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Baseline Data from a Belgium-Wide Survey of *Campylobacter* Species Contamination in Chicken Meat Preparations and Considerations for a Reliable Monitoring Program▽

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From February to November 2007, chicken meat preparations ($n = 656$) were sampled at 11 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 frequency distribution range of *Campylobacter* counts provided chicken meat preparations with higher *Campylobacter* incidences and concentrations. There was no significant difference between the counts of *Campylobacter* spp. in various preparation types. However, the *Campylobacter* counts and incidences 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. Recovery of *Campylobacter* spp. recovery by direct plating was higher (41.0%) 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 in the *Campylobacter* concentration in the sample. In the present study, we provide the first enumeration data on *Campylobacter* contamination in Belgian chicken meat preparations and address proposals for improving *Campylobacter* monitoring programs.

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 (22). 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 (16, 18, 24, 27). 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 (41).

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 for Health, Food Chain Safety, and Environment initiated a risk assessment exercise concerning contamination of poultry meat preparations by *Campylobacter* spp. (41). 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 semiquantitative nature of concentration data due to exclusive dependence on presence/absence testing (20, 41). 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 (29). 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, 36). Therefore, our research goal was to execute a Belgium-wide qualitative and quantita-

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tive microbiological survey of *C. jejuni* and *C. coli* (addressed collectively as *Campylobacter* spp.) 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. The 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, 41). Sampling was done at meat processing plants; final packages were taken from either production lines or factory chill rooms before distribution. We chose 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 list of operators. The list of processors 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 11 companies are distributed across Belgium in a way that allows the sampling team to visit them equally over randomized sampling days each month; the biggest 3 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 of 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 at different companies in different months and sampling different product forms, a total of 656 samples were tested 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 were evaluated in preliminary experiments and shown to fit the purpose of the present survey (23).

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 nonmeat components as the presence of seasonings and marinades may interfere with the analysis (4). For 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% [vol/vol] 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. (i) For enumeration, 10 ml (~1 g) was transferred to a sterile tube and 1 ml of it (10^{-1}) was spread plated over four (0.3, 0.3, 0.3, and 0.1 ml) modified charcoal cefoperazone 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 under a sterile laminar-flow hood. A microaerobic atmosphere was achieved by introducing a gas mixture consisting of 5% CO_2, 5% O_2, 5% H_2, and 85% N_2 in stainless steel jars (10-liter 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 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 bottle with a sealing ring and a 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 41.5°C. 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 compared to enrichment portions in tubes incubated microaerobically. Thereafter, we performed the rest of the qualitative detection after enrichment by using a portion of 10 ml (~1 g) from the initial homogenate in tubes incubated microaerobically.

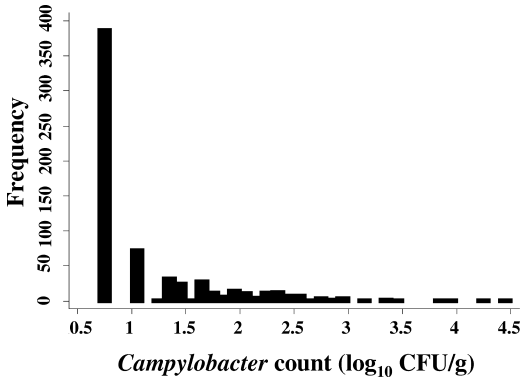

FIG. 1. Frequency distribution of *Campylobacter* counts in 656 chicken meat preparation samples. The scale on the *y* axis shows the number of samples that fall within the range of *Campylobacter* counts represented by the bars on the *x* axis.

Confirmation and species identification. Enumeration and confirmation of presumptive *Campylobacter* 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 restreaked on Muller-Hinton-based blood agar plates (Muller-Hinton agar base CM337 [Oxoid, Basingstoke, England] supplemented with 5% [vol/vol] full horse blood [E&O Laboratories, England]) and incubated microaerobically at 41.5°C for 24 h. Isolated colonies were then restreaked for purity on mCCDA and incubated microaerobically at 41.5°C overnight. From confluent growth on mCCDA, crude DNA lysates for PCR were prepared by using the previously described simple boiling protocol (19) 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* with the primers and running protocol described by Vandamme et al. (42).

