004

consumption or handling of contaminated raw or undercooked poultry meat is believed to be an important vehicle of infection (Anonymous, 2009). Gellynck et al. (2008) estimated the cost of illness associated with *Campylobacter* infection and sequelae in Belgium as € 27.3 million (estimate for the year 2004). According to their estimation, 40% of these costs are attributable to the consumption of poultry meat. This is in concordance with other source-attribution studies, outbreak investigations and case–control reports that incriminate chicken meat as a major source of the foodborne transmission of *Campylobacter* (Butzler, 2004; Friedman et al., 2004; Humphrey et al., 2007; Mullner et al., 2009).

Campylobacter inevitably finds its way to the chicken meat surface when carcasses are contaminated with intestinal contents during plucking and evisceration (Guerin et al., 2010; Rasschaert et al., 2006). In addition, the defeathering and cross-contamination between *Campylobacter*-positive and negative broiler batches at slaughter have been speculated to contribute to the overall carcasses contamination (Normand et al., 2008; Takahashi et al., 2006). Added to that, it has been shown that *Campylobacter* prevalence and concentration in chicken meat varies significantly in correlation with processors (Habib et al., 2008; Sampers et al., 2010). To understand factors that govern *Campylobacter* contamination variability, there is a need for intensive microbiological baseline studies across national broiler slaughterhouses. Quantitative (enumeration) and qualitative (presence/absence) microbiological data from such baseline studies can be important for evaluating different slaughterhouses and their food safety management programs (Lindblad et al., 2006). Additionally, a national survey across broiler slaughterhouses is an important tool for investigating processing conditions that must be controlled to prevent, eliminate, or reduce *Campylobacter* contamination (Sampers et al., 2008).

In addition to insights gained from microbiological baseline surveys on *Campylobacter* contamination in broiler slaughterhouses, results from official (e.g. by governmental bodies) food control inspections could add further insights about the status of quality management and operational hygiene in slaughterhouses (Hudson et al., 1996). In its essence, official food control inspection is a tool for directing enforcement efforts, as well as a tool for monitoring hygienic standards. These standards could be related to staff, premises, equipment and implementation of hygienic requirement for slaughter and handling of fresh carcasses. Contrasting the official inspection score for a slaughterhouse with *Campylobacter* contamination level in its carcasses seems an obvious area for evaluating the usefulness of such inspection scores for assuring safe product.

The aim of this study was to investigate factors associated with carcasses contamination with *Campylobacter* in nine Belgian slaughterhouses, using survey data collected in 2008 during the European Union baseline study (Anonymous, 2010). Moreover, the relation was investigated between results of the slaughterhouses control inspection checks, routinely performed by the Belgian Federal Agency for the Safety of the Food Chain (FASFC), and the status of *Campylobacter* contamination among the surveyed slaughterhouses.

2. Materials and methods

2.1. Survey design and sampling

The survey was executed in accordance with the requirements for a European Union-wide baseline survey on *Campylobacter* contamination in broiler carcasses (Anonymous, 2007). In Belgium, the population concerned was 9 poultry slaughterhouses that each process more than 10,000,000 carcasses per year. These were selected to fulfill an inclusion criterion for a targeted sampling approach that includes slaughterhouses supplying more than 85% of the Belgian broiler distribution chain (Anonymous, 2006a). The slaughterhouses were certified for operating under HACCP (hazard analysis critical control points) principles. Added to that, the major operational procedures were similar; for instance, all of the slaughterhouse use soft scalding procedures and all use air chilling systems. The analysis unit was one post-chill carcass (before further processing such as freezing, cutting or packaging) sampled from independent broiler batch. Here, a batch is a term used to define a group of broilers which have been raised in the same holding (farm of origin) and which are delivered and slaughtered on one single day. The sample size was based on a harmonized guideline across the European Union, and assuming annual prevalence of ~50% with desired confidence interval of 95% and 5% accuracy (Anonymous, 2007). In total, 389 carcasses were sampled proportional to slaughterhouse production. Sampling was done between January and December 2008, with equal number of samples collected per month. Samples were collected by trained inspectors from the Belgian FASFC, and were sent to the analysis laboratory under controlled temperature condition within 24 h of collection. Analysis of carcasses took place in two laboratories licensed by the Belgian Federal Service for Public Health and accredited in accordance with the requirements of ISO standard 17025. The two laboratories were located in two Belgian provinces and samples were distributed among them according to their geographical location.

2.2. Microbiological analysis

Campylobacter enumeration and qualitative detection were performed according to the guidelines of the ISO 10272:2006 methods (part 1 and 2) (Anonymous, 2006b,c). Neck skin was removed together with skin from one side of the carcass (breast skin), and avoiding as much visible fat as possible. A test portion of ~27 g was homogenized with 243 ml (~9*x* volume) of buffered peptone water (BPW, CM0509 [Oxoid, Basingstoke, England]). From this initial homogenate, testing was carried out in parallel as follows:

(I) For enumeration, 10 ml (~1 g) was transferred to a sterile tube and 1 ml of it (10^{-1}) was spread plated over three (0.3, 0.3, 0.4 ml) modified charcoal cefoperazone deoxycholate agar plates (mCCDA; *Campylobacter* blood-free selective medium CM739 plus selective supplement SR155 [Oxoid, Basingstoke, England]) in duplicate, in addition to spread plating of 0.1 ml from the same dilution on mCCDA. Two further serial dilutions (10^{-2} and 10^{-3}) were made in BPW, and 0.1 ml from each was spread plated on mCCDA. Plates were incubated in a microaerobic atmosphere achieved by introducing a gas mixture of 5% CO_2, 5%O_2, 5%H_2, and 85% N_2 in stainless steel jars (10-L size; Don Whitley Scientific, West Yorkshire, United Kingdom). Agar plates were incubated at 41.5 °C and counted after 48 h.

