Campylobacter Prevalence in the Broiler Supply Chain in the Netherlands

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ABSTRACT After a national control program, data on Campylobacter prevalence in the broiler supply chain in the Netherlands were gathered for 3 sampling points: departure of broiler farm and arrival and departure of the slaughterhouse. Monitoring data from this control program for 2002 to 2005 were analyzed to find correlations and possible trends in the data. As expected, the greatest correlations were found between adjacent sampling points. A high correlation was expected between number of animals slaughtered and Campylobacter prevalence, because it is assumed that larger companies will have greater hygiene standards due to better implementation of food safety regulations. However, statistical analysis showed that there was no clear correlation between company size and Campylobacter prevalence. Data analysis further identified an increasing trend in Campylobacter prevalence at departure of slaughterhouse from 2002 to 2005 with strong seasonality at all 3 sampling points. Measures to control Campylobacter, therefore, need to be reconsidered and possibly intensified to achieve a reduction in Campylobacter positives.

Key words: trend analysis, correlation, monitoring data, zoonotic pathogen

INTRODUCTION Campylobacteriosis is the most frequently reported zoonotic disease in humans in the European Union (EU). In 2004, an incidence of 51.6 cases per 100,000 inhabitants was reported by 24 EU member states. The incidence varied widely between countries, ranging from <0.1 to 303 cases per 100,000 inhabitants (EFSA, 2006a). In the Netherlands, the estimated number of cases is 80,000 per year (in a population of 16 million; Havelaar et al., 2007). Poultry meat is assumed to be one of the main sources of foodborne Campylobacter infections in accordance with the greatest proportion of Campylobacter-positive food samples (EFSA, 2006a; Humphrey et al., 2007).

European data on Campylobacter spp. are collected based on (the former) Council Directive 2003/99/EC, the community system for monitoring and collection of information on zoonoses (EC, 2003). Data on Campylobacter spp. provided by the Netherlands to the EU are based on a national control program on Salmonella spp. and Campylobacter spp. (Action Plan), which was set up and implemented by the Dutch Product Board for Livestock, Meat and Eggs (PVE) in 1997. The PVE is a so-called public law organization (Board), based on the Dutch Law on Industrial Organizations. It effectively operates as a consultative platform for employers and employee organizations. One of its features is that it has a co-governance task and can formulate regulations and decisions on specific and designated areas that have the power of law and are recognized as such by the national authority. Sanctioning on violation of these regulations is often defined in a specified disciplinary action. In the Netherlands, the EU Council Directive 92/117/EC was implemented through several regulations and decisions of the PVE. The PVE developed the control program on behalf of the Dutch authorities and in cooperation with representatives of all stages in the broiler meat production chain. At the start of the control program, data on the levels of Salmonella and Campylobacter contamination in the broiler supply chain were scarce and fragmented. Thus, as a reference for the control program, expert estimates on the Salmonella and Campylobacter prevalence in 1997 were used. For Campylobacter, an estimated 50% of the broiler flocks were contaminated with Campylobacter spp. in 1997, and in 2000, the estimated percentage was about 40% (PVE, 2002). Monitoring of products at retail level in 2004 by the National Food and Consumer Product Safety Authority showed that 18% of the products were contaminated with Campylobacter spp. (van der Zee et al., 2005). In 2001, a more rigorous

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approach was chosen, which led to the introduction of a more stringent control program (Action Plan 2000+). Its aim was to further reduce the level of Salmonella spp. in broiler meat. After the control program, reduction in pathogen prevalence was to be achieved by surveillance of the pathogens and implementation of hygiene measures at various stages of the broiler supply chain. For this purpose, the PVE issued 2 regulations on behalf of the Dutch authorities. One was aimed at the general broiler meat production chain, the other specifically on the slaughtering and cutting and deboning units (PVE, 1999a, b). The 2 regulations were each elaborated in different subregulations on the various types of units of poultry production. In broiler meat production, these regulations focused on hygiene and monitoring microbial quality of raw materials (broilers) and products leaving the slaughterhouse (meat and transport crates for broilers). The regulations define minimum standards for measures concerning hygiene, cleaning and disinfection (of buildings and equipment), monitoring of microbial quality at arrival and departure from the unit, exchange of monitoring results, and measures to be taken in case a contamination is found. The regulations also describe the requirements for organizations that provide services to the different production units, such as laboratories, inspection organizations, veterinarians, and organizations specialized in (microbial) sampling, cleaning, and disinfection. Results of the monitoring activities of these regulations were, and are, collected and stored by the PVE. The aim of this paper is to analyze Campylobacter monitoring data from the Dutch control program during the period from January 1, 2002 to December 31, 2005 and to determine possible trends in Campylobacter prevalence during this time.