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 CFU per gram. Samples were clustered within each company, and this was accounted for in the analysis by using the procedures xtlogit (random-effects logistic regression model) and xtpoisson (random-effects Poisson regression model) in the Stata statistical software, version 8.0. (39). 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* contamination. Almost half (48.02% [315/656]) of the chicken meat preparation samples tested 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 the samples were contaminated with <10 CFU/g. On the other hand, 29.38% of the samples were contaminated with a range of ≥10 to <100 CFU/g and 11.63% of the samples were contaminated with ≥100 CFU/g. The average *Campylobacter* concentration was 1.68 $\log_{10}$ CFU/g, with a standard deviation of ± 0.64 $\log_{10}$ CFU/g.

Variation in *Campylobacter* contamination between producers. Results in Table 1 and Fig. 2 reveal considerable variability in *Campylobacter* contamination levels between producers. All producers provided *Campylobacter*-positive samples, although

TABLE 1. Distribution of *Campylobacter* contamination in chicken meat preparation samples from 11 Belgian producers[a]

Company[b]	No. of samples	Total no. (%) positive	Mean $\log_{10}$ CFU/g ± SD
A	54	18 (34.62)	1.39 ± 0.40
B	38	29 (76.32)	1.62 ± 0.45
C	52	35 (67.31)	1.76 ± 0.68
D	79	67 (84.81)	1.86 ± 0.54
E	70	38 (54.29)	1.50 ± 0.58
F	43	4 (8.89)	1.25 ± 0.44
G	70	25 (35.71)	1.27 ± 0.48
H	45	8 (17.78)	1.27 ± 0.38
I	77	45 (58.44)	1.67 ± 0.63
J	77	25 (32.47)	1.72 ± 0.67
K	51	21 (41.18)	2.21 ± 1.08

[a] n = 656, February to November 2007.
[b] Company identification letters were assigned arbitrarily.

they ranged from 8.89% for producer F 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, $P < 0.0001$) in providing *Campylobacter*-contaminated samples, followed by producer B (OR = 5.9, $P < 0.0001$) and then producer C (OR = 3.7, $P = 0.001$). On the other hand, the incidence of *Campylobacter* in samples from producer F was significantly lower (OR = 0.13, $P = 0.003$). The three producers with the highest *Campylobacter* incidences (B, C, and D) also exhibited significantly higher *Campylobacter* counts and provided a wide frequency distribution range of *Campylobacter* concentrations 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 the samples obtained from this company tended to exceed a contamination level of 2 $\log_{10}$ CFU/g (Fig. 2).

Variation in *Campylobacter* contamination in relation to product forms and preparation types. The descriptive results in Table 2 were modeled by using random-effects logistic regression. In biological terms, almost 1.5 times as many portioned-form products (breasts, legs, and wings) as minced-form products were *Campylobacter* positive (OR = 1.7; $P = 0.002$; 95% CI, 1.2 to 2.5). Significantly higher *Campylobacter* counts ($P = 0.002$) were associated with chicken wings (Fig. 3A), with a mean count of 2.21 $\log_{10}$ CFU/g.

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

Variation in *Campylobacter* contamination in relation to sampling months. Figure 4 shows that there was a gradual increase, although it was 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 to 11.2) in July. There was no significant change in *Campylobacter* quantification in positive samples over the sampling months.