(II) For presence/absence testing, 10 ml (~1 g) of the same sample homogenate was added to 90 ml of Bolton broth (BB; Bolton broth CM0983 plus supplement SR183 [Oxoid, Basingstoke, England] with 5% [vol/vol] lysed horse blood [E&O Laboratories, England]). The enrichment broth was then incubated in a microaerobic atmosphere for 4 h at 37 °C and then for 44 h at 41.5 °C. Subsequently, 10 μl of the enrichment culture was plated onto mCCDA and incubated for 48 h at 41.5 °C.

Confirmation of presumptive *Campylobacter* colonies at the genus level was performed according to the ISO 10272:2006 principles (Anonymous, 2006b,c).

2.3. The control inspection checks and scoring of slaughterhouses

Results of the official inspection in the year 2008 for 8 out of the 9 slaughterhouses were provided by the Belgian FASFC (referred to in the following text as "FASFC-check"). "FASFC-check" was based on an audit, completed by trained inspectors, addressing different aspects related to general quality management, infrastructure and operational hygiene (Table 1). Inspectors visit these slaughterhouses regularly and perform a control audit according to a published set of guidance notes and using a nationally harmonized checklist referred to as "DPA-2286: inspection checklist for infrastructure, equipment and hygiene of poultry and small game slaughterhouses". This checklist is designed to assure satisfactory supervision of the slaughter hygiene in accordance with requirements of EU and Belgian regulations (for examples, EC regulation No 852/2004 on the hygiene of foodstuffs, EC regulation No 853/2004 laying down specific hygiene rules for food of animal origin, EC regulation No 1935/2004 on materials and articles intended to come into contact with food, EC regulation No 999/2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies, the Belgian Royal Decrees on premises registration (16/01/2006), on the hygiene of food of animal origin (22/12/2005) and on quality of water intended for use in food establishments (14/01/2002)).

In total 138 items are assessed and scored according to the standards observed. The main sections of the "FASFC-check" are listed in Table 1, and the full checklist could be downloaded through the following link: [http://www.favv.be/checklists-fr/_documents/FAVVChecklist-2286v3fr.pdf]. The output of "FASFC-check" indicates the number of severe deficiencies in the slaughterhouse's quality management, operational hygiene and infrastructure requirements; these are referred to with the term "non-conformity". Follow up of non-conformity scores on frequent inspection visits provides useful management data indicating whether the premises are improving or whether, whilst still meeting legal requirements, might be failing to maintain selected aspects of slaughterhouse operational hygiene and quality standards. The number of recorded nonconformities and the associated number of inspected items for each broiler slaughterhouse were extracted from the "FASFC-check" database.

2.4. Statistical analysis

Campylobacter survey results and data on "FASFC-check" for slaughterhouses were received in a Microsoft Excel spreadsheet format. Data were transferred to, and all statistical analysis carried out using the software STATA v.11.0 (StataCorp LP, College Station, TX). The explanatory variables included in the regression models

Table 1
Main sections of the checklist control conducted the Belgian Federal Agency for the Safety of the Food Chain ("FASFC-check") for broiler slaughterhouses.

Controlled items	General description	Number of requirements
Part 1: Requirements for food processing establishments		
1. General requirements on premises infrastructure	e.g. the premises space is appropriate, processing flow to prevent cross-contamination, separate locker room, separate and sufficient lavatories, sinks with appropriate taps and hygienic hand drying, etc.	17
2. Special requirements for premises sections in which foods are processed, handled, prepared	e.g. appropriate floor covering, walls and ceilings, smooth and easy to clean surfaces, separate storage facility for cleaning material, overall cleanliness of the locations, etc.	21
3. Requirements for tools and apparatus in contact with foods	e.g. hygienic design, maintenance, cleaning and disinfection frequency, the use of good practices and appropriate food grade chemical maintenance products, etc.	5
4. Hygiene requirements applicable to food handling	e.g. no entry of domestic animals, pest control, adequate storage of dangerous chemicals, use of appropriate labeling and application of biocides, insecticides etc.	11
5. Hygiene requirements applicable to packaging and pack material of foods	e.g. packaging material not to be exposed to contamination, availability of clean recipients and storage facility, etc.	6
6. Infrastructure and equipments for water supply	e.g. availability of sufficient potable water, frequency of control of water quality in accordance to legal requirements, non potable water distributed in separate tubing, etc.	3
7. Personnel Hygiene and training	e.g. medical certificate for personnel, appropriate and clean uniform, respect of personnel hygiene by personnel, training in personnel hygiene foreseen according to a defined plan, etc.	8
8. Handling of food waste, non consumable byproducts and other waste	e.g. no accumulation of waste in locations in which foods are handled, hygienic removal of waste, the use of closed containers for waste storage, appropriate and timely cleaning of waste containers, location for storage of waste are well maintained, treatment of wastewater, etc.	9
9. Auto-control systems	Documented HACCP plan in place	1
Part 2: Slaughterhouse specific requirements		
1. Registration is approved by competent authority	Administrative checks	4
2. Infrastructure of slaughterhouse	e.g. separated roofed location for receipt of animals, facilities for prevention of cross-contamination, availability of methods to disinfect utensils during slaughter, separate locations for offal, separate locations for cleaning and disinfection of crates and other transport material, control of temperature in refrigerators and freezers, etc.	23
3. Hygiene during slaughter	e.g. preventive measures taken to prevent contamination during evisceration, no use of wooden materials, no slaughter of sick animals, cool down to less than 4 °C as soon as possible after the evisceration step, timely communication on slaughter time and batch size to the official veterinary officer, etc.	29
4. Presence of Identification stamp/mark	To ensure traceability of slaughter-batches	1

were; Slaughterhouse of origin (categorical variable, A to I), month of sampling (categorical variable, January to December), age of birds (continuous variable, in days), status of thinning (dichotomous variable, yes or no) time between sampling and testing (dichotomous variable, testing in the same day or in a following day), and the testing laboratories (dichotomous variable, laboratory A or laboratory B). Univariate analyses were carried out to identify the main trend, variability and distribution of each individual variable. Variables with more than 20% of missing data (e.g. the status of thinning was reported for only one slaughterhouse) and those for which there was no variability were removed (e.g. the time between sampling and laboratory testing was the same in all samples) (Hue et al., 2010). Only factors significantly associated with *Campylobacter* contamination (likelihood ratio test $p < 0.20$) were considered for further analysis.

