Food Microbiology 27 (2010) 992–999

Contents lists available at ScienceDirect

Food Microbiology

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

Prevalence of and risk factors for *Campylobacter* spp. contamination of broiler chicken carcasses at the slaughterhouse

Olivier Hue [a], Sophie Le Bouquin [b], Marie-José Laisney [a], Virginie Allain [b], Françoise Lalande [a], Isabelle Petetin [b], Sandra Rouxel [a], Ségolène Quesne [a], Pierre-Yves Gloaguen [a], Mélanie Picherot [d], Julien Santolini [d], Gilles Salvat [a], Stéphanie Bougeard [c], Marianne Chemaly [a,*]

[a] *Hygiene and Quality of Poultry and Swine Products Unit, AFSSA, French Agency for Food Safety, BP53 22440 Ploufragan, France*
[b] *Epidemiology and Welfare of Poultry and Rabbit Unit, AFSSA, French Agency for Food Safety, BP53 22440 Ploufragan, France*
[c] *Epidemiology and Welfare of Swine Unit, AFSSA, French Agency for Food Safety, BP53 22440 Ploufragan, France*
[d] *Office for foodborne zoonoses and food microbiology French Ministry of agriculture and fisheries, General directorate for food, Division for food safety, 251 rue de Vaugirard 75732 PARIS Cedex15, France*

ARTICLE INFO

Article history:
Received 25 February 2010
Received in revised form
11 June 2010
Accepted 11 June 2010
Available online 23 June 2010

Keywords:
Chicken
Carcasses
Campylobacter
Contamination
Slaughter
Risk factors

ABSTRACT

A study was conducted in 2008 to estimate the prevalence and identify the risk factors for *Campylobacter* spp. contamination of broiler carcasses during the slaughtering process. A pool of 10 caeca and one carcass were collected from 425 batches of broiler chickens slaughtered in 58 French slaughterhouses over a 12-month period. Potential risk factors were identified according to the *Campylobacter* contamination status of carcasses and processing variables identified from questionnaires. The statistical analysis took into account confounding factors that have already been associated with the presence of *Campylobacter* on carcasses such as the slaughter age of the chicken or seasonal variations. *Campylobacter* spp. were isolated from 77.2% of caeca (95% CI 73.2 to 81.2) and from 87.5% of carcasses (95% CI 84.4 to 90.7). A multiple logistic regression showed 4 parameters as significant risk factors ($p < 0.05$) for contamination: (I) batches were not the first to be slaughtered in the logistic schedule (OR = 3.5), (II) temperature in the evisceration room was higher than 15 °C (OR = 3.1), (III) dirty marks on carcasses after evisceration were visible (OR = 2.6) and (IV) previous thinning of the flocks, from which slaughtered batches came, had occurred at the farm (OR = 3.3). This last result highlighted the need for sanitary precautions to be taken when catching birds for transport. At the slaughterhouse, evisceration seemed to be the operation contributing most to the spread of contamination. Effective risk management solutions could include the systematic external rinsing of carcasses after evisceration and the implementation of slaughtering schedules according to the *Campylobacter* contamination status of flocks.

1. Introduction

Campylobacteriosis is a foodborne disease associated with the infection of humans by thermophilic *Campylobacter* spp., mainly *Campylobacter jejuni* and *C. coli* (Lastovica and Skirrow, 2000). The incidence of human infections caused by thermophilic *Campylobacter* in the European Union (EU) has been continuously increasing in recent years and the bacteria is still the most commonly reported gastrointestinal zoonotic pathogen in humans in the EU (EFSA, 2009). Common signs and symptoms of *Campylobacter* infection include fever, diarrhea and abdominal pain (Butzler, 2004). In a few cases, the infection can evolve into severe complications and develop the Guillain-Barré syndrome which is an acute autoimmune neuropathy with ascending paralysis (Zilbauer et al., 2008). *Campylobacter* infections can acquired from sources such as untreated raw milk (Heuvelink et al., 2009) or water (Abe et al., 2008), but the consumption of poultry meat, particularly fresh broiler meat, constitutes the main risk factor for infection (Wingstrand et al., 2006).

Control of campylobacteriosis is therefore commonly focused on reducing the occurrence of *Campylobacter* in broiler meats. Most reports of work on control of *Campylobacter* have emphasized the need to decrease the prevalence and numbers of *Campylobacter* within poultry flocks. Indeed, it has been estimated that a 2-log reduction of *Campylobacter* in poultry faeces could lead to a 30-fold reduction in human campylobacteriosis (Rosenquist et al., 2003). Reducing carcasses contamination at the slaughterhouse has also been identified as a mean of reducing *Campylobacter* infections of

* Corresponding author. Tel.: +33 2 96 79 61 52; fax: +33 2 96 79 85 38.
E-mail address: m.chemaly@afssa.fr (M. Chemaly).

0740-0020/$ – see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.fm.2010.06.004

human. However, possible procedures for control of *Campylobacter* on poultry meat at slaughterhouses are limited by their practicality, legality or acceptability to consumers (Anonymous, 2005), although in Scandinavian countries freezing has been proven effective for reducing *Campylobacter* contamination of carcasses. It can be reduced by the scalding of carcasses but cross-contamination can still occur, particularly during the defeathering and evisceration processes (Rosenquist et al., 2006). Thus, the surfaces of carcasses from a *Campylobacter*-free flock can be contaminated when they were processed after an infected batch of birds. Because of the pathogen's ability to survive in water, in aerosols and on equipment (Figueroa et al., 2009), it is necessary to identify the critical points at which contamination of carcasses during the process can be controlled. Therefore, a European Union baseline study was carried out in member states to estimate the prevalence of *Campylobacter* contamination at the slaughterhouse. In France, an additional survey was conducted to identify risk factors associated with *Campylobacter* contamination of broiler carcasses.

Thus, the aims of the present study were to (i) assess thermotolerant *Campylobacter* contamination in chicken caeca and on carcasses collected during the slaughtering process, and (ii) to identify and quantify factors in slaughterhouse associated with the presence of *Campylobacter* spp. on carcasses.

2. Materials and methods

2.1. Experimental design

Unit for the statistical analysis was the "slaughter batch" defined as a group of chickens from the same flock, delivered at the same time to the same slaughterhouse. The study dealt with 58 EC approved French poultry slaughterhouses which each process more than 250 000 chickens per year. Data for this study were collected between January 1st and December 15th, 2008.

