Belgium-Wide Survey Of Campylobacter spp. Contamination In Chicken Meat Preparations: Baseline Data, And Considerations For A Reliable Monitoring Program

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Running title: Campylobacter in Belgian chicken preparations

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ABSTRACT

During the period from February to November 2007, chicken meat preparations (n=656) were sampled from eleven processing companies across Belgium. All samples were tested for *Campylobacter* by enrichment culture and by direct plating according to standard methods. Almost half (48.02%) of the samples were positive for *Campylobacter* spp. The mean *Campylobacter* count was 1.68 log$_{10}$ CFU/g with a standard deviation of ± 0.64 log$_{10}$ CFU/g. The study revealed a statistically significant variation in *Campylobacter* contamination levels between companies; processors with a wider range of frequency distribution of *Campylobacter* counts provided chicken meat preparations with higher *Campylobacter* incidence and concentrations. There was no significant difference between various preparation types on the counts of *Campylobacter* spp. However, *Campylobacter* count and incidence in chicken wings were the highest, and portioned form products (legs, wings, and breasts) showed a higher probability of being *Campylobacter*-positive compared to minced form products (sausages, burgers, and minced meat). The proportion of *Campylobacter*-positive samples was significantly higher in July than in other months. *Campylobacter* spp. recovery by direct plating was higher (41.0%) when compared to detection after enrichment (24.2%). Statistical modeling of the survey data showed that the likelihood of obtaining a positive result by enrichment culture increases with an increase of *Campylobacter* concentration in the sample. In the present study, we provide the first enumeration data on *Campylobacter* contamination in Belgian chicken meat preparations, and proposals for improving *Campylobacter* monitoring programs are addressed.


INTRODUCTION

Campylobacter jejuni and Campylobacter coli are recognized as the leading zoonotic causes of human gastrointestinal disease in Europe (6). A few hundred of these bacteria can induce clinical gastrointestinal symptoms (10). C. jejuni has recently been identified as an important infectious trigger for Guillain-Barré syndrome, the most common cause of acute flaccid paralysis in polio-free regions (24). In Belgium, 54.9 cases of human campylobacteriosis per 100,000 people were reported in 2006 by the National Reference and the Sentinel Laboratories (6). The major risk factor for human infection is believed to be consumption or mishandling of raw or undercooked chicken meat (17, 19, 26, 29). The contamination of Belgian poultry carcasses and meat with Campylobacter has been monitored since the year 2000 by the Federal Agency for the Safety of the Food Chain (FASFC), and the rate of positive samples is regarded as stable, but high (44).

Chicken meat preparations span a range of ready-to-cook products. These products include meat reduced to fragments or minced, and presented as marinated, stuffed or seasoned. All of these items have in common that they have been manipulated extensively during processing. As such, they have a potential for Campylobacter contamination not only on the surface of the meat, but also in the interior. In 2003, the Superior Health Council of the Belgian Federal Public Service (FPS) for Health, Food Chain Safety and Environment initiated a risk assessment exercise concerning Campylobacter spp. contamination in poultry meat preparations (44). This risk assessment exercise highlighted the limitation of data on Campylobacter contamination levels in chicken meat preparations as an important information gap; more precisely, the semi-quantitative nature of concentration data due to exclusive dependence on presence/absence testing (22, 44). The gap in quantitative data on
Campylobacter contamination in chicken meat preparations was also identified as a major risk analysis challenge across Europe (4).

Information gained from baseline studies can be used as a reference when setting food safety objectives, for developing microbiological criteria, and for evaluating different producers and their food safety management programs (32). Additionally, a national survey across the chicken meat industry is an important tool for investigating the conditions of processing that must be controlled to prevent, eliminate, or reduce Campylobacter contamination (4, 39). Therefore, our research goal was to execute a Belgium-wide qualitative and quantitative microbiological survey of C. jejuni and C. coli (addressed collectively as Campylobacter) contamination in chicken meat preparations, in order to enable the use of the study results as a reliable input for Campylobacter risk assessment in Belgium and similar settings if appropriate.