**MATERIALS AND METHODS**

**Sampling Strategy**

Based on the national control program of 2001, monitoring of Campylobacter was based on presence-absence testing of the pathogen both on arrival and departure of birds at various stages of the broiler supply chain. Because high numbers of positive samples were expected, monitoring of all units of birds for Campylobacter was considered not useful. Hence, samples were taken from each broiler farm twice a year, and these units were also sampled at arrival of the slaughterhouse. Samples were taken throughout the year such to avoid a seasonal influence on contamination. Campylobacter presence was analyzed by fecal samples at departure of the broiler farm, cecal samples at arrival of the slaughterhouse, and breast skin samples at departure of the slaughterhouse. Table 1 gives an overview of the number and type of samples taken at every stage, starting at the broiler farm.

**Microbial Analyses**

Samples were taken by the farmers and employees of the slaughterhouse and sent for analysis to accredited laboratories. The laboratories had to be accredited as ISO 17025, and the analyses performed should have been covered by the accreditation. Moreover, the laboratories should have participated in ring surveys organized by the National Reference Laboratory. Samples were analyzed for Campylobacter prevalence within 48 h using the methods commissioned by the PVE, which consists of the following. A sterile swab is used to sample the fecal and cecal samples. This swab is then used for inoculation of a modified charcoal cefoperazone deoxycholate agar (mCCDA) plate to obtain single colonies. Before 2004, breast skin samples of minimally 10 g were incubated in a 1:10 dilution of charcoal cefoperazone deoxycholate broth for 24 h at 41.5°C in a microaerobic environment (ca. 6% O2, 10% CO2, and 84% N2). After incubation, a sample from the broth was spread on a mCCDA plate. From 2004 onward, breast skin sampling was slightly altered using a different enrichment broth. Breast skin samples of 25 g were homogenized with 100 mL of Preston selective enrichment broth for 1 min. Then, the sample material was removed, and the broth was incubated for 24 h at 41.5°C. After incubation, a sample from the broth was inoculated on mCCDA. All mCCDA plates were incubated for 48 h at 41.5°C under microaerobic conditions. Suspected colonies from the plates were confirmed using oxidase reaction and microscopy. Part of the isolates was additionally confirmed with latex agglutination tests.

**Data Analysis**

**Data Organization.** It was mandatory for all chain steps in the broiler supply chain to communicate the test results to the next step in the production chain. The monitoring results for Campylobacter accompanied a flock of broilers from broiler farm to slaughterhouse. The slaughterhouse reported this information
per unit to the PVE for central collection and analyses. A format was established to ensure information reporting was uniform. Campylobacter data were collected in Microsoft Excel spreadsheets. Data gathering was standardized in 2002; therefore, data before this date were not used for further analysis. For central storage and analyses of the data, a Microsoft SQL database was defined and set up. For extraction of the data from the spreadsheets into the database, a custom import filter was written in C++ (Microsoft Visual Studio 2005, Microsoft Corporation, Redmond, WA). This allowed automatic import of the data into the database. After importation of the data, the monitoring results were checked for inconsistencies. Data that did not meet the following criteria were left out for further analyses: a valid slaughter date and a Campylobacter prevalence of 0 (unknown), 1 (negative), or 2 (positive). Double entries for thinned birds were omitted in the analysis. One assumption was made for the cecal samples. According to the control program, the slaughterhouse did not need to report the cecal samples if fecal samples were tested positive. Therefore, it was assumed that if cecal samples were reported as unknown and the corresponding fecal sample was positive, the cecal sample was positive as well. In total, around 60,000 fecal and cecal results and 73,000 breast skin results were used for further analyses.