Effect of direct culture versus selective enrichment on *Campylobacter* isolation from chicken meat preparations. Table 3 indicates that *Campylobacter* recovery by direct plating was dramatically higher; 41.0% detected by direct plating compared to 24.2% by enrichment culture. However, 7% (46/656)

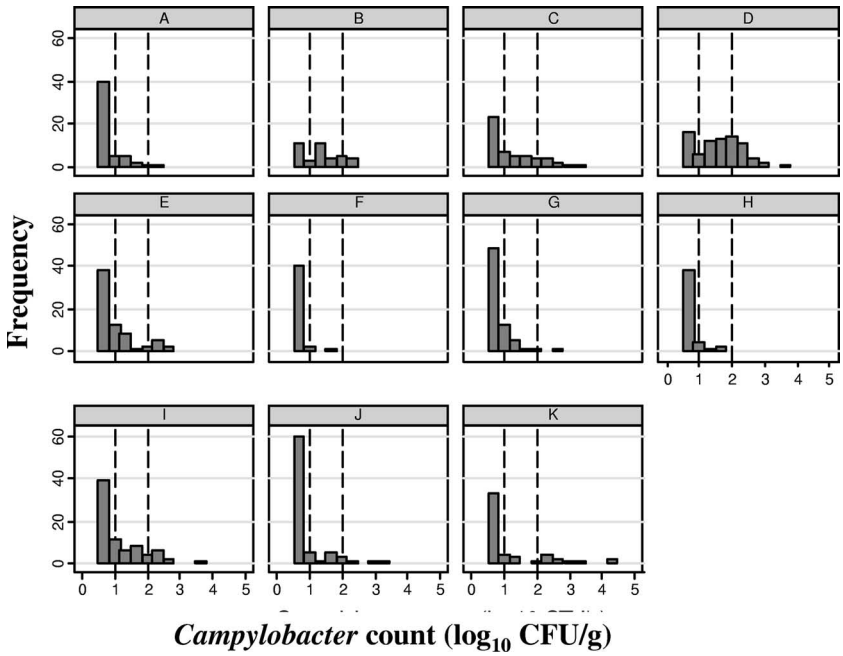

FIG. 2. Variation in frequency distribution of *Campylobacter* counts in chicken meat preparation samples over companies. The 11 companies are identified by the letters A to K, and the two dashed lines denote contamination levels of 1 and 2 $\log_{10}$ CFU/g. The scale on the y axis shows the number of samples that fall within the range of *Campylobacter* counts represented by the bars on the x axis.

TABLE 2. Distribution of *Campylobacter* contamination in chicken meat preparations in relation to product forms and preparation types[a]

Sample form or type	No. of samples	Total no. (%) positive
Minced forms		
Burgers	71	28 (39.44)
Minced meat	99	42 (42.42)
Sausages	146	58 (39.73)
Total minced forms	316	128 (40.51)
Portioned forms		
Breasts[b]	153	68 (44.44)
Legs[c]	139	83 (59.71)
Wings[d]	48	36 (75.00)
Total portioned forms	340	187 (55.00)
Preparation types		
None (without)	189	71 (37.57)
Coated	80	30 (37.50)
Marinated	306	166 (54.25)
Seasoned	81	48 (59.26)

[a] n = 656, 11 Belgian producers, February to November 2007.
[b] All breast samples were fillets but one sample that included skin.
[c] No. of chicken legs with skin, 105/139; no. of chicken legs without skin, 34/139.
[d] All wing samples were presented with skin.

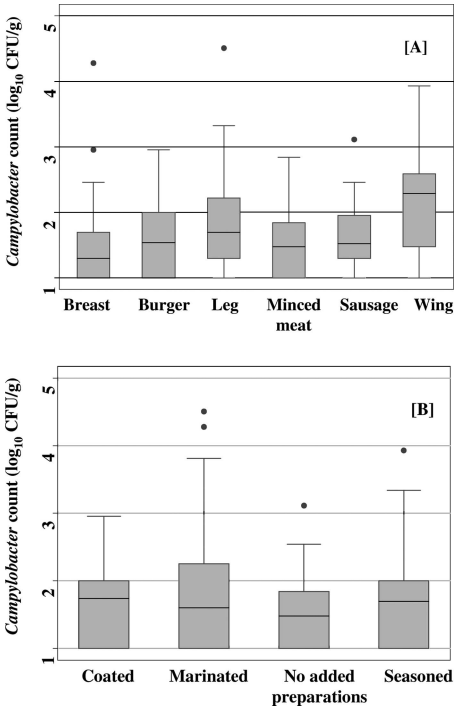

FIG. 3. Variation in *Campylobacter* counts in 656 chicken meat preparation samples from 11 Belgian producers, distributed according to product forms (A) and preparation types (B). The line inside each box represents the median value, and the upper and lower hinges represent the 75th and the 25th percentiles, respectively. The highest *Campylobacter* contamination counts (values over the 90th percentile) are shown as circles.

of the samples were found to be positive by enrichment but were below the limit of quantification by direct culture.