MATERIALS AND METHODS

The population concerned. Target products were chicken meat preparations. By definition, this refers to portioned, cut or minced meat to which spices or other ingredients (seasoning, marinade, coating, sauce, etc.) are added to improve sensory properties or texture, but the cut surface retains the characteristics of fresh meat (1, 44). Sampling was done from meat processing plants; final packages were sampled from either production lines or from factory chill rooms before distribution. We choose the processing level as a sampling point in order to target food businesses supplying a majority of the market.

Sampling frame and selection procedures. The sampling frame was based on the FASFC operators list. The processors list was updated and verified by consulting collaborators in the national poultry union and distribution sector. We adopted a targeted sampling approach (5)
by selecting 11 out of the 61 companies on the list in order to assure the following criteria: the
eleven companies are distributed across Belgium and in a way that allows the sampling team
to visit equally over randomized sampling days in each month; the biggest (three) companies,
supplying more than 85% of the Belgian distribution chains, are included; and, the companies
selected allow the sampling of different batches of portioned and minced products, and from
different preparation types. All processing plants were visited, and all product forms were
sampled each month over the sampling period.

Sample size determination. The number of samples was estimated based on an assumed
annual prevalence of ~50%, with a desired confidence interval (CI) of 95% and 5% accuracy
(4). Matching these criteria with capacity and feasibility of sampling and laboratory testing,
and taking into consideration sampling different companies over different months and
sampling different product forms, a total of 656 samples were tested during the period from
February to November 2007.

Microbiological analysis. Enumeration and qualitative detection were performed according
to the guidelines of the ISO 10272:2006 methods (2, 3). The performance characteristics and
measurement uncertainty of the enumeration method was evaluated in preliminary
experiments, and shown to be fit for the purpose of the present survey (25).

For meat preparations made from whole pieces of meat, such as marinated or ready-to-
cook stuffed and seasoned chicken portions, the sample was taken as much as possible from
the surface of the meat, starting with the skin if present, but scraping away any sauce or non-
meat components as the presence of seasonings and marinades may interfere with the analysis
(4). For meat preparations made from minced chicken meat, a portion was taken throughout
the sample as a cross-section. A test portion of 12 g was transferred to 9 volumes (108 ml) of
Bolton enrichment broth (BB) (Bolton broth CM0983 plus supplement SR183 [Oxoid,
Basingstoke, England] with 5% v/v lysed horse blood [E&O laboratories, England]) and homogenized in a stomacher blender for 1 min. From this initial homogenate, testing was carried out in parallel, as follows: a) For enumeration: 10 ml (~1 g) was transferred to a sterile tube, and 1 ml from it (10^{-1}) was spread plated over four (0.3, 0.3, 0.3, and 0.1 ml) modified charcoal Cefoperazon Deoxycholate agar plates (mCCDA) (Campylobacter blood free selective medium CM739 plus selective supplement SR155 [Oxoid, Basingstoke, England]). One further serial dilution (10^{-2}) was made in 0.1% Peptone water (1 g Bacteriological peptone [Oxoid, Basingstoke, England] in 1 liter of sterile deionized water) and 0.1 ml was spread plated on mCCDA. To obtain easily countable colonies, plates were air-dried for 35 min at room temperature with the cover partly opened in a sterile laminar flow. A microaerobic atmosphere was achieved by introducing a gas mixture consisting of 5% CO₂, 5% O₂, 5% H₂ and 85% N₂ in stainless steel jars (10 liter size, Don Whitley Scientific, West Yorkshire, UK). Agar plates were incubated at 41.5ºC and counted after 48 h; b) For presence/absence testing: 10 ml (~1 g) from the same sample of homogenate was transferred to a sterile tube, while the remaining 100 ml (~10 g) of sample homogenate was transferred to a sterile Schott bottle (100 ml volume bottle with sealing ring and screw cap) and filled up to ca. 2 cm below the mouth with added BB. Tubes were incubated microaerobically, as described above, and Schott bottles were incubated aerobically, both at 41.5ºC. After 48 h of incubation, 10 µl aliquots were subsequently plated onto mCCDA plates (plates were allowed to warm to room temperature without being dried). However, after testing 75 samples, it was notable that the enrichment in Schott bottles incubated aerobically was associated with less Campylobacter recovery when compared to enrichment portions in tubes incubated microaerobically. Thereafter, we continued the rest of the qualitative detection after enrichment using a portion of 10 ml (~1 g) from the initial homogenate in tubes incubated microaerobically.
Confirmation and species identification. Enumeration and confirmation of presumptive Campylobacter spp. colonies were performed according to the ISO 10272:2006 principles (1, 2), but with the following modifications: presumptive and suspected colonies, based on colony morphology, were re-streaked on Muller-Hinton based blood agar plates (Muller-Hinton agar base CM337 [Oxoid, Basingstoke, England] supplemented with 5% v/v full horse blood [E&O laboratories, England]) and incubated microaerobically at 41.5°C for 24 h. Isolated colonies were then re-streaked for purity on mCCDA and incubated microaerobically at 41.5°C overnight. From confluent growth on mCCDA, crude DNA lysates for PCR were prepared using the previously described simple boiling protocol (21), and the rest was stored at –80°C. From each positive sample, up to three isolates were subjected to multiplex PCR for identification of C. jejuni and C. coli utilizing the primers and running protocol described by Vandamme et al. (45).