2.4.1. For "FASFC-check" data

Non-conformity scores based on "FASFC-check" were used as a global indicator for the status of quality of management and operational hygiene. The scores were calculated for each slaughterhouse, as: (Number of recorded nonconformities/Number of audited items) × 100. The correlation between slaughterhouse ranking based on inspection scores and their ranking based on *Campylobacter* prevalence was evaluated using Spearman's rank correlation coefficient. Ranking starts from 1 (lowest; in inspection scores (nonconformity score)/and in *Campylobacter* prevalence) to 8 (highest).

2.4.2. For qualitative detection results

Presence of *Campylobacter* in a carcass was recorded as positive if detected by direct-plating and/or enrichment culture. Logistic regression analysis was used to examine the association between presence/absence (binary variable) of *Campylobacter* and the following explanatory variables; the slaughterhouse (categorical variable), age of birds in days (continuous variable) and month of sampling (categorical variable).

2.4.3. For enumeration data

Results with no recovered *Campylobacter* count were reported as "less than 10 CFU/g" (below the limit of *Campylobacter* quantification by direct plating method). Hence, the count data are left-censored due to limitations of the testing method that prevents knowing the true value of the dependent variable despite having some measurement of it. The summary statistics (mean and standard deviation) of *Campylobacter* concentrations over the 389 carcasses was calculated using maximum likelihood estimation (MLE) for left-censored data. A description of the application of the MLE method for censored microbiological data can be found in Busschaert et al. (2010). Tobit regression analysis was used to examine the association between *Campylobacter* concentrations and the explanatory variables of slaughterhouse, age of birds and month of sampling. The Tobit regression approach was recommended for handling food microbiology data containing left-censored values (below the limit of Quantification) (Lorimer and Kiermeier, 2007). It is similar to ordinary least square regression except the coefficients are fit by MLE (Lubin et al., 2004).

Both the logistic regression analysis (for qualitative detection results) and Tobit regression analysis (for concentration data) were run with incorporated random-effects to account for a possible clustering at the holding level (from which the slaughtered-batch originates). Statistical significance was declared at *P*-value of <0.05.

3. Results

Results in Table 2 show that 51.9% (202/389) (95% CI, 46.8%–56.9%) of the tested carcasses were positive for *Campylobacter*. The overall status of positive is a combination of all positive results obtained by direct plating and/or enrichment cultures. More *Campylobacter* positive samples were recovered by direct plating compared to enrichment culture; 134 samples were positive by direct plating but were negative by enrichment culture, while 6 samples were positive by enrichment culture but were negative by direct plating, and 62 samples were positive by both culture methods. The concentration data showed a skewed distribution to the right, as *Campylobacter* concentrations in almost half (49.6%) of the tested carcasses were <1 $\log_{10}$ CFU/g, while 20.6% were contaminated with ≥3 $\log_{10}$ CFU/g (Fig. 1). The mean *Campylobacter* concentration, as modeled by maximum likelihood estimation for left-censored data, was 1.8 $\log_{10}$ CFU/g, with a standard deviation of 1.9 $\log_{10}$ CFU/g.

Table 2
Distribution of *Campylobacter* contamination in chicken carcasses from 9 Belgian slaughterhouses.[a]

Slaughter house	No. of samples	Total no. (%) positive[b]	Frequency of count bands/no. (%)		
			<1 $\log_{10}$ CFU/g[c]	≥ 1 & <3 $\log_{10}$ CFU/g	≥3 $\log_{10}$ CFU/g
A	20	13 (65.0)	7 (35.0)	6 (30.0)	7 (35.0)
B	66	39 (59.1)	28 (42.3)	27 (41.0)	11 (16.7)
C	50	28 (56.0)	23 (46.0)	11 (22.0)	16 (32.0)
D	30	23 (76.6)	7 (23.3)	14 (46.7)	9 (30.0)
E	38	17 (44.7)	22 (57.9)	12 (31.6)	4 (10.5)
F	47	29 (61.7)	18 (38.3)	18 (38.3)	11 (23.4)
G	50	21 (42.0)	31 (62.0)	12 (24.0)	7 (14.0)
H	64	23 (35.9)	41 (64.1)	11 (17.2)	12 (18.7)
I	24	9 (37.5)	16 (66.7)	5 (20.9)	3 (12.4)

[a] $n = 389$, January to December 2008.
[b] number of all positive results as obtained by direct plating and/or enrichment cultures.
[c] below limit of quantification for the direct plating method.

There was considerable variability in *Campylobacter* contamination between slaughterhouses (Table 2). Worth highlighting, ≥30% of broiler carcasses sampled from slaughterhouses A, C, and D had a *Campylobacter* contamination level of ≥3 $\log_{10}$ CFU/g. Slaughterhouse D provided the highest percentage of *Campylobacter* positive carcasses (Table 2). Careful investigation of the data (Fig. 2) showed that birds slaughtered in slaughterhouse D were significantly (*t*-test, $P < 0.001$) (mean age of 81 days) older than birds from the other slaughterhouses (mean age of 41 days).

