Human risk from thermotolerant Campylobacter on broiler meat in Denmark

Louise Boysen *, Maarten Nauta, Ana Sofia Ribeiro Duarte, Hanne Rosenquist

Technical University of Denmark, National Food Institute, Division for Epidemiology and Microbial Genomics, Moerkhøj Bygade 19B, 2860 Soeborg, Denmark

ARTICLE INFO

Article history:
Received 10 July 2012
Received in revised form 12 December 2012
Accepted 5 January 2013
Available online 20 January 2013

Keywords:
Risk assessment
Chicken meat
Campylobacteriosis

ABSTRACT

This paper describes a new approach by which changes over time in the relative risk of human campylobacteriosis from broiler meat are evaluated through quantitative microbiological risk assessment modelling. Danish surveillance data collected at retail from 2001 to 2010 on numbers of thermotolerant Campylobacter spp. on Danish produced and imported chilled and frozen broiler meat were the basis for the investigation. The aim was to explore if the risk from the different meat categories had changed over time as a consequence of implemented intervention strategies. The results showed a slight decrease from 2005 to 2008 in the human risk from Danish produced broiler meat, and a decrease from 2005 to 2010 in the risk from imported chilled meat. This risk reduction coincides with control measures implemented to reduce Campylobacter in Danish and imported chilled broiler meat. The human risk of campylobacteriosis from Danish frozen meat increased but remained lower compared to chilled meat. In total, the relative risk from broiler meat available for sale in Denmark increased from 2001 to 2005 after which the risk decreased to a level similar to the period 2001–2002. The use of QMRA in the evaluation of intervention strategies based on monitoring data provided an added value, compared to the traditional approach of only using changes in prevalence. The estimated human health risk is a function of prevalence and the distribution of concentrations, and therefore takes best usage of the available data, while providing the most relevant outcome for food safety risk managers.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Thermotolerant Campylobacter is the most frequently reported gastrointestinal bacterial pathogen in Denmark as well as in EU (European Food Safety Authority, 2011; Anonymous, 2012). Chilled broiler meat is considered to be the largest single source of human Campylobacter cases in Denmark; with an estimated population attributable risk of 24% (CI 8–53%) (Wingstrand et al., 2006). Monitoring of several different retail products in Denmark has furthermore shown that thermotolerant Campylobacter is rarely detected on pork (0.2% of 2413 samples of minced meat in 2002) and beef (0.1% of 3046 samples of minced meat in 2002) (Anonymous, 2003) but more frequently in turkey meat (44% in chilled imported retail meat in 2007) (Anonymous, 2009) and broiler meat (39% and 53% in Danish produced and imported chilled meat, respectively, in 2011) (Anonymous, 2012). Similar observations have been reported across Europe (European Food Safety Authority, 2009). In Denmark, the occurrence of thermotolerant Campylobacter in broiler meat has been monitored at retail since 1995. Campylobacter is, furthermore, found on occasion in a variety of vegetables (0% in baby maize, sprouts, etc. to 3% in leafy greens in 2009–2010) (The Danish Veterinary and Food Authority, 2011). Further investigation regarding the quantitative level could be interesting to evaluate the significance in relation to human exposure and risk.