Statistical analysis. For a descriptive summary of enumeration results, Campylobacter counts were converted to a logarithmic scale to approximate the results to normal distribution. Results of Campylobacter detection after enrichment were recorded as binary variables in terms of Campylobacter presence or absence, and enumeration results were recorded as numbers of colonies forming units per gram. Samples were clustered within each company and this was accounted for in the analysis using the procedures xtlogit (random-effects logistic regression model) and xtpoisson (random-effects Poisson regression model) in STATA® statistical software, version 8.0. (42). The enumeration data exhibited a skewed distribution and Poisson regression was not always the best fit model. Therefore, a negative binomial model was used to account for extra-Poisson variation.
RESULTS

Overview of *Campylobacter* spp. contamination. Almost half (48.02% (315/656)) of chicken meat preparation samples were positive for *Campylobacter* spp. The status of contaminated samples is presented as a combination of all positive results obtained by direct plating and/or enrichment cultures. The count data (Fig. 1) showed a skewed distribution to the left, as 58.99% of samples were contaminated with less than 10 CFU/g. On the other hand, 29.38% of samples were contaminated with a range from $\geq 10$ to $< 100$ CFU/g, and 11.63% of samples were contaminated with $\geq 100$ CFU/g. The average *Campylobacter* concentration was 1.68 $\log_{10}$ CFU/g, with a standard deviation of $\pm 0.64$.

Variation in *Campylobacter* spp. contamination between producers. Results in Table 1 and Figure 2 reveal considerable variability in *Campylobacter* contamination levels between producers. All producers provided *Campylobacter* positive samples, although they ranged from 8.89% for producer [F] up to 84.81% for producer [D]. Random-effects logistic regression analysis indicates that producer [D] was by far the most significant (Odds ratio (OR)= 10.2, $P$ value $< 0.0001$) in providing *Campylobacter* contaminated samples, followed by producer [B] (OR= 5.9, $P$ value $< 0.0001$), and then producer [C] (OR= 3.7, $P$ value= 0.001). On the other hand, the incidence of *Campylobacter* in samples from producer [F] was significantly lower (OR= 0.13, $P$ value= 0.003). The three producers ([B],[C],[D]) with highest *Campylobacter* incidence also exhibited significantly higher *Campylobacter* counts, and provided a wide frequency distribution range in *Campylobacter* concentration in their tested samples (Fig. 2). However, the highest average of *Campylobacter* counts was associated with producer [K] (Table 1), as 21.5% (11/51) of samples obtained from this company tended to exceed a contamination level of $2 \log_{10}$ CFU/g (Fig. 2).
Variation in *Campylobacter* spp. contamination in relation to product forms and preparation types. The descriptive results in table 2 were modeled using Random-effects logistic regression. In biological terms, portioned form products (breasts, legs, wings) compared with minced form products were almost one and half fold higher (OR= 1.7; *P* value= 0.002; 95% CI: 1.2-2.5) for being *Campylobacter* positive. With regard to the counts of *Campylobacter* spp., a significantly higher count (*P* value= 0.002) was associated with chicken wings (Fig. 3A), with a mean count 2.21 log_{10} CFU/g.