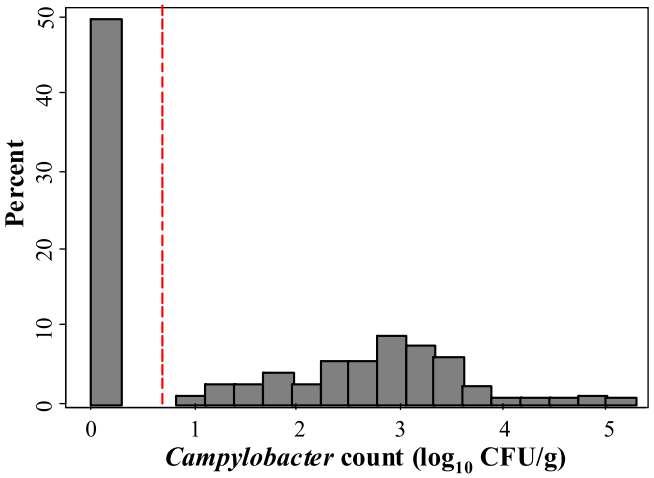

Fig. 1. Distribution of *Campylobacter* counts in 389 broiler carcasses. The scale on the *y* axis shows the percent of samples that fall within the range of *Campylobacter* counts represented by the bars on the *x* axis. The dashed line separates samples with enumerable results from those below the limit of quantification (<1 $\log_{10}$ CFU/g).

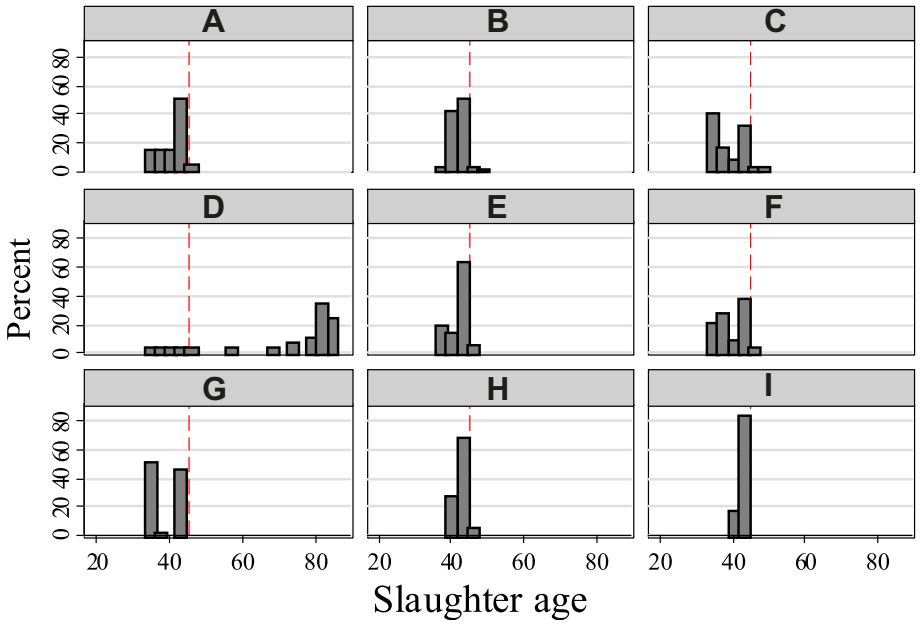

Fig. 2. Distribution of slaughter bird's age (in days) in broiler carcasses sampled from the 9 slaughterhouses (A to I). The dashed line denotes bird age of 45 days. The scale of the *y* axis shows the percent of carcasses that fall within the range of age represented by the bar on *x* axis.

Slaughterhouse D was the only premise among the 9 abattoirs that slaughtered free-range and organic birds. These birds tend to be older at slaughter as they differ from the common intensively-produced broilers in their rearing and management systems.

Results of the effect of slaughterhouse, sampling months and broiler's age as risk factors for *Campylobacter* contamination in broiler carcasses are given in Table 3. The initial regression models also included interactions between the variables of interest. However, none of these interactions were statistically significant ($P > 0.05$). Slaughterhouses H and I had significantly lower odds of providing *Campylobacter* positive carcasses, while slaughterhouses E, G, H and I provided significantly lower *Campylobacter* concentrations compared to carcasses from slaughterhouse A (the reference category in the regression model). The increase in age of birds was significantly associated with an increase in the odds of a carcass becoming *Campylobacter* positive, however was not associated with a significant increase in *Campylobacter* concentrations (Table 3). Both *Campylobacter* prevalence and concentration were significantly higher in carcasses sampled during June and September (but not in July or August) than carcasses sampled in January. As presented in Fig. 3, the significant increase in *Campylobacter* concentration in June and September was associated with a change in the pattern of counts frequency distribution. Compared to other months, the frequency of carcasses with <1 $\log_{10}$ CFU/g was much lower in June and September; that accounted for $\sim$20% of samples, compared to at least 45% in any other month (Fig. 3).

With regard to the official inspection of slaughterhouses, "FASFC-check", Fig. 4 indicates that there was tangible variability in non-conformity scores reported over the eight slaughterhouses (slaughterhouse E was not part of this check). "FASFC-check" evaluated 138 items, and in 6 out of the 8 slaughterhouses this inspection was performed twice. Highest non-conformity score rate was 14.8% (41/276) for slaughterhouse D, followed by 13.4% (37/276) for slaughterhouse A, then 11.6% (16/138) for slaughterhouse F, and 9.4% for slaughterhouse B, then 9.1% (25/276) for slaughterhouse H, followed by 6.2% (17/276) for slaughterhouse C, followed by 5.4% (15/276) for slaughterhouse G, and the lowest was slaughterhouse I with a score of 3.3% (9/276). Interestingly, the rank of slaughterhouses based on their official control inspection scores, "FASFC-check", and the rank based on prevalence of *Campylobacter* in their carcasses were strongly correlated (Spearman's rank correlation coefficient = 0.857).

4. Discussion

Assuring that slaughterhouses are under controlled operational standards is an important pillar in the integrated management of *Campylobacter* in the poultry meat chain. In the present study, *Campylobacter* contamination in broiler carcasses varied significantly between the 9 Belgian slaughterhouses. It was evident that certain premises produce carcasses with both higher concentrations ($\geq$3 $\log_{10}$ CFU/g) and prevalence of *Campylobacter* compared to other operators. This is in agreement with a previous study on *Campylobacter* contamination in Belgian chicken meat preparations, which concluded a significant variation in *Campylobacter* counts and prevalence among 11 processing companies (Habib et al., 2008).