2.2. Sample size

The sample size was set on the bases of an expected prevalence of 50% and a confidence interval of 95%. A harmonised procedure was set up by the European Union for each member state. In France, the General Directorate of Food (Ministry of Agriculture) established the sample size by reference to the French annual chicken production figures in 2003. The sample therefore constituted of 425 batches of which: 327 were batches of conventional reared broiler chickens (76.9%), 68 were batches of label chickens (16.0%), 21 were batches of heavy chickens (4.94%), 6 were batches of free-range chickens (1.41%) and 3 were batches of organic chickens (0.71%). This distribution is in agreement with French annual chicken production in 2008 (ITAVI, 2010). Conventional and heavy chickens correspond to intensive rearing where birds are kept in building and slaughtered after about 40 days (after about 44 days for heavy chickens). Label and organic chickens are slow growing birds that are slaughtered after at least 81 days. These birds are kept inside with a lower density of birds/m^2 than standard chickens with an outdoor access and with a different feed from that provided for standard chickens.

2.3. Sampling design

We relied on data collected during the European investigation of *Campylobacter* in foods (Anonymous, 2007). The sampling scheme, representative of the target population during the year, was based on two-level stratification. These levels were (I) the annual slaughter volume for a slaughterhouse, with the number of batches to be investigated per slaughterhouse being proportional to the number of chickens slaughtered annually at the slaughterhouse and (II) the month, being spread homogeneously over 12 months with 36 batches being investigated per month. The sampling days by month and by slaughterhouse were arranged to avoid investigation of two batches on the same day at the same slaughterhouse.

2.4. Sample collection

Caeca were randomly collected from 10 birds per batch during evisceration and pooled into a sterile bag. One carcass was also taken from the processing line after chilling with use of a clean pair of latex gloves and a sterile bag. The samples were sent in an insulated box, within 24 h, to the National Reference Laboratory for *Campylobacter* (AFSSA-LERAPP, Ploufragan, France), where detection and enumeration of *Campylobacter* were performed.

2.5. Sample analysis

Campylobacter from caecal contents were recovered using both direct plating and enumeration. Direct plating was performed by putting 10 μl of each pooled caecal sample onto a selective modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) (Oxoid, Dardilly, France), followed by incubation for 44 ± 4 h at 41.5 ± 1 °C under an atmosphere of 5% O_2, 10% CO_2 and 85% N_2. For each positive plate, up to 5 typical *Campylobacter* colonies were then subcultured onto plates of blood agar plates (AES, Bruz, France) for further characterization according to the NF EN ISO 10272-1 standard (Anonymous, 2006). A flock was considered *Campylobacter*-positive if at least one confirmed *Campylobacter* was isolated. The enumeration of *Campylobacter* was performed using a 1:10 dilution of pooled caeca in tryptone salt broth (Biomerieux, Craponne, France). The suspensions were then homogenized for 1 min in a peristaltic homogenizer (AES, Bruz, France) and 1:10 serial dilutions of the homogenate in tryptone salt broth were prepared. All dilutions were plated on mCCDA and incubated for 44 ± 4 h at 41.5 ± 1 °C under the microaerobic atmosphere. The limit for enumeration was 200 CFU/g.

Enumeration and sample enrichment were both used to recover *Campylobacter* from carcasses. Twenty seven grams of skin from the wishbone and the neck were put into a sterile bag and diluted at 1:10 in a tryptone salt broth. The mix was then homogenized for 1 min in a peristaltic homogenizer. Detection by enrichment and enumeration was performed according to part 1 and 2 respectively of the NF EN ISO 10272 standard. For detection purposes, 10 ml of the homogenate was added to 90 ml of Bolton broth (Oxoid, Dardilly, France). The inoculated broth was then incubated under microaerobic conditions for 4 h at 37 °C and then for 44 ± 4 h at 41.5 ± 1 °C. Subsequently, 10 μl of the culture was plated onto mCCDA and Butzler agar (Virion N°2) (Oxoid, Dardilly, France) and incubated for 44 ± 4 h at 41.5 ± 1 °C. For each positive plate, up to 2 typical *Campylobacter* colonies were subcultured onto plates of blood agar for further characterization according to the NF EN ISO 10272-1 standard. *Campylobacter* were enumerated by duplicate plating 1 ml of the homogenate onto three plates of mCCDA. Ten-fold serial dilutions of the homogenate in tryptone salt broth were also prepared and plated onto one plate of mCCDA. All plates were incubated under microaerobic conditions for 44 ± 4 h at 41.5 ± 1° C. The limit for enumeration was 10 CFU/g.

2.6. Statistical analysis

Prevalence of *Campylobacter*-positive batches of caeca or carcasses were calculated using Systat®9 software (SPSS Inc., Chicago, IL, USA). A batch was considered positive if *Campylobacter* was detected and/or enumerated. For enumeration purposes,

bacterial counts were log10 transformed to obtain approximately normally distributed data. Samples in which *Campylobacter* was detected below the enumeration threshold were assigned a log value of one to allow calculation of means.

Personnel from each official veterinary service completed a questionnaire about the structural and functional characteristics of the slaughterhouse. Also, questionnaires about sampling and the broiler flock were completed for every flock from which samples were obtained.

Univariate analyses were carried out, using the PROC MEANS and PROC FREQ procedures in SAS® 9.1 software (SAS Institute Inc., Cary, NC, USA) to identify the main trend, variability and distribution of each individual variable. Variables with more than 20% of missing data and those for which there was no variability were removed.

Prior to studying relationships between independent variables and *Campylobacter* contamination, it was necessary to consider the variables already known to be confounding factors in terms of the presence or absence of *Campylobacter*.

Explanatory variables were selected while assessing the relationships between the presence of *Campylobacter* and each explanatory variable. These relationships were assessed using Generalized Estimating Equations (Liang and Zeger, 1986) including repeated effects, carried out using the PROC GENMOD procedure in SAS® 9.1 software. The GEE models were used with a binomial probability distribution and a logit link function. The statistical unit was the slaughter batch, the slaughterhouse being considered as a repeated measurement factor in order to take into account the within slaughterhouse covariability, specifying an exchangeable structure for the covariance matrix. The GEE models allow consideration of both quantitative and categorical explanatory variables. Only factors significantly associated with *Campylobacter* contamination (likelihood ratio test $p \leq 0.20$) were considered for further analysis.