On the other hand, incidence of *Campylobacter* in seasoned products was slightly higher (Table 2), but only border-line statistically significant (*P* value= 0.088). There was no notable difference in *Campylobacter* concentration between various preparation types (marinade, seasoning sauce, and coated (e.g., with herbs, cheese, etc.) (Fig. 3B).

Variation in *Campylobacter* spp. contamination in relation to sampling months. Figure 4 shows that there was a gradual increase, although not statistically significant, in the number of *Campylobacter*-positive samples during May and June. Random-effects logistic regression analysis showed that this increase became statistically significant (OR=4.0; *P* = 0.007; CI: 1.4-11.2) in July. There was no significant change in *Campylobacter* quantification from positive samples over sampling months.

Effect of direct culture versus selective enrichment on the isolation of *Campylobacter* from chicken meat preparations. Table 3 indicates that *Campylobacter* recovery using direct plating was dramatically higher; 41.0% detected by direct plating compared to 24.2% by enrichment culture. However, 7% (46/656) of the samples were found to be positive by enrichment but were below the limit of quantification for direct culture.

The relation between direct plating results (count; continuous variable) and enrichment results (presence/absence; binary variable) was evaluated statistically;
Interestingly, there was a significant positive relation (negative binomial regression analysis; coefficient= 0.82, P value= 0.007) indicating that the likelihood of obtaining a positive result by selective enrichment increases with an increase of *Campylobacter* concentration in the sample. Thus, samples with lower *Campylobacter* concentrations had a lower likelihood of giving a positive result by selective enrichment compared to direct plating.

Isolates from positive samples (by direct and enrichment culture) were identified using multiplex PCR for *C. jejuni* and *C. coli* concurrently. *C. jejuni* was dominant, amounting to 73.1% of direct plating isolates, and to 66.4% of selective enrichment isolates. Both species were concurrently present in isolates from 25 positive samples detected by direct plating, and in 16 positive samples detected by enrichment culture.