Results from the present study show the value of *Campylobacter* monitoring using enumeration (quantitative) data in evaluating the risk posed by different broiler slaughterhouses. Such evaluation should not be based only on the mean value of concentration levels, but basically should consider the shape of the concentrations distribution in the samples from these slaughterhouses. Numerous risk assessment studies showed that the risk of human campylobacteriosis posed by broiler meat is largely determined by tails of the concentration distribution (Nauta et al., 2009). In addition, a recent study in Iceland showed that the risk of human illness is correlated with carcasses that originated from slaughter-batches with higher *Campylobacter* concentrations (Callicott et al., 2008). Thus, risk managers can benefit from the present survey data by allocating inspection and management efforts toward slaughterhouses that tend to provide carcasses with higher *Campylobacter* prevalence and concentrations.

We attempted to test a hypothesis that "part" of the slaughterhouse effect on *Campylobacter* carcasses contamination could be

Table 3
Comparison by random-effects logistic and Tobit regression models of the risk of *Campylobacter* carcasses ($n = 389$) contamination in Belgian broiler slaughterhouses.

Variable	Detection data			Enumeration data		
	logistic regression model			Tobit regression model		
	Odd ratio (95% CI)	S.E	*P*-value	Coefficient (95% CI)	S.E	*P*-value
Slaughterhouse						
A[a]	1.00	—	—	1.00	—	—
B	0.66 (0.22–1.96)	0.36	0.460	−0.56 (−1.92, 0.78)	0.68	0.410
C	0.70 (0.22–2.16)	0.40	0.539	−0.28 (−1.69, 1.12)	0.71	0.689
D	0.41 (0.06–2.57)	0.38	0.343	−0.12 (−2.38, 2.13)	1.14	0.914
E	0.35 (0.11–1.15)	0.21	0.086	−1.52 (−3.03, −0.02)	0.76	0.047[b]
F	0.86 (0.28–2.68)	0.50	0.807	−0.17 (−1.57, 1.23)	0.71	0.809
G	0.38 (0.12–1.18)	0.22	0.096	−1.61 (−3.06, −0.16)	0.73	0.029[b]
H	0.25 (0.08–0.74)	0.13	0.013[b]	−1.65 (−3.05, −0.25)	0.71	0.020[b]
I	0.25 (0.07–0.93)	0.16	0.040[b]	−2.01 (−3.71, −0.30)	0.86	0.021[b]
Sampling month						
January[a]	1.00	—	—	1.00	—	—
February	0.81 (0.31–2.10)	0.39	0.670	0.10 (−1.17, 1.39)	0.65	0.872
March	0.73 (0.27–1.99)	0.37	0.547	−0.43 (−1.78, 0.91)	0.68	0.525
April	0.50 (0.18–1.40)	0.26	0.190	−1.03 (−2.45, 0.37)	0.71	0.149
May	1.04 (0.38–2.83)	0.53	0.931	−0.01 (−1.37, 1.34)	0.69	0.986
June	4.46 (1.50–13.25)	2.47	0.007[b]	1.70 (0.44, 2.97)	0.64	0.008[b]
July	1.04 (0.38–2.87)	0.53	0.932	0.48 (−0.87, 1.84)	0.69	0.484
August	1.09 (0.39–2.97)	0.55	0.866	0.39 (−0.93, 1.73)	0.68	0.557
September	3.70 (1.28–10.73)	2.01	0.016[b]	1.59 (0.30, 2.87)	0.65	0.015[b]
October	1.17 (0.41–3.35)	0.62	0.757	0.55 (−0.84, 1.95)	0.71	0.437
November	1.40 (0.50–3.90)	0.73	0.519	0.70 (−0.66, 2.07)	0.69	0.311
December	0.98 (0.36–2.70)	0.50	0.984	0.20 (−1.51, 1.57)	0.69	0.765
Broilers age	1.04 (1.00–1.09)	0.05	0.041[b]	0.02 (−0.04, 0.07)	0.02	0.429

[a] Reference category in the model.
[b] variable with a significant difference (<0.05).

related to the quality management and operational hygienic standards of the slaughterhouse operations. Therefore we used the score obtained from a Belgian official control inspection (FASFC-check) as a proxy for the operational hygiene and quality management status in a slaughterhouse. Theoretically, such kind of proxy score awards poor values (e.g. non-conformity score) to those premises with poor operational hygiene standards and higher values to better premises (Pinillos and Jukes, 2008). Poor non-conformity score, as a collective indicator for poor operational hygiene, might be reflected on the microbiological status of carcasses. For instance, in a study in UK using a comparable mandatory check, there was a significant negative correlation between the mean Hygiene Assessment System (HAS) scores and the total viable counts for different beef abattoirs (Hudson et al., 1996). Our results provide evidence that deficiencies in slaughterhouse quality management and operational hygiene, as globally