In 2003, an action plan against Campylobacter in broilers was adopted in Denmark. The action plan focused primarily on improvement of biosecurity in the primary production, scheduling of Campylobacter positive broiler flocks to frozen production (where practical and possible), reduction of the Campylobacter concentration on broiler meat at slaughterhouses by freezing, and reduction of cross-contamination in domestic kitchens through consumer campaigns (Anonymous, 2004a). In 2008, a new four year action plan was initiated with the aim to decrease the prevalence and the concentration of Campylobacter in broilers and broiler meat. The key elements included initiatives at all levels of the production chain. At farm level, focus was directed towards development and implementation of an industry code of practice for farmers to increase attention to biosecurity measures and development of fly screens for broiler houses, which have proven to be very effective in preventing introduction of Campylobacter in the broiler houses under Danish conditions; eliminating the peak in Campylobacter flock prevalence in summer (Hald et al., 2007). Investigation of applicable methods for decontamination (steam-ultrasound, crust-freezing, air-chilling, freezing) and improved hygiene were the key initiatives at abattoir level. Initiatives directed towards consumers included launching of consumer information campaigns and development of educational material for school...
children, in the middle school, to improve awareness on kitchen hygiene (Anonymous, 2004a). Development and intensified "case-by-case control" was also part of the new action plan. This "case-by-case control" was implemented in Denmark in 2007 and aims at reducing high-risk batches of fresh meat entering the market. In practice, a number of imported and Danish batches of fresh meat are examined for the presence of Salmonella and numbers of Campylobacter and based on these results the relative risk from a batch is assessed using risk modelling. If a batch is considered injurious according to article 14 in the EU food law (Regulation (EC) 178/2002), the food producing establishments cannot market the batch and already marketed batches must be withdrawn (Anonymous, 2009).

As mentioned above different initiatives have been implemented in the broiler production chain in Denmark, but the effect of measures has not been evaluated. Observing changes in the number of registered human Campylobacter cases to evaluate the effect of initiatives in the broiler production will probably not convey the exact result, as several reservoirs harbour Campylobacter and pose risk to humans via different pathways, and accordingly influence the number of human campylobacteriosis cases. Broiler meat is considered the largest source of domestically acquired campylobacteriosis; however, it is only one source of many. The exact proportion of cases attributable to this source/pathway combination as well as the temporal epidemiology is not known. Instead of evaluating the registered number of human cases, the Campylobacter status is traditionally assessed based on prevalence in for example broiler flocks and broiler meat. However, the number of bacteria ingested is believed to be of great importance in relation to human illness (Nauta et al., 2009). Using risk assessment models to evaluate changes in relative human risk from different meat categories could be a way to evaluate effects of action plans.

Several risk assessments on Campylobacter in broiler meat have been performed within the last ten years. The risk assessments are used for different purposes: i.e. to evaluate potential effects of control measures in the broiler production chain and to assess the human risk due to Campylobacter in broiler meat (Nauta et al., 2009). In Denmark, risk assessment models are also used on a day to day basis in the case-by-case control (see above) (Anonymous, 2009).

In this study, we use quantitative microbiological risk assessment (QMRA) modelling as a novel tool to evaluate changes over time in the relative risk of human campylobacteriosis from broiler meat. The data evaluated are surveillance data on numbers of thermotolerant Campylobacter spp. on Danish produced and imported chilled and frozen retail broiler meat from the period 2001–2010.

2. Materials and methods

2.1. Sampling

In the period 2001–2010, samples of fresh chilled and frozen imported and Danish produced broiler meat were randomly collected nationwide from local retail establishments. Before 2007 samples only included whole broiler carcasses; while from 2007, samples included whole broiler carcasses, fillets and legs. The change in sampling plan was a consequence of changing consumer purchasing behaviour. The samples collected should, according to the sampling plan, reflect the products available in the counter at retail. Data showed disequilibrium between the four categories of meat in the proportion of samples with and without skin. The frozen meat (Danish produced and import) and Danish produced chilled meat were predominantly comprised of samples with skin, while the majority of the imported chilled meat samples were without skin.

The samples were collected and analysed by the Danish Regional Veterinary and Food Authorities. All samples were collected at random with no knowledge of Campylobacter status. The number of samples collected for each category within years is presented in Table 1.

2.2. Microbiological analysis

Samples of frozen meat were defrosted in the package before analysis.