**DISCUSSION**

*Campylobacter prevalence and considerations while comparing survey data.* The finding that almost half of tested chicken meat preparations were contaminated with *Campylobacter* (Table 1) is similar, to an extent, with prevalence data from the Belgian monitoring program conducted by the FASFC. Ghafir et al. (22) indicated that *Campylobacter* prevalence in broiler meat preparations from Belgian retail establishments was 49.4% (39/79) and 44.9% (44/98) in 2002 and 2003, respectively. Their data were based on a laboratory methodology in which results were recorded after testing a 25 g test portion by enrichment in Preston broth and subsequent isolation on mCCDA. Surprisingly, the data of Ghafir et al., and our survey results, greatly contradict monitoring data from the same Belgian agency (FASFC) conducted in the following years (7, 8); the Belgian monitoring data indicated a *Campylobacter* prevalence of 3.7% (10/269) and 2.5% (4/162) for broiler meat preparations sampled at processing plants in 2005 and 2006, respectively, while for broiler meat preparations at retail
level a prevalence of 3.4% (3/87) and 2.0% (2/102) was determined in 2005 and 2006, respectively, as well. Such a dramatic drop in *Campylobacter* prevalence based on the FASFC monitoring data for 2005 and 2006 is likely a reflection of a change in laboratory methodology that replaced the enrichment of 25 g test portions (used in 2002-3 monitoring) with a lower enrichment volume equal to 0.01 g (on which 2005-6 data were based), but still using Preston enrichment broth and subsequent isolation on mCCDA (7, 8). Modeling quantitative versus qualitative detection results from our survey showed that the lower the *Campylobacter* concentration in the sample is, the lower the likelihood of obtaining a positive result using selective enrichment is. Additionally, the present survey showed that *Campylobacter* concentration was less than 10 CFU/g in 60% of chicken meat preparation samples (Fig. 1). Thus, we anticipate that a testing protocol based on *Campylobacter* detection in an enrichment volume equal to 0.01 g would not be appropriate for chicken meat preparations; such a testing approach is expected to detect more samples with relatively higher *Campylobacter* concentrations, while its diagnostic sensitivity might not be suitable for relatively low level contamination. Our hypothesis could be supported by recent laboratory findings by Rosenquist and colleagues (41), as they indicated that the sensitivity of qualitative detection using Bolton enrichment broth declines significantly with lower concentrations of *Campylobacter* in artificially inoculated samples.

A reliable analysis of a national prevalence trend should be based on stable testing procedures. Unnecessary changes in laboratory testing procedures may hinder the comparison of monitoring data between countries (4, 6), and may also hinder such comparisons within the same country.

*Campylobacter counts and risk assessment.* The quantitative data concluded by the present survey are considered to be the first on *Campylobacter* contamination levels in Belgian chicken meat preparations. The counts distribution (Fig. 1) is based on microbiological testing
procedures with pre-evaluated performance characteristics and estimated measurement uncertainty (25). Therefore, the present survey data could provide a contribution to the future optimization of a quantitative risk assessment of *Campylobacter* in Belgium.

The *Campylobacter* load in chicken meat preparations showed a concentration average of ~50 CFU/g. Nevertheless, it might not be correct to assume that the infection risk of *Campylobacter* through chicken meat preparations is low because of such “relatively” low counts. Dose-response studies have shown that the infective dose of *C. jejuni* may be quite low (10, 16, 33). In a restaurant associated outbreak, the number of *C. jejuni* in the causative chicken meal was estimated to range from 53 to 750 *C. jejuni* per gram (40). Additionally, in-vitro models indicate that the invasion efficiency of some *Campylobacter* strains to intestinal cell lines is optimal at the lowest range of multiplicities of infection, which suggests that *Campylobacter* is a highly efficient solitary invader. This means that a single *C. jejuni* can induce its own uptake into host cells (27).

**Considerations related to product forms and preparation types.** Statistical modeling indicated that the odds of *Campylobacter* incidence in minced form products are less when compared to portioned form products (Table 2). This finding shows that balancing sample forms should be accounted for when designing a chicken meat preparation survey. Improper balance of sample forms might introduce an unpredicted bias into prevalence and count results. The variation in *Campylobacter* incidence between minced and part forms might be attributed to the fact that the processing of minced meat preparations implies progressive exposure of *Campylobacter* to air during portioning, grinding and dicing of meat taken from whole carcasses. Bostan et al. reported a progressive decrease in *Campylobacter* counts starting at 2.8×10^5 to 4.3×10^5 in whole meat, 1.1×10^4 CFU/g in ground meat, 3.8×10^3 CFU/g in cubed meat, and <10 CFU/g in meatballs (11).
On the other hand, significantly higher incidence and counts of *Campylobacter* were attributed to chicken wing samples (Table 2 and Fig. 3.A). Chicken wings can be identified as a particularly high-risk product group, since the high load of *Campylobacter* in chicken wings could increase the probability of pathogen transfer to other surfaces through cross-contamination and inappropriate handling during meal preparation and cooking (12, 36).