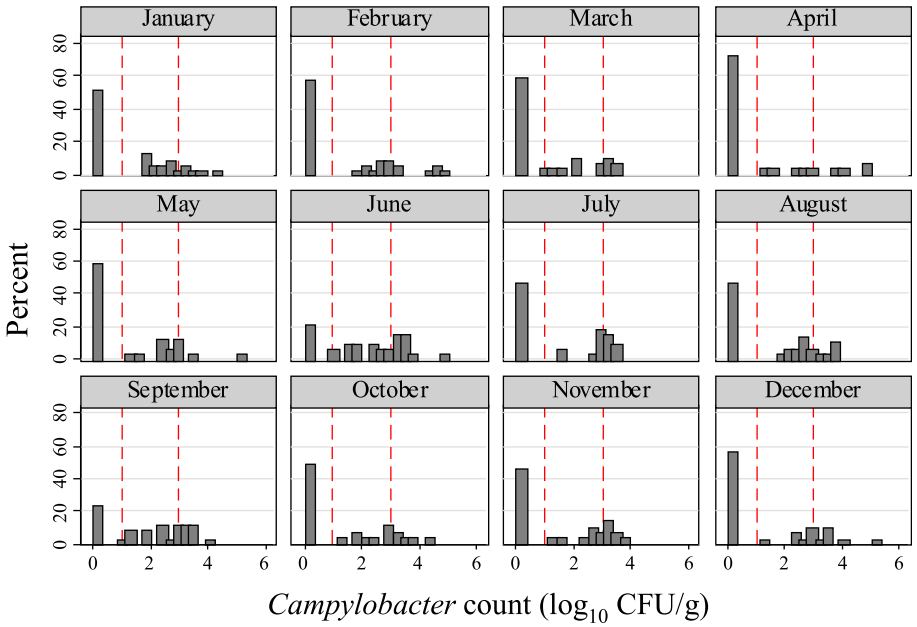

Fig. 3. Distribution of *Campylobacter* counts in broiler carcasses in relation to sampling months (January to December). The two dashed lines denote contamination levels of 1 and 3 $\log_{10}$ CFU/g. The scale of the *y* axis shows the percent of carcasses that fall within the range of *Campylobacter* counts represented by the bar on *x* axis.

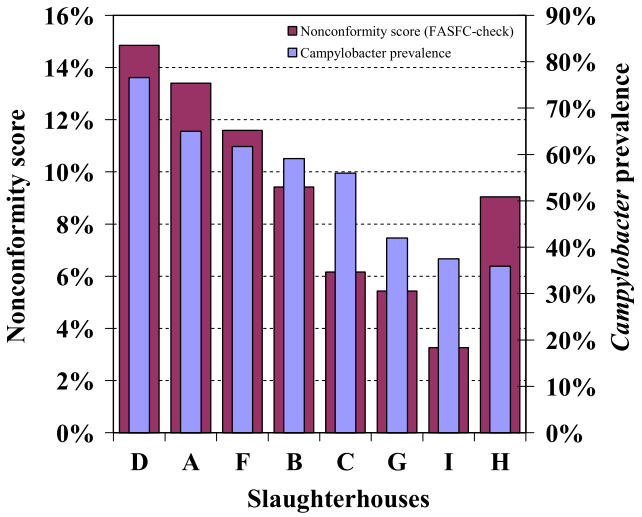

Fig. 4. Slaughterhouses hygiene inspection rank (based on FASFC-check non-conformity scores in 2008) and rank based on *Campylobacter* prevalence in carcasses (based on EU baseline data in 2008). Ranking starts from 1 (lowest; in hygiene non-conformity score/*Campylobacter* prevalence) to 8 (highest).

indicated by "FASFC-check" scores, are correlated with *Campylobacter* contamination status in broiler carcasses (Fig. 4). However, this finding might not be stated as a "causal" relationship between the slaughterhouse operational hygiene and *Campylobacter* contamination; the effect of the slaughterhouse is likely related to several other factors, though our findings indicate that hygienic operations are among these factors. It has been reported that *Campylobacter* positivity status in the flocks from which carcasses originates could be an important factor that affect *Campylobacter* prevalence in broiler carcasses (Berrang et al., 2004; Malher et al., 2011; Smith et al., 2007). Due to data and resources limitation the correlation between flock status and carcasses contamination was not investigated thoroughly in the present *Campylobacter* baseline survey. On the other hand, other studies pointed to or speculated a relation between food quality and hygiene management and *Campylobacter* contamination in the meat processing sector. Sampers et al. (2010) compared the scores of food safety management system (FSMS) activities in two chicken meat preparation plants in Belgium. They concluded that the scores of FSMS are correlated to the overall contamination with *Campylobacter*, as well as with some other microbial indicators (e.g. *Escherichia coli* and *Enterobacteriaceae*). Stern and Robach (2003) speculated that HACCP implementation in U.S slaughterhouses might have led to a reduction in *Campylobacter* spp. on processed poultry products. Also a recent French study pointed that factors related to poor performance of slaughter operations, like increase of temperature in the evisceration room and presence of visible dirty marks, increased the odds of producing *Campylobacter* positive broiler carcasses (Hue et al., 2010). These studies, together with our conclusion on a possible relationship between slaughterhouse operational hygiene inspection scores and *Campylobacter* contamination in broiler carcasses, show that enhancing slaughterline operations could offer an opportunity for *Campylobacter* risk mitigation. Such proposed enhancement can contribute to the risk management goal of reducing human illnesses, notably attributed to carcasses from premises with insufficient operational hygienic performance.

A careful investigation of the present baseline data could allow us to define a correlation between slaughterhouse D and older age birds. These older birds were either from organic or free-range flocks. Van Overbeke et al. (2006) indicated that *Campylobacter* infections at slaughter were significantly higher in Belgian organic flocks compared to conventional broilers. In their study, organic flocks were most probably infected with *Campylobacter* between week 7 and week 10. The production system of free-range or organic birds exposes them to increased contact with environment and other possible sources of acquiring *Campylobacter* infection; for example, to wild and domestic animals, insects and soil (Newell and Fearnley, 2003; Rivoal et al., 2005; Wieland et al., 2005).

Our results show that there was a significant increase in the prevalence and concentrations of *Campylobacter* in broiler carcasses in relation to only selected months in the year 2008 (June and September) rather than an association with the whole summer period (as compared to carcasses sampled in January). There is no clear justification for such finding, and based on it we can not conclude a seasonal trend (rather than monthly peaks) in *Campylobacter* contamination in the year 2008. However, other studies showed an increasing trend of *Campylobacter* prevalence in chicken meat in association with the summer periods (Guerin et al., 2008; Habib et al., 2008; Jore et al., 2010). Some studies speculate that the seasonal variation in *Campylobacter* colonization in broilers would be temperature-related or associated with the abundance of flies (serving as mechanical carriers) in warm months (Guerin et al., 2008). Also the tendency of increasing ventilation rate of broiler houses in the summer months (which implies increased contact with outdoor environment) might play a role (Hald et al., 2008; Hansson et al., 2007; Jore et al., 2010).