**Statistical Analysis.** For each sampling point, the number of positive and negative samples was counted per slaughterhouse and quarter of the year for the time period 2002 to 2005, but excluding data from the second and third quarter of 2003 (which were assumed to be atypical due to an outbreak of avian influenza). To perform trend analysis, the fraction of positives per quarter i (ni) was estimated using a logistic model:

$$logit(p_i) = \log\left(\frac{p_i}{1-p_i}\right) = \mu_{\text{Season}(i)} + \text{Spline}(t; df)$$  \hspace{1cm} [1]

with $\mu_{\text{Season}(i)}$ = the average value, dependent on season. Seasonal variation is only incorporated when a significant distinction between the 4 seasons was found (using a likelihood ratio test at significance level 0.05). The fraction of positives was modeled as a function of time using a smoothing spline function (Hastie and Tibshirani, 1990). The smoothness of the function can be adjusted with the degrees of freedom (df). When df = 1, this gives a straight line; greater degrees of freedom will give more curvature of the function. The optimal degrees of freedom were estimated using significance tests (with a significance level of 0.05) in a forward selection procedure. Apart from a trend analysis in time and effects of seasons, the effect of slaughterhouse company size was tested using the number of slaughtered animals (in millions) per company as an additional predictor variable in the model.

Furthermore, Pearson correlation coefficients were calculated between sampling points using counts per quarter and company transformed by the empirical logistic function (McCullagh and Nelder, 1989):

$$logit(p) = \log\left(\frac{\text{n}_{\text{pos}} + 0.5}{\text{n}_{\text{neg}} + 0.5}\right).$$ \hspace{1cm} [2]

Data pairs with $n_{\text{pos}} = n_{\text{neg}} = 0$ for one or both of the sampling points were excluded from the calculation of correlation. All calculations were performed with Genstat Release 9.2 (VSN International, Hemel Hempstead, UK).

**RESULTS AND DISCUSSION**

**Correlations in the Poultry Production Chain**

Samples are taken at various points in the poultry production chain, and results from one unit are communicated to the next. The underlying assumption is that there is a correlation between production steps. To test the hypothesis of positive correlations between sampling points, correlation coefficients were estimated using the empirical logistic function (equation [2]). This resulted in a correlation coefficient of 0.34 between fecal samples at farm level and cecal samples at the start of the slaughterhouse. Between fecal samples and breast skin samples at departure from the slaughterhouse, a correlation coefficient of 0.25 was found, and for cecal and breast skin samples, the correlation coefficient was 0.61. This indicates that correlation is greatest between adjacent sampling points in the chain, which is to be expected. The correlation was not perfect, which was probably caused by the large scatter in the data set (as can be seen in Figure 1). This scatter was caused by variation in Campylobacter prevalence but also by uncertainty in the data set. Uncertainty can be diminished by increasing the amount of samples taken. For example, in the current PVE regulations, only 1 breast skin sample is taken per day, whereas multiple batches are slaughtered each day. Furthermore, each broiler farm is only sampled twice a year. To improve insight of Campylobacter contamination in the poultry production chain, each unit should be sampled individually, and the number of samples per unit should be optimized to obtain representative data. Sampling per batch instead of per day also improves traceability options, because retail samples of Dutch products taken by the Food and Consumer Product Safety Authority can then be related to batch samples at the slaughterhouse.

**Effect of Company Size**

To obtain more detailed insight in Campylobacter prevalence, the results per company were analyzed further. Figure 2 shows a large variation between
companies per quarter of the year. Variation in the application of hazard analysis and critical control points and good manufacturing practice principles will influence product contamination (Humphrey et al., 2007) and may thus explain the variation found between the various companies. Because data on this were not available, company size was used as an explanatory factor. In general, larger businesses are usually on top of implementing food safety regulations. Smaller companies, however, may lack trained technical employees to adopt good hygienic practices and may thus react slower to new regulations (Holt and Henson, 2000). Therefore, a large slaughterhouse (with high numbers of slaughtered animals) is assumed to give lower Campylobacter prevalences due to greater hygiene standards. Numbers of slaughtered animals per quarter of the year ranged between 2000 for a small company to 10 million for a large company. Statistical analyses were performed on the cecal and breast skin samples, because these samples were taken at the slaughterhouse. The results showed that correlation between company size and Campylobacter prevalence was only marginally significant for breast skin samples (parameter estimated as $-4.2$, standard error $2.5$, $P = 0.07$), and no correlation was found for cecal samples. Although the cecal samples are taken at the slaughterhouse, they are taken at arrival. Therefore, company size of the slaughterhouse is not expected to influence Campylobacter status at arrival at the slaughterhouse. More detailed analysis of companies with low prevalences may reveal measures that are effective in reduction of Campylobacter prevalence at the slaughterhouse level.