During laboratory testing, it was notable that traces of feathers or feather shafts were commonly still connected to wing samples. *Campylobacter* originally associated with feathers might be transferred to the skin through the action of the picker’s rubber fingers during mechanical feather removal in the slaughterhouse (13). Also, the high count of *Campylobacter* spp. in chicken wings (Fig. 3.A) might be attributed to imperfect scalding, post-scalding contamination, or a combination of both (15).

**Direct plating-or-enrichment, versus, direct plating-and-enrichment.** The technique used in our survey was recommended by the scientific working group of the EFSA for an EU-wide monitoring program for *Campylobacter* in chicken meat preparations (4). This EFSA recommended approach is based on performing quantitative and qualitative detection of *Campylobacter* in parallel from the same test portion. This allows better control over sub-sampling bias arising from the heterogeneous nature of bacterial distribution in food. Moreover, we used a highly specific and sensitive multiplex PCR, instead of biochemical tests, for result confirmation and species identification. This multiplex PCR was evaluated to be of 93% sensitivity and 100% specificity for *C. jejuni* subspecies *jejuni* identification, and of 100% sensitivity and 100% specificity for *C. coli* identification (38).

Surprisingly, the variation between direct culture and selective enrichment was very evident in our chicken preparation survey. Nevertheless, variation between direct and enrichment culture in *Campylobacter* recovery was previously cited in different food and environmental samples. Musgrove et al. (34) indicated a decrease in detection of 36.7% of
Campylobacter spp. in chicken caecal samples with enrichment procedures. Gharst et al. (23) tested 143 fecal samples from mature cattle colon at slaughter. Campylobacter was detected in 50.3% by selective enrichment, 12.6% by direct plating and 37.1% by both methods. In another study, comparing both culture methods for the recovery of Campylobacter from bootsocks, feces and cloacal swabs from broiler flocks, Jørgensen et al. (30) concluded that enrichment was not significantly better than direct plating. Finally, in 2006, using the same ISO method as we used, data from the monitoring system in the Netherlands indicated a Campylobacter prevalence of 14.5% (199/1368) in broiler meat using enrichment procedures alone; however, Campylobacter prevalence increased to 34% after adding positive enumeration results from the same samples (20). The previous findings from the Dutch monitoring is very close to our survey results, as combining the results for detection by direct plating with those detected by enrichment culture led to an almost doubling of the apparent prevalence of Campylobacter in chicken meat preparations (Table 3).

Campylobacter spp. has a slower growth rate than many other bacterial species and competes poorly outside of its intestinal niche (14, 28). The enrichment step might provide an opportunity for rapidly growing Campylobacter strains to be selected and perhaps overgrow slow-growing strains (34). However, enrichment using Bolton broth provides a reasonable compromise between the selectivity of Campylobacter and suppression of competitor flora (18). Nevertheless, some meat flora could still survive during enrichment in Bolton broth. Baylis et al. revealed a high incidence of E. coli spp. and Pseudomonas spp. in Bolton broth after the enrichment of artificially and naturally contaminated meat samples (9). In our study, we frequently encountered background flora on mCCDA plates despite the preceding enrichment in Bolton Broth. In fact, the overgrowth of indigenous flora on mCCDA plates after 48 h of enrichment might cause a false negative confirmation of a positive sample (31).
Thus, as the initial density of Campylobacter decreases, the antagonistic interaction of the
dominant indigenous micro-biota is expected to increase.

In our survey, both culture methods are considered to be complimentary to each other.
Therefore, in the case of chicken meat preparations, we recommend a combination of both
culture procedures in parallel in order to obtain the best estimate of Campylobacter
prevalence.

**Seasonality.** We cannot claim that our study proves an absolute seasonality pattern of
Campylobacter, rather it indicates a possible peak in the so-called “warm months”,
resembling those indicated in other European countries (35, 37, 43). In our study, the number
of samples varied over months due to logistical reasons. However, we maintained a
proportional distribution of sampling visits to all companies every month, and all product
forms were sampled from each company.