Finally, a multiple logistic regression including all the previously selected explanatory variables was performed. A downward selection, PROC LOGISTIC procedure in SAS®9.1 was used, with variables introduced if $p < 0.20$ and excluded if $p > 0.05$. Moreover, contrasts estimate results were used to determine the significance of the link between the various explanatory variable modalities and the dependent, associated with the odds ratio estimation.

3. Results

3.1. Prevalence

Overall prevalence of *Campylobacter*-positive batches based on caeca was 77.2% ($CI_{95\%} = [73.2; 81.2]$). The mean count of *Campylobacter* recovered from caecal contents was 8.04 ± 1.0 $\log_{10}$ CFU/g and values ranged between 4.2 $\log_{10}$ and 10.6 $\log_{10}$ CFU/g.

In the case of carcasses, 372 out of 425 (87.5%; $CI_{95\%} = [84.4; 90.7]$) batches were positive for *Campylobacter*. Contamination ranged from 1.0 up to 4.39 $\log_{10}$ CFU/g with a mean value of 2.39 ± 0.8 $\log_{10}$ CFU/g.

3.2. Consideration of confounding factors

Slaughter age can have distorting effects on results (Newell et al., 2008). In our study, slaughter age was very strongly related to the presence of *Campylobacter* on carcasses ($p < 0.001$). We observed that the older the animal, the higher the prevalence of *Campylobacter* contamination both in caeca and carcasses (Fig. 1A). The percentage of positive batches became significantly different for a slaughter age higher than 68 days. However, the numbers of *Campylobacter* detected in the samples was independent of slaughter age (Fig. 1B). Slaughter age was a function of the type of chicken. Table 1 shows that "label" and organic chickens were

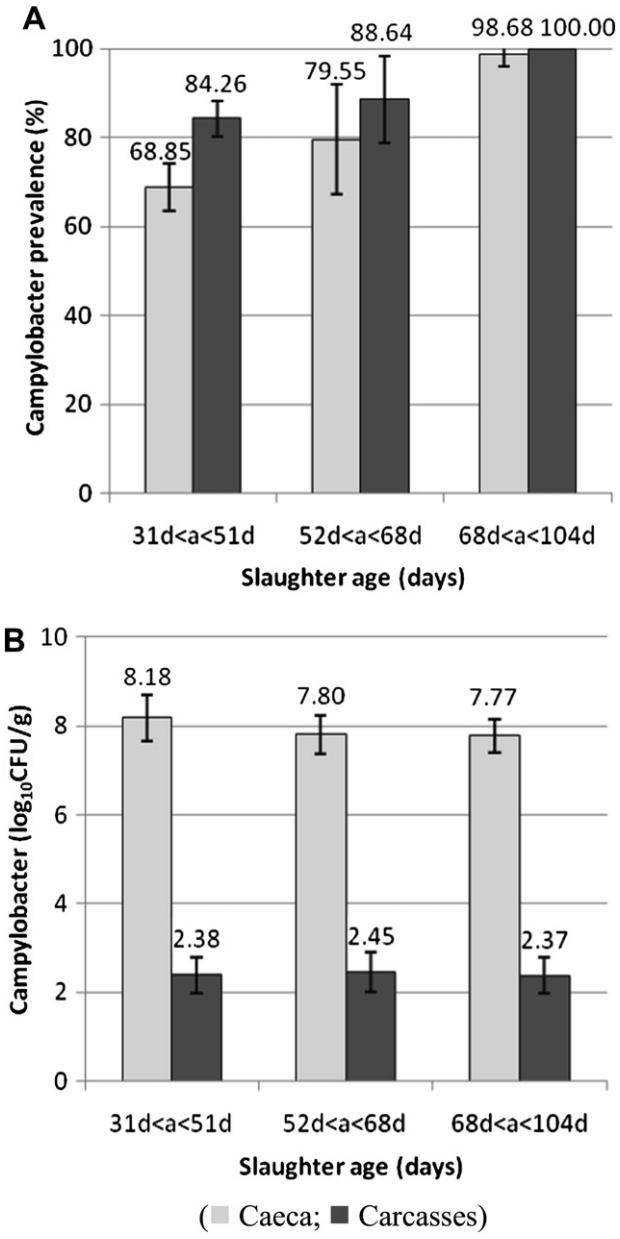

Fig. 1. Numbers of *Campylobacter* in caecal contents and on carcasses in 425 batches of chickens of different age at slaughter.

Table 1
Campylobacter contamination according to the type of chicken in the slaughtered batch ($n = 425$).

Chicken type	n	Slaughter age (days)	Mean age (days)	Positive batches (%)		Mean numbers (CFU/g)	
				Caecal contents	Carcasses	Caecal contents	Carcasses
Standard	327	<44	42	69.7	84.7	8.11	2.39
Free-range	6	>56	78	66.7	83.3	7.61	1.88
Label*	68	>81	88	100.0	100.0	7.82	2.46
Organic	3	>81	88	100.0	100.0	7.03	1.51
Others	21	–	53	81.0	90.5	8.32	2.47
Total	425	–	51	75.3	87.5	8.05	2.39

*Label chickens are slow growing birds kept inside with a low density of birds and with an outdoor access.

slaughtered after 81 days and were always *Campylobacter*-positive. However the type of chicken had no influence on the numbers of *Campylobacter* detected in samples.

Season was also considered as a confounding factor. The prevalence of *Campylobacter* in caeca and on carcasses increased significantly ($p < 0.05$) between May and October (Fig. 2A). However, the season had no influence on the numbers of *Campylobacter* in the samples (Fig. 2B).

3.3. Selection of variables

The univariate analysis allowed the selection of 43 variables which were considered for bivariate analysis. The variables related to similar practices of the slaughterhouses and to similar characteristics of chicken batches were eliminated (Table 2).

The bivariate analysis of explanatory variables allowed the selection of the variables most related with presence of *Campylobacter* on carcasses (Table 3). Thirty one variables, significantly related to the presence of *Campylobacter* in carcass were selected for multivariate logistic regression. Variables related to the number of carcasses per chilling trolley were eliminated because of insufficient data. However, the number of carcasses on a trolley tended to be correlated with the number of positive samples.

