Baseline Data from a Belgium-Wide Survey of Campylobacter Species Contamination in Chicken Meat Preparations and Considerations for a Reliable Monitoring Program\(^\dagger\)

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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 \(1.68 \log_{10} \text{CFU/g}\) with a standard deviation of \(\pm 0.64 \log_{10} \text{CFU/g}\). The study revealed a statistically significant variation in Campylobacter contamination levels between companies; processors with a higher frequency distribution range of Campylobacter counts had a higher probability of being Campylobacter positive compared to minced-form products \(\text{sauces, burgers, and minced meat}\). The proportion of Campylobacter-positive samples was significantly higher in July than in other months. Recovery of Campylobacter spp. 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.

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\text{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).}
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\text{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).}
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\text{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, distributed in a way that allows the sampling team to visit them equally over randomized sampling days each month; the biggest 3 companies, 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, Basingstoke, England]) with 5% [vol/vol] lysed horse blood [E&O Laboratories, Basingstoke, England]) and incubated microaerobically at 41.5°C for 48 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 Van-damme 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 xilogit (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 log10 CFU/g, with a standard deviation of ≥ 0.64 log10 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
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 $2 \log_{10} \text{CFU/g}$ (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 $2.21 \log_{10} \text{CFU/g}$.

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)

### Table 1. Distribution of Campylobacter contamination in chicken meat preparation samples from 11 Belgian producers

<table>
<thead>
<tr>
<th>Company</th>
<th>No. of samples</th>
<th>Total no. (%) positive</th>
<th>Mean log$_{10}$ CFU/g $\pm$ SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>54</td>
<td>18 (34.62)</td>
<td>$1.39 \pm 0.40$</td>
</tr>
<tr>
<td>B</td>
<td>38</td>
<td>29 (76.32)</td>
<td>$1.62 \pm 0.45$</td>
</tr>
<tr>
<td>C</td>
<td>52</td>
<td>35 (67.31)</td>
<td>$1.76 \pm 0.55$</td>
</tr>
<tr>
<td>D</td>
<td>79</td>
<td>67 (84.81)</td>
<td>$1.86 \pm 0.54$</td>
</tr>
<tr>
<td>E</td>
<td>70</td>
<td>38 (54.29)</td>
<td>$1.50 \pm 0.58$</td>
</tr>
<tr>
<td>F</td>
<td>43</td>
<td>4 (8.89)</td>
<td>$1.25 \pm 0.44$</td>
</tr>
<tr>
<td>G</td>
<td>70</td>
<td>25 (35.71)</td>
<td>$1.27 \pm 0.48$</td>
</tr>
<tr>
<td>H</td>
<td>45</td>
<td>8 (17.78)</td>
<td>$1.27 \pm 0.38$</td>
</tr>
<tr>
<td>I</td>
<td>77</td>
<td>45 (58.44)</td>
<td>$1.67 \pm 0.63$</td>
</tr>
<tr>
<td>J</td>
<td>77</td>
<td>25 (32.47)</td>
<td>$1.72 \pm 0.67$</td>
</tr>
<tr>
<td>K</td>
<td>51</td>
<td>21 (41.18)</td>
<td>$2.21 \pm 1.08$</td>
</tr>
</tbody>
</table>

$^a$ $n = 656$, February to November 2007.

$^b$ Company identification letters were assigned arbitrarily.

**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 $2 \log_{10} \text{CFU/g}$. 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.
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 $\beta_1 = 0.82$, $P = 0.007$), indicating that the likelihood of obtaining a positive result by selective enrichment increases with an increase in the \textit{Campylobacter} concentration in the sample. Thus, samples with lower \textit{Campylobacter} concentrations had a lower likelihood of giving a positive result by selective enrichment compared to direct plating.

\textbf{DISCUSSION}

\textit{Campylobacter} prevalence and considerations while comparing survey data. The finding that almost half of the chicken meat preparations tested were contaminated with \textit{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 \textit{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 \textit{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 \textit{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. 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.](http://aem.asm.org/)

![FIG. 4. Occurrence of Campylobacter bacteria in Belgian chicken meat preparations from February to November 2007. A, percentage of positive samples; dotted columns, number of samples tested; squared columns, number of Campylobacter-positive samples.](http://aem.asm.org/)
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 $2.8 \times 10^5$ to $4.3 \times 10^9$ CFU/g in whole meat, $1.1 \times 10^4$ CFU/g in ground meat, $3.8 \times 10^3$ 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 95% 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. Ghart 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 after 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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