**General remarks on survey design and sampling procedures.** In the present study, we
choose the processing sector as our sampling point. Results in Table 1 and Figure 1 show the
potential of sampling at the industry level in revealing the variability in Campylobacter
contamination in the processing chain. The value of an effective process control system is
most evident when data are organized and used to further increase knowledge about the extent
of variability in the distribution of microbial contamination. Based on the present survey data,
we can conclude that processors with a high degree of quantitative variability (Fig. 2) are
more likely to produce hazardous chicken meat preparations with higher Campylobacter
concentrations and incidence. Thus, a quantitative Campylobacter monitoring program could
be of value in prioritizing a Campylobacter risk-based inspection, as well as tracing sources
of unacceptable contamination. In conclusion, the present study provides a comprehensive
quantitative data set on Campylobacter contamination in chicken meat preparations. Careful
analysis of this data highlights certain issues that need to be considered for reliable
Campylobacter monitoring in chicken meat preparations, mainly; (i) the need to account for
the variability in contamination between processors and its impact on the risk to the public.
Such variation can be overlooked by targeting major retailers or supermarket chains as the
only sampling points; (ii) the need to account for the bias of detection methods on survey
conclusions and on the comparison of contamination trends.

It will be investigated in subsequent work if, and how, certain processing practices
could influence the risk profile of Campylobacter contamination. In view of the imperfect
sensitivity of the routine testing methods, it will be of interest to consult available statistical
modeling approaches in order to update our knowledge on the true Campylobacter prevalence
estimate.

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REFERENCES


FIGURE LEGENDS

Figure 1. Frequency distribution of Campylobacter spp. counts in 656 chicken meat preparation samples. The scaling of the Y-axis shows the number of samples that fall within the range of log_{10} CFU/g of X-axis represented by the bar.

Figure 2. Variation in frequency distribution of Campylobacter spp. counts in chicken meat preparation samples over companies. The eleven companies are identified by letters from [A] to [K], the figure include two dashed lines denote to contamination levels of 1 log_{10} CFU/g and 2 log_{10} CFU/g. The scaling of the Y-axis shows the number of samples that fall within the range of log_{10} CFU/g of X-axis represented by the bar.

Figure 3. Variation in Campylobacter spp. counts in 656 chicken meat preparation samples from eleven Belgian producers, distributed according to (A) product forms, and (B) preparation types. The line inside the box represents the median value, upper and lower hinges represent the 75th and the 25th percentiles, respectively. Highest values of Campylobacter contaminated counts (values over the 90th percentile) are shown as circles.

Figure 4. Occurrence of Campylobacter spp. in Belgian chicken meat preparations over the months from February to November 2007. Symbols: ▲, % of positives; dotted column, number of samples tested; squared column, number of Campylobacter positive samples.
Fig. 1. (One column size)
Fig. 2. (Two columns)

Campylobacter count (log$_{10}$ CFU/g)
Fig. 3. (One column size)

![Graph A: Campylobacter count (log_{10} CFU/g)]

- Breast
- Burger
- Leg
- Minced meat
- Sausage
- Wing

![Graph B: Campylobacter count (log_{10} CFU/g)]

- Coated
- Marinated
- No added preparations
- Seasoned

[ACCEPTED]
Fig. 4. (One column size)
Table 1. Distribution of *Campylobacter* spp. contamination in chicken meat preparations sampled from 11 Belgian producers (*n* = 656, February to November 2007).