Because of the close relationship between the independent variable and the variable "presence of *Campylobacter* in caeca", we needed to consider a model with and without this variable as it might mask other factors. The results of these two models are strictly different. The first model indicated that the risk of carcass contamination increased when *Campylobacter* was isolated in a caecal sample (OR = 77.3) and when the batch was not slaughtered first in the logistic schedule (OR = 11.3) (data not shown). The second model (excluding "presence of *Campylobacter* in caeca") showed four risk factors, three of which were new (Table 4).

The comparison of these two models showed that the second one (deviance $p = 0.308$ and Pearson χ^2 $p = 0.550$) was a better fit

Table 2
Common characteristics of slaughterhouse excluded for statistical analysis.

Slaughterhouse characteristics	
Use of detergent to clean the slaughterhouse	100%
Practice of daily draining the scalding bath	100%
Cleaning crates or containers between batches	100%
No control for *Campylobacter* disinfection efficiency	98.36%
Cleaning trucks between batches	98.36%
Slaughterhouse with a carving workshop	96.72%
Scheduling for salmonella-positive batches at farm	93.44%
Anesthesia using electronarcosis	93.44%
Partial disinfection of scalding bath between two batches	0%
Chicken batch characteristics	
Campylobacter status unknown	100%
No anomaly during ante-mortem inspection	96%
Batch vaccinated for *Salmonella* spp.	0.47%

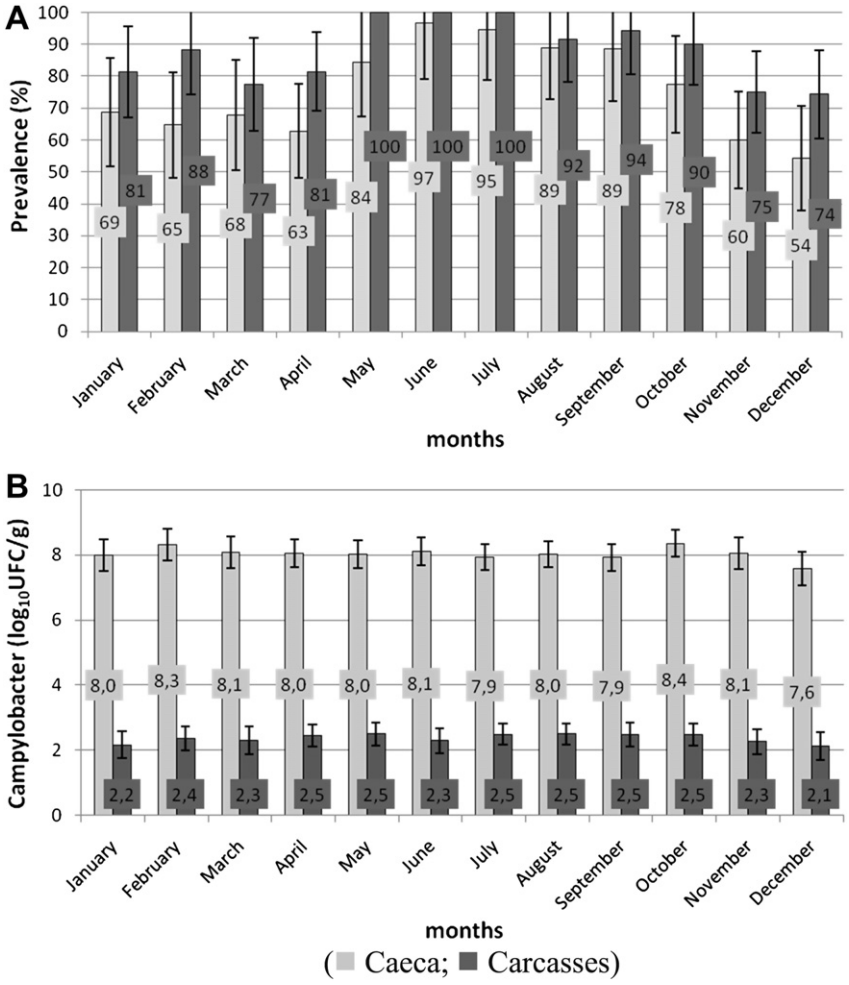

Fig. 2. Prevalences of *Campylobacter*-positive samples from caecal contents or carcasses, and mean of numbers of *Campylobacter* in samples of caecal contents or from carcasses obtained from 425 batches of carcasses at slaughterhouse for each month in 2008.

Table 3
Selected variables (threshold of 20%) entered in the Generalized Linear Model used to explain *Campylobacter*-positive carcasses ($n = 425$).