The relationship between direct-plating results (count, a continuous variable) and enrichment results (presence/absence, a binary variable) was evaluated statistically; interestingly, there was a significant positive relationship (negative binomial regression analysis; coefficient = 0.82, P = 0.007), indicating that the likelihood of obtaining a positive result by selective enrichment increases with an increase in the *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 plating and enrichment culture) were identified by multiplex PCR for *C. jejuni* and *C. coli* concurrently. *C. jejuni* was dominant, amounting to 73.1% of the direct-plating isolates and 66.4% of the 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 the chicken meat preparations tested were contaminated with *Campylobacter* (Table 1) is similar, to an extent, to prevalence data from the Belgian monitoring program conducted by the FASFC. Ghafir et al. (20) indicated that the *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 *Campylobacter* prevalences 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 the retail level prevalences of 3.4% (3/87) and 2.0% (2/102) were determined in 2005 and 2006, respectively, as well. The dramatic drop in *Campylobacter* prevalence based on the FASFC monitoring data for 2005 and 2006 is likely a reflection of the change in laboratory methodology, in which

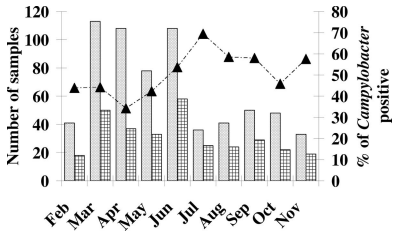

FIG. 4. Occurrence of *Campylobacter* bacteria in Belgian chicken meat preparations from February to November 2007. ▲, percentage of positive samples; dotted columns, number of samples tested; squared columns, number of *Campylobacter*-positive samples.

TABLE 3. *Campylobacter* detection in chicken meat preparations by direct plating versus enrichment culture[a]

No. of samples	Result obtained by:	
	Direct plating	Enrichment culture
341	−	−
113	+	+
156	+	−
46	−	+

[a] n = 656, 11 Belgian producers, February to November 2007. For direct plating, + indicates a countable result and − indicates below quantification limit; for enrichment culture, + indicates *Campylobacter* recovered and − indicates below detection limit.

the enrichment of 25-g test portions (used in 2002 and 2003 monitoring) was replaced with a lower enrichment volume equal to 0.01 g (on which 2005 and 2006 data were based) but still with Preston enrichment broth and subsequent isolation on mCCDA (7, 8). Modeling of 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 by selective enrichment is. Additionally, the present survey showed that the *Campylobacter* concentration was less than 10 CFU/g in 60% of the chicken meat preparation samples tested (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 (38), as they indicated that the sensitivity of qualitative detection with BB declines significantly at lower *Campylobacter* concentrations 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 produced by the present survey are considered to be the first on *Campylobacter* contamination levels in Belgian chicken meat preparations. The count distribution (Fig. 1) is based on microbiological testing procedures with preevaluated performance characteristics and estimated measurement uncertainty (23). Therefore, the present survey data could provide a contribution to the future optimization of a quantitative *Campylobacter* risk assessment 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 risk of *Campylobacter* infection 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, 15, 30). In a restaurant-associated outbreak, the number of *C. jejuni* bacteria in the causative chicken meal was estimated to range from 53 to 750/g (37). Additionally, in vitro models indicate that the efficiency with which some *Campylobacter* strains invade 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* bacterium can induce its own uptake into host cells (25).