<table>
<thead>
<tr>
<th>ID</th>
<th>No. sampled</th>
<th>Total positive no. (%)</th>
<th>Mean (log(_{10}) CFU/g) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>54</td>
<td>18 (34.62)</td>
<td>1.39 ± 0.40</td>
</tr>
<tr>
<td>B</td>
<td>38</td>
<td>29 (76.32)</td>
<td>1.62 ± 0.45</td>
</tr>
<tr>
<td>C</td>
<td>52</td>
<td>35 (67.31)</td>
<td>1.76 ± 0.68</td>
</tr>
<tr>
<td>D</td>
<td>79</td>
<td>67 (84.81)</td>
<td>1.86 ± 0.54</td>
</tr>
<tr>
<td>E</td>
<td>70</td>
<td>38 (54.29)</td>
<td>1.50 ± 0.58</td>
</tr>
<tr>
<td>F</td>
<td>43</td>
<td>4 (8.89)</td>
<td>1.25 ± 0.44</td>
</tr>
<tr>
<td>G</td>
<td>70</td>
<td>25 (35.71)</td>
<td>1.27 ± 0.48</td>
</tr>
<tr>
<td>H</td>
<td>45</td>
<td>8 (17.78)</td>
<td>1.27 ± 0.38</td>
</tr>
<tr>
<td>I</td>
<td>77</td>
<td>45 (58.44)</td>
<td>1.67 ± 0.63</td>
</tr>
<tr>
<td>J</td>
<td>77</td>
<td>25 (32.47)</td>
<td>1.72 ± 0.67</td>
</tr>
<tr>
<td>K</td>
<td>51</td>
<td>21 (41.18)</td>
<td>2.21 ± 1.08</td>
</tr>
</tbody>
</table>

*Companies identification letters are assigned arbitrary.*
**Table 2.** Distribution of *Campylobacter* spp. contamination in chicken meat preparations in relation to product forms and preparation types (n= 656, 11 Belgian producers, February to November 2007).

<table>
<thead>
<tr>
<th>Product forms</th>
<th>No. sampled</th>
<th>Total positive no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burgers</td>
<td>71</td>
<td>28 (39.44)</td>
</tr>
<tr>
<td>Minced meat</td>
<td>99</td>
<td>42 (42.42)</td>
</tr>
<tr>
<td>Sausages</td>
<td>146</td>
<td>58 (39.73)</td>
</tr>
<tr>
<td>Total minced forms</td>
<td>316</td>
<td>128 (40.51)</td>
</tr>
<tr>
<td>Breast&lt;sup&gt;a&lt;/sup&gt;</td>
<td>153</td>
<td>68 (44.44)</td>
</tr>
<tr>
<td>Legs&lt;sup&gt;b&lt;/sup&gt;</td>
<td>139</td>
<td>83 (59.71)</td>
</tr>
<tr>
<td>Wings&lt;sup&gt;c&lt;/sup&gt;</td>
<td>48</td>
<td>36 (75.00)</td>
</tr>
<tr>
<td>Total portioned forms</td>
<td>340</td>
<td>187 (55.00)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Preparation types</th>
<th>No. sampled</th>
<th>Total positive no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (without)</td>
<td>189</td>
<td>71 (37.57)</td>
</tr>
<tr>
<td>Coated</td>
<td>80</td>
<td>30 (37.50)</td>
</tr>
<tr>
<td>Marinated</td>
<td>306</td>
<td>166 (54.25)</td>
</tr>
<tr>
<td>Seasoned</td>
<td>81</td>
<td>48 (59.26)</td>
</tr>
</tbody>
</table>

<sup>a</sup> All breast samples were fillet except one sample contained skin; <sup>b</sup> chicken legs with skin= 105/139, and chicken legs without skin= 34/139; <sup>c</sup> All wing samples were presented with skin.
Table 3. Detection of *Campylobacter* spp. in chicken meat preparations by direct plating versus enrichment culture (*n* = 656, 11 Belgian producers, February to November 2007). For direct plating, positive = countable result and negative = below quantification limit; for enrichment culture, positive = *Campylobacter* recovered and negative = below detection limit.

<table>
<thead>
<tr>
<th>Direct plating</th>
<th>Enrichment culture</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>–</td>
<td>–</td>
<td>341</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>113</td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>156</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>46</td>
</tr>
</tbody>
</table>