Variable*	Variable modality	Size	% positive	p value ($\chi 2$ test)
Presence of *Campylobacter* in caeca	Yes	–	–	<0.001
	No			
Numbers of *Campylobacter* in caecal contents		–	–	<0.001
Official veterinary inspection	**All of the chain**	**69**	**76.8**	**0.185**
	Part of the chain	**248**	**86.7**	
	occasional	**68**	**94.1**	
	other**	**40**	**100.0**	
Number of slaughter chains	**1**	**349**	**86.5**	**0.111**
	2	**76**	**92.1**	
Traffic between 2 workrooms during slaughter	**Regular**	**209**	**82.8**	**0.127**
	Occasional	**144**	**92.4**	
	None	**71**	**91.5**	
Possibility for operators to go from one workroom to another	Yes	117	92.3	0.179
	No	308	85.7	
Operators must pass in a demarcation zone before entering to slaughter room	**Yes**	**361**	**86.4**	**0.078**
	No	**64**	**93.4**	
Hygiene equipment in the demarcation zone *wash-hand basin (WHB), boot cleaner (BC), boot sole cleaner (BSC)*	**WHB, BC and SC**	**181**	**87.8**	**0.100**
	no SAS	**64**	**93.8**	
	BC and/or SC and/or WHB (2/3)	**180**	**85.0**	
People who washed and disinfected (W/D) aprons at the end of the production day	**Specialized internal team**	**65**	**80.0**	**0.167**
	Slaughter chain employee	**275**	**89.5**	
	Disposable aprons	**54**	**88.9**	
Type of disinfection	**Spray**	**95**	**83.2**	**0.186**
	foam	**295**	**88.8**	
Composition of basic disinfectant agent *Chlorine (Cl) , sodium hypochlorite (NaCl), Glutaraldehyde (Glut.), Didecyl Dimethyl Ammonium Chloride (DDAC), Lauryl Dimethyl Benzyl Ammonium Chloride (LDBAC)*	**Cl or NaCl**	**85**	**85.9**	**0.155**
	Glut. and DDAC	**73**	**82.2**	
	Glut. and CLDBA	**107**	**86.0**	
	DDAC and/or LDBAC	**82**	**91.5**	
	others	**36**	**97.2**	
Frequency of the control of W/D slaughter machine (times per month)	**[0; 4]**	**102**	**88.2**	**0.041**
	[4; 30]	**228**	**84.2**	
	≥30	**52**	**96.2**	
Frequency of the control of W/D conveyors belt (times per month)	[0; 4]	104	88.5	0.042
	[4; 30]	229	84.3	
	≥30	49	95.9	
Frequency of the control of W/D small slaughtering tools (times per month)	[0; 4]	15	100.0	0.160
	[4; 30]	319	85.0	
	≥30	40	5.0	
Frequency of the control of W/D wall and floor (times per month)	[0; 4]	141	87.2	0.135
	[4; 30]	209	85.2	
	≥30	32	96.9	
Presence of *Salmonella spp.* on carcasses	**Yes**	**32**	**93.8**	**0.111**
	No	**393**	**87.0**	
Type of lairage area for animals	**Fully close**	**230**	**87.4**	**0.176**
	Covered	**46**	**93.5**	
	Not covered	**42**	**95.2**	
	Other	**107**	**82.2**	
Number of pluckers on the first chain	**≤3**	**232**	**92.7**	**0.012**
	4	**105**	**82.9**	
	≥5	**88**	**79.5**	
Number of pluckers on the second chain	**0**	**349**	**86.5**	**0.111**
	3	**76**	**92.1**	
Number of scalding baths on the second chain	**0**	**349**	**86.5**	**0.167**
	1	**76**	**92.1**	
Number of plucking fingers ordered (or changed) per year	**[0; 30 000]**	**187**	**93.6**	**0.086**
	[30 000; 100 000]	**153**	**82.4**	
	> 100 000	**78**	**84.6**	
Frequency of external rinsing of eviscerated carcasses	**Systematically**	**379**	**86.3**	**0.159**
	Occasionally if need be or no external rinsing	**46**	**97.8**	
Possible contact with organic matter from another carcass	**Yes**	**124**	**79.0**	**0.012**
	No	**301**	**91.0**	
Taking down/re-hanging between workrooms 2 and 3 requires manual intervention	**Yes**	**46**	**87.0**	**0.119**
	No	**345**	**86.4**	
Location of the farm providing batches	**Brittany**	**149**	**91.3**	**0.020**
	North West Dept	**111**	**87.4**	
	Others	**165**	**84.2**	
Attribution of a reference number to the flock	Yes	352	88.6	0.171
	No	70	81.4	
Thinning of the flock	**Yes**	**152**	**94.1**	**0.006**
	No	**262**	**83.6**	
Other species slaughtered the same day in the same slaughterhouse	**Yes**	**195**	**85.1**	**0.080**
	No	**230**	**89.6**	

(*continued on next page*)

Table 3 (*continued*)

Variable*	Variable modality	Size	% positive	*p* value (χ2 test)
Turkeys slaughtered the same day in the same slaughterhouse	Yes	117	80.3	0.153
	No	308	90.3	
Other type of chicken slaughtered the same day in the same slaughterhouse	Yes	59	78.0	0.113
	No	366	89.1	
Some "others" species slaughtered the same day in the same slaughterhouse	Yes	31	67.7	0.078
	No	394	89.1	
Rank of batch in the slaughter program (among all species)	**First half**	**194**	**86.6**	**0.039**
	Second half	**231**	**88.3**	
Batch was slaughtered first in the slaughter program (among all species)	**No**	**383**	**89.8**	**0.037**
	Yes	**42**	**66.7**	
Investigated batch was slaughtered first in the slaughter program (among chickens)	No	368	89.9	0.086
	Yes	57	71.9	
Average time for chilling of carcasses	**[0; 1h]**	**58**	**91.4**	**0.051**
	[1h; 3h]	**183**	**87.4**	
	≥ 3h	**183**	**86.3**	
Temperature in evisceration room (°C)	**[0; 15]**	**166**	**79.5**	**0.003**
	[15; 20]	**204**	**91.7**	
	> 20	**44**	**95.5**	
Temperature of scalding bath n°1 (°C)	**[0; 51°C]**	**84**	**88.1**	**0.102**
	[51°C; 55°C]	**261**	**85.8**	
	≥ 55°C	**78**	**92.3**	
Temperature of chilling room (or tunnel) for investigated chicken batch (°C)	**≤0**	**180**	**88.9**	**0.153**
	[0; 3]	**200**	**85.5**	
	≥ 3	**45**	**91.1**	
Process was stopped during slaughter of investigated batch	**Process was stopped**	**58**	**94.8**	**0.026**
	No anomaly	**367**	**86.4**	
Presence of dirty marks on eviscerated carcasses	**Yes**	**221**	**91.4**	**0.028**
	No	**197**	**82.7**	
Minimum number of carcasses per chilling trolley***	[0; 128]	34	79.4	0.110
	[128; 160]	58	91.4	
	≥ 160	52	88.5	
Maximum number of carcasses per chilling trolley***	[0; 128]	33	78.8	0.141
	[128; 160]	38	89.5	
	≥ 160	73	90.4	
Number of classes of size of carcasses within the batch	**0**	**321**	**89.7**	**0.062**
	[1; 10]	**62**	**82.3**	
	≥ 10	**41**	**78.0**	

*All variables are significantly related to the presence of *Campylobacter* in carcass ($p < 0.20$) but only those in bold are selected because they are independent from each other.
**Official veterinary inspection was conducted during the same specific time slot of the day.
***2 Variables were deleted because there was insufficient data.

with the data than the first one (deviance $p = 0.019$ and Pearson χ^2 $p = 0.050$). All second degree interactions have been tested and none were significant at a threshold of 5%.

3.4. Risk factors

The risk of *Campylobacter* contamination was increased (OR = 3.3) when the flock had previously been depopulated. For 152 batches from such flocks, the contamination rate was 94.1% as compared with 83.6% for the 262 batches from flocks that had not been depopulated.