### Trend Analysis

Campylobacter prevalences in feces, cecal, and breast skin samples from 2002 to 2005 were used for

### Table 2. Average Campylobacter prevalence and number of samples (total, positives, and negatives) taken in each sampling point at the broiler farm and the slaughterhouse

<table>
<thead>
<tr>
<th>Sampling point</th>
<th>2002</th>
<th>2003 $^1$</th>
<th>2004</th>
<th>2005</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Feces (farm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17,420</td>
<td>11,669</td>
<td>15,882</td>
<td>14,524</td>
</tr>
<tr>
<td>Positives</td>
<td>546</td>
<td>134</td>
<td>283</td>
<td>241</td>
</tr>
<tr>
<td>Negatives</td>
<td>3,209</td>
<td>1,603</td>
<td>2,494</td>
<td>1,756</td>
</tr>
<tr>
<td>Prevalence $^2$</td>
<td>14.5</td>
<td>7.7</td>
<td>10.2</td>
<td>12.1</td>
</tr>
<tr>
<td><strong>Cecum (slaughterhouse)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17,420</td>
<td>11,669</td>
<td>15,882</td>
<td>14,524</td>
</tr>
<tr>
<td>Positives</td>
<td>832</td>
<td>338</td>
<td>572</td>
<td>744</td>
</tr>
<tr>
<td>Negatives</td>
<td>2,546</td>
<td>1,400</td>
<td>1,964</td>
<td>1,636</td>
</tr>
<tr>
<td>Prevalence</td>
<td>24.6</td>
<td>19.5</td>
<td>22.6</td>
<td>31.3</td>
</tr>
<tr>
<td><strong>Breast skin (slaughterhouse)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>19,155</td>
<td>14,781</td>
<td>19,970</td>
<td>19,327</td>
</tr>
<tr>
<td>Positives</td>
<td>472</td>
<td>346</td>
<td>1,169</td>
<td>1,447</td>
</tr>
<tr>
<td>Negatives</td>
<td>3,745</td>
<td>3,004</td>
<td>3,019</td>
<td>2,206</td>
</tr>
<tr>
<td>Prevalence</td>
<td>11.2</td>
<td>10.3</td>
<td>27.9</td>
<td>39.6</td>
</tr>
</tbody>
</table>

$^1$Results for all quarters are given including data influenced by avian influenza.

$^2$Prevalence is calculated as the fraction of positives on the total number of positive and negative samples.
trend analysis. Average prevalences and number of samples taken for the 3 sampling points are given in Table 2. Figure 2 shows *Campylobacter* prevalence per company per quarter of the year. In the summer of 2003, the Netherlands coped with an avian influenza outbreak. Although the effect of this outbreak is not visible in the average prevalence for 2003, comparison of individual data per company did reveal decreased prevalences. Therefore, results for the second and third quarter of 2003 are depicted in Figure 2 but were not incorporated in the statistical analysis. Results of the analysis showed a strong seasonal effect for all 3 sampling points ($P < 0.001$), with the greatest prevalence in the third quarter of the year (Figure 2). Seasonal variation for *Campylobacter* with peaks in the summer and fall has also been found by others (Wallace et al., 1997; van der Zee et al., 2005; McCrea et al., 2006; Meldrum et al., 2006; van de Giessen et al., 2006). Human *Campylobacter* infections in the EU also follow a seasonal pattern with peaks in wk 22 and 23. More northern countries have later peaks (Nylen et al., 2002). The increased prevalence in (late) summer coincides well with the fact that temperature is found to be a significant factor in the prevalence of *Campylobacter* (Wallace et al., 1997; van der Zee et al., 2005).