Considerations related to product forms and preparation types. Statistical modeling indicated that the odds of *Campylobacter* incidence are lower in minced-form products than in portioned-form products (Table 2). This finding shows that balancing sample forms should be accounted for when designing a survey of chicken meat preparations. 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, from 2.8×10^5 to 4.3×10^5 CFU/g 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 [K. Bostan, H. Aksu, O. Ozgen, and M. Ugur, Proc. World Congr. Food Hyg. (WAVFH), 1997].

On the other hand, significantly higher *Campylobacter* incidences and counts were attributed to chicken wing samples (Table 2 and Fig. 3A). Chicken wings can be identified as a particularly high-risk product group, since the high *Campylobacter* load in chicken wings could increase the probability of pathogen transfer to other surfaces through cross-contamination and inappropriate handling during meal preparation and cooking (11, 33). 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 (12). Also, the high *Campylobacter* count in chicken wings (Fig. 3A) might be attributed to imperfect scalding, postscalding contamination, or a combination of both (14).

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 a European-Union-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 with the same test portion. This allows better control over subsampling 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 found to have 93% sensitivity and 100% specificity for *C. jejuni* subsp. *jejuni* identification and 100% sensitivity and 100% specificity for *C. coli* identification (35).

Surprisingly, the variation between direct culture and selective enrichment was very evident in our chicken preparation survey. Nevertheless, variation between direct culture and selective enrichment in *Campylobacter* recovery was previously cited in different food and environmental samples. Musgrove et al. (31) indicated a decrease in the detection of *Campy-*

lobacter bacteria of 36.7% in chicken cecal samples by enrichment procedures. Gharst et al. (21) tested 143 fecal samples from mature cattle colons at slaughter. *Campylobacter* was detected in 50.3% by selective enrichment, in 12.6% by direct plating, and in 37.1% by both methods. In another study comparing the two culture methods for *Campylobacter* recovery from bootsocks, feces, and cloacal swabs from broiler flocks, Jørgensen et al. [F. Jørgensen, J. Corry, L. Williams, and C. Barbedo-Pinto, poster 101, Proc. 14th Int. Workshop *Campylobacter Helicobacter* Relat. Organisms (CHRO), 2007] concluded that enrichment was not significantly better than direct plating. Finally, in 2006, on the basis of the same ISO method as we used, data from the monitoring system in The Netherlands indicated a *Campylobacter* prevalence of 14.5% (199/1,368) in broiler meat by enrichment procedures alone; however, the *Campylobacter* prevalence increased to 34% after adding positive enumeration results from the same samples [E. De Boer and B. Wit, poster 373, Proc. 14th Int. Workshop *Campylobacter Helicobacter* Relat. Organisms (CHRO), 2007]. The previous Dutch monitoring findings are very close to our survey results, as combining the results of detection by direct plating with those of detection by enrichment culture almost doubled the apparent *Campylobacter* prevalence in chicken meat preparations (Table 3).

Campylobacter spp. have a slower growth rate than many other bacterial species and compete poorly outside of their intestinal niche (13, 26). The enrichment step might provide an opportunity for rapidly growing *Campylobacter* strains to be selected and perhaps overgrow slow-growing strains (31). However, enrichment with BB provides a reasonable compromise between the selectivity of *Campylobacter* and suppression of competitor flora (17). Nevertheless, some meat flora could still survive during enrichment in BB. Baylis et al. revealed a high incidence of *E. coli* spp. and *Pseudomonas* spp. in BB 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 BB. 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 (28). Thus, as the initial density of *Campylobacter* decreases, the antagonistic interaction of the dominant indigenous microbiota is expected to increase.

In our survey, the two culture methods are considered to be complementary 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 (32, 34, 40). In our study, the number of samples varied over months for 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 chose the processing sector as our sampling point. Results in Table 1 and Fig. 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 incidences. 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 set of data on *Campylobacter* contamination in chicken meat preparations. Careful analysis of these 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) and (ii) the need to account for the biasing effect 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 *Campylobacter* contamination risk profile. 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 of the true *Campylobacter* prevalence estimate.

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