Table 4
Risk factors for contamination of broiler chicken carcasses by *Campylobacter* spp. at slaughterhouse ($n = 425$).

Variable	Estimated parameters	Standard deviation	Odds Ratio	CI 95%	*p* value
Thinning of the flock					
• Yes	0.597	0.197	3.302	1.523–7.157	0.002
• No	–	–	1.000		–
Batch was slaughtered first in the slaughter program (among all species)					
• No	0.626	0.202	3.497	1.586–7.710	0.002
• Yes	–	–	1.000		–
Temperature in evisceration room (°C)					
• >15 °C	0.558	0.162	3.050	1.616–5.755	<0.001
• ≤15 °C	–	–	1.000		–
Presence of dirty marks on eviscerated carcasses					
• Yes	0.482	0.162	2.625	1.393–4.945	0.003
• No	–	–	1.000		–

Intercept = 1.820 ($p < 0.001$).

Batches that were first to be slaughtered in the logistic schedule benefited from a protective factor because of a reduced contamination by *Campylobacter*. The batches not slaughtered in these conditions indeed showed a higher probability to be contaminated by *Campylobacter* (OR = 3.5). The 42 batches that were slaughtered first in the day presented a contamination rate of 66.7%, which is significantly lower ($p < 0.05$) than the 89.8% rate of contamination of others batches.

The proportion of *Campylobacter*-positive carcasses fluctuated according to the temperature in the evisceration room. When the temperature was higher than 15 °C (median temperature in our database), carcass contamination increased (OR = 3.1). Batches slaughtered in these conditions showed a higher contamination rate (92.34%) than the others (79.5%). This variable may be related to seasonal variability. Indeed, in most slaughterhouses, the air in the evisceration room is not conditioned in any way. We can therefore assume that the inside temperatures were proportional to those outside. Fig. 3 shows this trend with average temperature ≤ 15 °C, measured only during winter month, with consequently low carcass prevalence (see "*Consideration of confounding factors/season*").

The probability of *Campylobacter* contamination was significantly increased if visible dirty marks were observed on carcasses following evisceration (OR = 2.6). In batches of chickens showing visible dirty marks, the proportion of *Campylobacter*-positive carcasses was higher (91.4%) than in batches without such marks (82.7%).

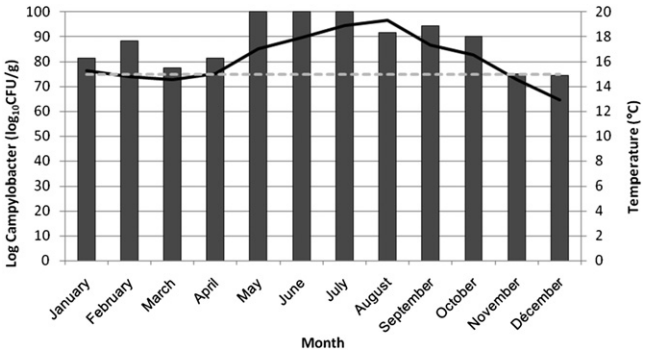

Fig. 3. Mean temperatures in slaughterhouse evisceration rooms (−) and prevalences of *Campylobacter*-positive carcasses (■) for each month during 2008.

4. Discussion

This study generated data representative for national production of chicken carcasses and showed the high prevalence of *Campylobacter* in caeca and carcass skin samples. Our findings for the prevalence of *Campylobacter* in batches, on the basis of caecal samples, are in agreement with a recent Belgian study in which a similar prevalence (73%) was found (Rasschaert et al., 2007). However, contamination with *Campylobacter* varies among European countries. To give examples, Danish study showed a prevalence 37% (Heuer et al., 2001) while a Dutch study indicated that prevalence ranged from 20% to 31% between 2002 and 2005 (Van Asselt et al., 2008). This disparity might be due to differences in sampling schemes, analytical methods, the ages of the birds that were sampled, etc. Moreover, the prevalence values given above were for only "standard" chickens; *i.e.* intensively reared birds of average age of 41 days whereas the birds sampled in our study included many "label" and free-range chickens, which can be more highly contaminated by *Campylobacter* (Avrain et al., 2003).

The prevalence of *Campylobacter*-positive carcasses, found in this study, is comparable to prevalence reports for chicken carcasses from other European countries (Jozwiak et al., 2006; King and Adams, 2008; Mackiw et al., 2008). But, as with caeca, prevalence on carcasses can vary substantially from one country to the other (Arsenault et al., 2007; Atanassova and Ring, 1999; Son et al., 2007). Even within a given study, results may vary between the slaughterhouses (Rasschaert et al., 2006). The scientific report of EFSA on the baseline survey on the prevalence of *Campylobacter* in broiler batches reported prevalences on carcasses varying from 4.9 to 100% among European country in 2008 (EFSA, 2010).

Although the prevalence of *Campylobacter* within a batch is often high, the sampling of one carcass per batch might underestimate prevalence values among batches. It should also be noted that the prevalence of *Campylobacter* on carcasses was higher than the prevalence on caeca. This showed that cross-contamination occurred during the slaughtering process, which justifies the need for the risk factor analysis performed in this study.

The number of carcasses on a trolley during chilling tended to be associated with the increase of contamination on carcasses. We can assume that chilling would lead to better results if trolleys carried fewer carcasses and therefore would be more efficient to eliminate *Campylobacter* from surfaces. Moreover, the risk of cross-contamination on trolleys increased when carcasses were in close contact with one another. However it would be necessary to develop specific studies (case/control) to confirm the impact of this factor on the presence of *Campylobacter*.

The contamination of caeca increased with the slaughter age, as was shown in previous studies (Newell et al., 2008; Russa et al., 2005). The prevalence of *Campylobacter* in caeca increased significantly between May and October as found in some studies (Denis et al., 2007; Hansson et al., 2007; Reich et al., 2008).

The risk for *Campylobacter* contamination increased when a previous batch thinning of broiler house had occurred. This practice is used to divide the flock into batches for staggered slaughter. This practice can indeed be a risk factor if the sanitary barrier was not strictly maintained (Adkin et al., 2006). In particular, transport crates can be a source of contamination even when they have been disinfected (Berrang et al., 2004a; Slader et al., 2002). *Campylobacter* prevalence depends on the length of time between thinning and slaughter because *Campylobacter* can rapidly spread within the flock (Van Gerwe et al., 2005).