Trend analysis on *Campylobacter* prevalences from 2002 to 2005 showed that there is a small declining trend in *Campylobacter*-positive fecal samples, whereas the cecal samples showed a small increasing trend (Figure 2). A more prominent increasing trend can be seen in the breast skin samples (Figure 2c). The simplest characterization of each trend function is given by the slope of the best-fitting linear function (Table 3). For fecal and cecal samples, the standard error in the model parameters was relatively large, indicating that this effect was not very distinct. For breast skin samples, the obvious trend was confirmed in the statistical analysis (the linear parameter was +0.63 with a standard error of 0.07). However, the latter results may be partially caused by a change in analyzing technique. From 2004 onward, a different broth was used as enrichment medium (see materials and methods section).

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Prevalence of *Campylobacter* between 2002 and 2005 and estimated trend function (solid line) excluding data from the second and third quarter of 2003 for (a) feces, (b) cecum, (c) and breast skin. Each triangle indicates *Campylobacter* prevalence of 1 company per quarter of the year.
which resulted in greater isolation rates for Campylobacter (Jacobs-Reitsma et al., 2003). However, allowing for this discontinuity in the model, the increasing trend was still found (linear parameter $-0.47$, standard error 0.15). Data from another study in the Netherlands from 1999 to 2002 did not show a significant trend in Campylobacter prevalence, but an increase in broiler flock prevalence at farm level was visible (van de Giesen et al., 2006). Retail samples of chicken breast meat show an increase in prevalence from 1999 to 2001 and a decrease from 2001 to 2004 (van der Zee et al., 2005), which contradicts our results. An explanation for these findings may be that removal of breast skins is performed more hygienically over the years, resulting in decreased prevalence in chicken breast meats over time. Overall within the EU, there is no clear trend in Campylobacter prevalence in finished chicken products, and strong fluctuations between years occur. In Denmark and the Netherlands, there seems to be a decreasing trend in finished products, whereas in Germany and Belgium, the trend is increasing from 2001 to 2005 (EFSA, 2006b). Other European data on Campylobacter prevalences at the slaughterhouse reveal a wide range of positive samples. For example, Danish studies showed a flock prevalence before slaughter of 52% in 2000 (Hald et al., 2000) and 37% in 2001 (Heuer et al., 2001). Skin samples at the end of the slaughter phase varied from 24% in Denmark (Hald et al., 2000) to 92% in Ireland (Moore, 2003) and 93% in Hungary (Jozwiak et al., 2006). In our study, we found a pre-slaughter prevalence of 20% to 31% over the years 2002 to 2005 and for breast skin samples a Campylobacter prevalence in the range of 10 to 40% for 2002 to 2005 (Table 2). These figures indicate that preslaughter prevalences in the Netherlands are lower than in Denmark, and postslaughter prevalences coincide with the Danish data. Our results indicate that despite the regulations set by the PVE, Campylobacter prevalence in the Dutch poultry production system has not declined over the years. Once an infection is established in a flock, close to 100% of the birds become colonized and shed high numbers of Campylobacter (Wagenaar et al., 2008). Therefore, Campylobacter infections of flocks in the broiler supply chain need to be prevented. Current hygiene measures taken to reduce Salmonella prevalence in the poultry chain seem to be ineffective in reducing Campylobacter prevalence, because breast skin samples show an increasing trend. Apparently, further actions are required to reduce Campylobacter prevalence throughout the poultry production chain. Possible cost-effective interventions for Campylobacter may be a combination of lowering the prevalence on the farm through strict hygiene measures, reduction of faecal leakage during processing, and chemical decontamination of finished products (Havelaar et al., 2007).

### CONCLUSIONS

This study showed that correlations for Campylobacter prevalence were greatest between adjacent sampling points in the chain, and no clear correlation was found between company size and Campylobacter prevalence. An increasing trend in Campylobacter prevalence in breast skin samples from 2002 to 2005 has been found as well as strong seasonality at all sampling points. Measures to control Campylobacter need to be reconsidered and possibly intensified to achieve a reduction in Campylobacter positives. Effectiveness of measures taken can be checked by increased sampling in the poultry production chain in such a way that data are representative and can be linked from the broiler farm up to the finished product.

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### REFERENCES