In conclusion, in the present study we demonstrate how outcomes from baseline survey could be coupled with other readily available data from national control authorities in order to have a better insight over *Campylobacter* contamination status from different broiler slaughterhouses. Our results demonstrate a relationship, however might not be causal, between slaughterhouse operational hygiene and quality management on one hand and *Campylobacter* carcasses contamination on the other. Findings from this work call for subsequent in-depth research on technical and hygiene-related factors that could impact *Campylobacter* contamination levels in broiler slaughterhouses. Added to that, the correlation between *Campylobacter* flock positivity status, intestinal contamination level, and the concentration of *Campylobacter* and proportion of positive carcasses needs to be studies further.

Acknowledgment

We are grateful to the Belgian Federal Agency for the Safety of the Food Chain for providing the national data on the *Campylobacter* baseline survey in and on the official hygiene inspection of slaughterhouses for the year 2008. The surveillance and laboratory analysis teams are acknowledged for their professional work. Ihab Habib is indebted to the Fund for Scientific Research-Flanders (FWO-Vlaanderen, project G.024.09N) for a position as a post-doctoral fellow.

References

Anonymous, 2006a. Report of Task Force on Zoonoses Data Collection on proposed technical specifications for a coordinated monitoring programme for *Salmonella* and *Campylobacter* in broiler meat in the EU. EFSA J. vol. 92, 1–33.

Anonymous, 2006b. ISO 10272 Microbiology of Food and Animal Feeding Stuffs —Horizontal Method for Detection and Enumeration of Campylobacter Spp — Part 1: Detection Method. International Organisation for standardization (ISO), Geneva, Switzerland.

Anonymous, 2006c. ISO 10272 Microbiology of Food and Animal Feeding Stuffs —Horizontal Method for Detection and Enumeration of Campylobacter Spp — Part 2: Enumeration Method. International Organisation for standardization (ISO), Geneva, Switzerland.

Anonymous, 2007. Directive 2003/99/CE of the European Parliament and the Council on the monitoring of zoonoses and zoonotics agents amending Council Decision 2007/516/CE. Off. J. Eur. Union, L190–L225.

Anonymous, 2009. Trends and Sources: Report on Zoonotic Agents in Belgium in 2008. Working Group on Foodborne Infections and Intoxications. FAVV-AFSCA, Brussels, Belgium.

Anonymous, 2010. Analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses in the EU, 2008. Part A: *Campylobacter* and *Salmonella* prevalence estimates. The EFSA J. 8, 22–34.

Berrang, M.E., Smith, D.P., Windham, W.R., Feldner, P.W., 2004. Effect of intestinal content contamination on broiler carcass *Campylobacter* counts. J. Food Prot. 67, 235–238.

Busschaert, P., Geeraerd, A.H., Uyttendaele, M., Van Impe, J.F., 2010. Estimating distributions out of qualitative and (semi)quantitative microbiological contamination data for use in risk assessment. Int. J. Food Microbiol. 138, 260–269.

Butzler, J.P., 2004. *Campylobacter*, from obscurity to celebrity. Clin. Microbiol. Infect. 10, 868–876.

Callicott, K.A., Hargardottir, H., Georgsson, F., Reiersen, J., Frigriksdottir, V., Gunnarsson, E., Michel, P., Bisaillon, J.R., Kristinsson, K.G., Briem, H., Hiett, K.L., Needleman, D.S., Stern, N.J., 2008. Broiler *Campylobacter* contamination and human campylobacteriosis in Iceland. Appl. Environ. Microbiol. 74, 6483–6494.

Friedman, C.R., Hoekstra, R.M., Samuel, M., Marcus, R., Bender, J., Shiferaw, B., Reddy, S., Ahuja, S.D., Helfrick, D.L., Hardnett, F., Carter, M., Anderson, B., Tauxe, R.V., 2004. Risk factors for sporadic *Campylobacter* infection in the United States: a case-control study in FoodNet sites. Clin. Infect. Dis. 3 (38 Suppl), S285–S296.

Gellynck, X., Messens, W., Halet, D., Grijspeerdt, K., Hartnett, E., Viaene, J., 2008. Economics of reducing *Campylobacter* at different levels within the Belgian poultry meat chain. J. Food Prot. 71, 479–485.

Godschalk, P.C., Heikema, A.P., Gilbert, M., Komagamine, T., Ang, C.W., Glerum, J., Brochu, D., Li, J., Yuki, N., Jacobs, B.C., van, B.A., Endtz, H.P., 2004. The crucial role of *Campylobacter jejuni* genes in anti-ganglioside antibody induction in Guillain-Barre syndrome. J. Clin. Invest. 114, 1659–1665.

Guerin, M.T., Martin, S.W., Reiersen, J., Berke, O., McEwen, S.A., Fridriksdóttir, V., Bisaillon, J.R., Lowman, R., 2008. Campy-on-Ice Consortium. Temperature-related risk factors associated with the colonization of broiler-chicken flocks with *Campylobacter* spp. in Iceland, 2001–2004. Prev. Vet. Med. 15, 14–29.

Guerin, M.T., Sir, C., Sargeant, J.M., Waddell, L., O'Connor, A.M., Wills, R.W., Bailey, R.H., Byrd, J.A., 2010. The change in prevalence of *Campylobacter* on chicken carcasses during processing: a systematic review. Poult. Sci. 89, 1070–1084.

Habib, I., Sampers, I., Uyttendaele, M., Berkvens, D., De Zutter, L., 2008. Baseline data from a Belgium-wide survey of *Campylobacter* species contamination in chicken meat preparations and considerations for a reliable monitoring program. Appl. Environ. Microbiol. 74, 5483–5489.

Hald, B., Skovgard, H., Pedersen, K., Bunkenborg, H., 2008. Influxed insects as vectors for *Campylobacter jejuni* and *Campylobacter coli* in Danish broiler houses. Poult. Sci. 87, 1428–1434.

Hansson, I., Vagsholm, I., Svensson, L., Olsson, E.E., 2007. Correlations between *Campylobacter* spp. prevalence in the environment and broiler flocks. J. Appl. Microbiol. 103, 640–649.