The statistical analysis showed that when a batch of chickens was not slaughtered first in a working day the probability of the batch being contaminated by *Campylobacter* increased. Several hypotheses can be advanced to explain this result. Due to the fact that the processing chain has been cleaned and disinfected before work begins, contamination risks will be low for that first batch. However, as the day progresses, cross-contamination will increase after contaminated batches have been slaughtered. This occurs more frequently during the evisceration operation (Figueroa et al., 2009) during which the rupture of viscera releases high numbers of *Campylobacter* onto the carcass. Even a small amount of faecal contents can significantly increase the numbers of *Campylobacter* on carcasses (Berrang et al., 2004b). A systematic washing procedure might be an effective mean of significantly reducing *Campylobacter* numbers on carcass surfaces. However, *Campylobacter* contamination can occur throughout the entire process, including in the chilling room (Berndtson et al., 1996). The contamination of equipment, work surfaces, process water and air increases the probability of contamination of initially *Campylobacter*-free carcasses. Evidently, the contamination of non infected batches of chickens depends on both the *Campylobacter* status of previously slaughtered batches and the amount of cross-contamination. Many works have shown that cross-contamination can occur in the course of a day and the implementation of slaughter schedule could preserve *Campylobacter*-free batches.

In our study, the contamination risk was observed to increase when the temperature in the evisceration room was higher than 15 °C. This result can be associated to the conditions in which *Campylobacter* survives in the air or on equipment. *Campylobacter* has previously been recovered in aerosol (Haas et al., 2005) and could be the cause of carcass contamination. However, this risk factor is difficult to interpret because *Campylobacter* survives at least as well at lower temperatures (Chan et al., 2001). Moreover, this variable may be related to seasonal differences in temperature. A case/control study would be necessary to validate this factor as it was not shown that room temperatures under 15 °C in summer reduce cross-contamination by *Campylobacter*.

The contamination risk by *Campylobacter* was found to increase when dirty marks were observed on eviscerated carcasses. Although many sanitary practices are applied at the slaughterhouse, the presence of such marks is frequently notified. The machines used for evisceration cannot adapt to the natural variation of carcass sizes within a given batch. Consequently, the rupture of viscera is common and the release of intestinal contents can contaminated the carcasses eviscerated. Moreover, the equipment and the machines could also be dirtied and therefore could contaminate the following carcasses. The systematic rinsing of eviscerated carcasses or the preliminary sorting of carcasses according to their size may help limit dirty marks and therefore preserve *Campylobacter*-free batches from further contamination.

The analysis of risk factor at the slaughterhouse showed how the maintenance of biosecurity measures during thinning and the

implementation of a schedule for slaughtering *Campylobacter*-free batches first each day are necessary for mitigation of *Campylobacter* contamination. The analysis also demonstrated that the evisceration process contributes to carcass contamination. Data obtained in this study could be used in HACCP and risk management implementation at slaughterhouses. It would be interesting to develop specific studies, (case/control) to separately test the impact of each factor on the load and the presence or absence of *Campylobacter*.

Acknowledgements

We are grateful to French Veterinary Authorities for collecting samples and questionnaires and the staff of slaughterhouses participating in the study for making this work possible. The Food Safety Department of the French Ministry of Agriculture and the European commission are gratefully acknowledged for their financial support.

References

Abe, T., Haga, S., Yokoyama, K., Watanabe, N., 2008. An outbreak of *Campylobacter jejuni* subsp. jejuni infection via tap water. Jpn. J. Infect. Dis. 61, 327.

Adkin, A., Hartnett, E., Jordan, L., Newell, D., Davison, H., 2006. Use of a systematic review to assist the development of *Campylobacter* control strategies in broilers. J. Appl. Microbiol. 100, 306–315.

Anonymous, 2005. Advisory Committee on the Microbiological Safety of Food: Second Report on *Campylobacter*. Food Standards Agency Available online: http://www.food.gov.uk/multimedia/pdfs/acmsfcampylobacter.pdf, 185 pp..

Anonymous, 2006. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for Detection and Enumeration of *Campylobacter* Spp. International Organisation for Standardisation, Geneva, Swiztzerland.

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. Official Journal of the European Union, L190/25.

Arsenault, J., Letellier, A., Quessy, S., Normand, V., Boulianne, M., 2007. Prevalence and risk factors for *Salmonella* spp. and *Campylobacter* spp. caecal colonization in broiler chicken and turkey flocks slaughtered in Quebec, Canada. Prev. Vet. Med. 81, 250–264.

Atanassova, V., Ring, C., 1999. Prevalence of *Campylobacter* spp. in poultry and poultry meat in Germany. Int. J. Food Microbiol. 51, 187–190.

Avrain, L., Humbert, F., L'Hospitalier, R., Sanders, P., Vernozy-Rozand, C., Kempf, I., 2003. Antimicrobial resistance in *Campylobacter* from broilers: association with production type and antimicrobial use. Vet. Microbiol. 96, 267–276.

Berndtson, E., Danielsson-Tham, M.L., Engvall, A., 1996. *Campylobacter* incidence on a chicken farm and the spread of *Campylobacter* during the slaughter process. Int. J. Food Microbiol. 32, 35–47.

Berrang, M.E., Northcutt, J.K., Cason, J.A., 2004a. Recovery of *Campylobacter* from broiler feces during extended storage of transport cages. Poult. Sci. 83, 1213–1217.

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

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

Chan, K.F., Le Tran, H., Kanenaka, R.Y., Kathariou, S., 2001. Survival of clinical and poultry-derived isolates of *Campylobacter* jejuni at a low temperature (4 degrees C). Appl. Environ. Microbiol. 67, 4186–4191.

Denis, M., Huneau-Salaun, A., Balaine, L., Salvat, G., 2007. Seasonal variation in the quantity of *Campylobacter* spp. excreted by chicken in broiler flocks. Zoonoses and public health 54 (Suppl. 1) 14th Int. Workshop on *Campylobacter*, *Helicobacter* and Related Organisms, Rotterdam, The Netherlands, 02–05.09.2007.

EFSA, 2009. The community summary report on trends and sources of zoonoses and zoonotic agents in the European Union in 2007. EFSA J. 223 Available online: www.efsa.europa.eu.

EFSA, 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. EFSA J. 8 (03), 1503, 99 pp. Available online: www.efsa.europa.eu.

Figueroa, G., Troncoso, M., Lopez, C., Rivas, P., Toro, M., 2009. Occurrence and enumeration of *Campylobacter* spp. during the processing of Chilean broilers. BMC Microbiol. 9.

Haas, D., Posch, J., Schmidt, S., Wüst, G., Sixl, W., Feierl, G., Marth, E., Reinthaler, F.F., 2005. A case study of airborne culturable microorganisms in a poultry slaughterhouse in Styria, Austria. Aerobiologia 21, 193–201.

Hansson, I., Plym Forshell, L., Gustafsson, P., Boqvist, S., Lindblad, J., Olsson Engvall, E., Andersson, Y., Vagsholm, I., 2007. Summary of the Swedish *Campylobacter* program in broilers, 2001 through 2005. J. Food Prot. 70, 2008–2014.

Heuer, O.E., Kpedersen, K., Jsandersen, J.S., Madsen, M., 2001. Prevalence and antimicrobial susceptibility of thermophilic *Campylobacter* in organic and conventional broiler flocks. Lett. Appl. Microbiol. 33, 269–274.

Heuvelink, A.E., van Heerwaarden, C., Zwartkruis-Nahuis, A., Tilburg, J.J., Bos, M.H., Heilmann, F.G., Hofhuis, A., Hoekstra, T., de Boer, E., 2009. Two outbreaks of campylobacteriosis associated with the consumption of raw cows' milk. Int. J. Food Microbiol. 134, 70–74.

ITAVI, 2010. Report of situation in April 2010, France – broiler chicken. Available online: http://www.itavi.asso.fr/economie/eco_filiere/volailles.php?page=prod#prod_fr.

Jozwiak, A., Reichart, O., Laczay, P., 2006. The occurrence of *Campylobacter* species in Hungarian broiler chickens from farm to slaughter. J. Vet. Med. Ser. B. Infect. Dis. Vet. Public Health 53, 291–294.

King, S., Adams, M.C., 2008. Incidence of *Campylobacter* in processed poultry: is it a concern for human health? J. Food Saf. 28, 376–388.

Lastovica, A.J., Skirrow, M.B., 2000. In: Nachamkin, I., Blaser, M.J. (Eds.), Clinical Significance of *Campylobacter* and Related Species Other than *Campylobacter jejuni* and *C. coli*. *Campylobacter*, pp. 89–120.

Liang, K.-Y., Zeger, S.L., 1986. Longitudinal data analysis using generalized linear models. Biometrika 73, 13–22.

Mackiw, E., Popowski, J., Szponar, L., 2008. Thermotolerant *Campylobacter* spp. – report on monitoring studies performed in 2004–2005 in Poland. Food Control 19, 219–222.

Newell, D.G., Allen, V., Elvers, K., James, S., Dorfper, D., Hanssen, I., Jones, P., Gittins, J., Stern, N., Sargeant, J., Davies, R., Connerton, I., 2008. A critical review of interventions and strategies (both biosecurity and non-biosecurity) to reduce *Campylobacter* on the poultry farm. Available on line: http://www.foodbase.org.uk//admintools/reportdocuments/384-381-682_Final_report_version_310.pdf.

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

Rasschaert, G., Houf, K., Van Hende, J., De Zutter, L., 2007. Investigation of the concurrent colonization with *Campylobacter* and *Salmonella* in poultry flocks and assessment of the sampling site for status determination at slaughter. Vet. Microbiol. 123, 104–109.

Reich, F., Atanassova, V., Haunhorst, E., Klein, G., 2008. The effects of *Campylobacter* numbers in caeca on the contamination of broiler carcasses with *Campylobacter*. Int. J. Food Microbiol. 127, 116–120.

Rosenquist, H., Nielsen, N.L., Sommer, H.M., Norrung, B., Christensen, B.B., 2003. Quantitative risk assessment of human campylobacteriosis associated with thermophilic *Campylobacter* species in chickens. Int. J. Food Microbiol. 83, 87–103.

Rosenquist, H., Sommer, H.M., Nielsen, N.L., Christensen, B.B., 2006. The effect of slaughter operations on the contamination of chicken carcasses with thermotolerant *Campylobacter*. Int. J. Food Microbiol. 108, 226–232.

Russa, A.D., Bouma, A., Vernooij, J.C.M., Jacobs-Reitsma, W., Stegeman, J.A., 2005. No association between partial depopulation and *Campylobacter* spp. colonization of Dutch broiler flocks. Lett. Appl. Microbiol. 41, 280–285.

Slader, J., Domingue, G., Jorgensen, F., McAlpine, K., Owen, R.J., Bolton, F.J., Humphrey, T.J., 2002. Impact of transport crate reuse and of catching and processing on *Campylobacter* and *Salmonella* contamination of broiler chickens. Appl. Environ. Microbiol. 68, 713–719.

Son, I., Englen, M.D., Berrang, M.E., Fedorka-Cray, P.J., Harrison, M.A., 2007. Prevalence of Arcobacter and *Campylobacter* on broiler carcasses during processing. Int. J. Food Microbiol. 113, 16–22.

Van Asselt, E.D., Jacobs-Reitsma, W.F., Van Brakel, R., Van Der Voet, H., Van Der Fels-Klerx, H.J., 2008. *Campylobacter* prevalence in the broiler supply chain in the Netherlands. Poult. Sci. 87, 2166–2172.

Van Gerwe, T.J.W.M., Bouma, A., Jacobs-Reitsma, W.F., Van Den Broek, J., Klinkenberg, D., Stegeman, J.A., Heesterbeek, J.A.P., 2005. Quantifying transmission of *Campylobacter* spp. among broilers. Appl. Environ. Microbiol. 71, 5765–5770.

Wingstrand, A., Neimann, J., Engberg, J., Nielsen, E.M., Gerner-Smidt, P., Wegener, H.C., Molbak, K., 2006. Fresh chicken as main risk factor for campylobacteriosis, Denmark. Emerg. Infect. Dis. 12, 280–285.

Zilbauer, M., Dorrell, N., Wren, B.W., Bajaj-Elliott, M., 2008. *Campylobacter* jejuni-mediated disease pathogenesis: an update. Trans. R. Soc. Trop. Med. Hyg. 102, 123–129.