Hudson, W.R., Mead, G.C., Hinton, M.H., 1996. Relevance of abattoir hygiene assessment to microbial contamination of British beef carcases. Vet. Rec. 139, 587–589.

Hue, O., Le, B.S., Laisney, M.J., Allain, V., Lalande, F., Petetin, I., Rouxel, S., Quesne, S., Gloaguen, P.Y., Picherot, M., Santolini, J., Salvat, G., Bougeard, S., Chemaly, M., 2010. Prevalence of and risk factors for *Campylobacter* spp. contamination of broiler chicken carcasses at the slaughterhouse. Food Microbiol. 27, 992–999.

Humphrey, T., O'Brien, S., Madsen, M., 2007. Campylobacters as zoonotic pathogens: a food production perspective. Int. J. Food Microbiol. 117, 237–257.

Jore, S., Viljugrein, H., Brun, E., Heier, B.T., Borck, B., Ethelberg, S., Hakkinen, M., Kuusi, M., Reiersen, J., Hansson, I., Engvall, E.O., Lofdahl, M., Wagenaar, J.A., van, P.W., Hofshagen, M., 2010. Trends in *Campylobacter* incidence in broilers and humans in six European countries, 1997-2007. Prev. Vet. Med. 93, 33–41.

Lindblad, M., Lindmark, H., Lambertz, S.T., Lindqvist, R., 2006. Microbiological baseline study of broiler chickens at Swedish slaughterhouses. J. Food Prot. 69, 2875–2882.

Lorimer, M.F., Kiermeier, A., 2007. Analysing microbiological data: Tobit or not Tobit? Int. J. Food Microbiol. 116, 313–318.

Lubin, J.H., Colt, J.S., Camann, D., Cerhan, J.R., Severson, R.K., Bernstein, L., Hartge, P., 2004. Epidemiological evaluation of measurement data in the presence of detection limits. Environ. Health Perspect. 112, 1691–1696.

Malher, X., Simon, M., Charnay, V., Déserts, R.D., Lehébel, A., Belloc, C., 2011. Factors associated with carcass contamination by *Campylobacter* at slaughterhouse in cecal-carrier broilers. Int. J. Food Microbiol. 150, 8–13.

Mullner, P., Spencer, S.E., Wilson, D.J., Jones, G., Noble, A.D., Midwinter, A.C., Collins-Emerson, J.M., Carter, P., Hathaway, S., French, N.P., 2009. Assigning the source of human campylobacteriosis in New Zealand: a comparative genetic and epidemiological approach. Infect. Genet. Evol. 9, 1311–1319.

Nauta, M., Hill, A., Rosenquist, H., Brynestad, S., Fetsch, A., van der Logt, P., Fazil, A., Christensen, B., Katsma, E., Borck, B., Havelaar, A., 2009. A comparison of risk assessments on Campylobacter in broiler meat. Int. J. Food Microbiol. 129, 107–123.

Newell, D.G., Fearnley, C., 2003. Sources of *Campylobacter* colonization in broiler chickens. Appl. Environ. Microbiol. 69, 4343–4351.

Normand, V., Boulianne, M., Quessy, S., 2008. Evidence of cross-contamination by *Campylobacter* spp. of broiler carcasses using genetic characterization of isolates. Can. J. Vet. Res. 72, 396–402.

Pinillos, R.G., Jukes, D.J., 2008. Hygiene assessment system (HAS) scores - An analysis of the available data from English slaughterhouses. Food Control 19, 806–816.

Rasschaert, G., Houf, K., Van Hende, J., De Zutter, L., 2006. *Campylobacter* contamination during poultry slaughter in Belgium. J. Food Prot. 69, 27–33.

Rivoal, K., Ragimbeau, C., Salvat, G., Colin, P., Ermel, G., 2005. Genomic diversity of *Campylobacter coli* and *Campylobacter jejuni* isolates recovered from free-range broiler farms and comparison with isolates of various origins. Appl. Environ. Microbiol. 71, 6216–6227.

Sampers, I., Habib, I., Berkvens, D., Dumoulin, A., Zutter, L.D., Uyttendaele, M., 2008. Processing practices contributing to *Campylobacter* contamination in Belgian chicken meat preparations. Int. J. Food Microbiol. 128, 297–303.

Sampers, I., Jacxsens, L., Luning, P.A., Marcelis, W.J., Dumoulin, A., Uyttendaele, M., 2010. Performance of food safety management systems in poultry meat preparation processing plants in relation to *Campylobacter* spp. contamination. J. Food Prot. 73, 1447–1457.

Smith, D.P., Northcutt, J.K., Cason, J.A., Hinton Jr., A., Buhr, R.J., Ingram, K.D., 2007. Effect of external or internal fecal contamination on numbers of bacteria on prechilled broiler carcasses. Poult. Sci. 86, 1241–1244.

Stern, N.J., Robach, M.C., 2003. Enumeration of *Campylobacter* spp. in broiler feces and in corresponding processed carcasses. J. Food Prot. 66, 1557–1563.

Takahashi, R., Shahada, F., Chuma, T., Okamoto, K., 2006. Analysis of *Campylobacter* spp. contamination in broilers from the farm to the final meat cuts by using restriction fragment length polymorphism of the polymerase chain reaction products. Int. J. Food Microbiol. 110, 240–245.

Van Overbeke, I., Duchateau, L., De Zutter, L., Albers, G., Ducatelle, R., 2006. A comparison survey of organic and conventional broiler chickens for infectious agents affecting health and food safety. Avian Dis. 50, 196–200.

Wieland, B., Regula, G., Danuser, J., Wittwer, M., Burnens, A.P., Wassenaar, T.M., Stark, K.D., 2005. *Campylobacter* spp. in dogs and cats in Switzerland: risk factor analysis and molecular characterization with AFLP. J. Vet. Med. B. Infect. Dis. Vet. Public Health 52, 183–189.