The number of thermotolerant Campylobacter spp. in the samples was determined semi-quantitatively according to NMKL method 119. Revisions of the method were applied when published (Anonymous, 2004b, 2005, 2007). In brief, one equivalent of broiler meat (minimum 15 g) was stomached for 120 s with 8 or 9 equivalents of broth (2001–2002: 9 equivalent of Mueller-Hinton broth (MHS) (Difco, Becton Dickinson, Sparks, MD, USA); 2003–2007: 8 equivalents in Bolton broth (Oxoid, Basingstoke, UK) with supplements (sodium pyrovate 0.25 mg/l, sodium metabisulphite 0.25 mg/l, ferro sulphate 0.25 mg/l (Oxoid)), cephalorzone 30 mg/l (Sigma-Aldrich, Saint Louis, MO, USA), and trimethoprim lactate 66 mg/l (Sigma-Aldrich)). A collaborative trial demonstrated no statistically significant difference between the method used in 2001–2002 and the method used since 2003 (Rosenquist et al., 2007).

As a result of this semi-quantitative method, the numbers of samples were obtained in the classes <0.1 cfu/g, 0.1–1 cfu/g; 1–10 cfu/g, 10–100 cfu/g, 100–1000 cfu/g, and ≥1000 cfu/g for each meat category, year and season. The observed prevalence (pobs) is the percentage of samples that is not falling within the first class, <0.1 cfu/g.

2.3. The proportion of meat available for sale

The proportion of broiler meat available for sale in Denmark (Table 1) was obtained from the Danish Agriculture and Food Council and Statistics Denmark.

2.4. Data analyses

The Campylobacter prevalence for broiler meat is influenced by season (Boysen et al., 2011). Therefore, the annual mean prevalence for retail samples was calculated as means of the quarterly prevalences to account for seasonality. To evaluate whether concentrations were influenced by season, the difference between quarterly concentrations (in logs) was tested using an unpaired t-test. An α-value of 0.05 was considered to be statistically significant in the statistical analyses. Analyses were performed using the GraphPad QuickCalc software, Inc.

The semi-quantitative method used provides interval data and has a limit of detection (LoD) of 0.1 cfu/g. As food products may be contaminated below this LoD, the observed prevalence will most likely not be the “true” prevalence of contaminated products. To facilitate the risk assessment, a lognormal distribution was fitted to the semi-quantitative data. The estimated prevalence (pest) indicates the proportion of food products for which the 10 based log of the concentrations (log cfu/g) can be described by a normal distribution with mean (μ) and standard deviation (σ). Estimates for pest, μ and σ were obtained for each meat category and year using maximum likelihood estimation (MLE). This allows the incorporation of censored data (Lorimer and Kiermeier, 2007).

---

Table 1

<table>
<thead>
<tr>
<th>Year</th>
<th>Domestic chilled</th>
<th>Domestic frozen</th>
<th>Import chilled</th>
<th>Import frozen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>prop</td>
<td>N</td>
<td>prop</td>
</tr>
<tr>
<td>2001</td>
<td>515</td>
<td>24%</td>
<td>336</td>
<td>56%</td>
</tr>
<tr>
<td>2002</td>
<td>247</td>
<td>24%</td>
<td>149</td>
<td>58%</td>
</tr>
<tr>
<td>2003</td>
<td>160</td>
<td>23%</td>
<td>187</td>
<td>54%</td>
</tr>
<tr>
<td>2004</td>
<td>178</td>
<td>21%</td>
<td>392</td>
<td>50%</td>
</tr>
<tr>
<td>2005</td>
<td>340</td>
<td>22%</td>
<td>547</td>
<td>43%</td>
</tr>
<tr>
<td>2006</td>
<td>62</td>
<td>25%</td>
<td>535</td>
<td>39%</td>
</tr>
<tr>
<td>2007</td>
<td>302</td>
<td>24%</td>
<td>365</td>
<td>41%</td>
</tr>
<tr>
<td>2008</td>
<td>757</td>
<td>23%</td>
<td>299</td>
<td>35%</td>
</tr>
<tr>
<td>2009</td>
<td>702</td>
<td>26%</td>
<td>548</td>
<td>31%</td>
</tr>
<tr>
<td>2010</td>
<td>767</td>
<td>26%</td>
<td>477</td>
<td>26%</td>
</tr>
</tbody>
</table>
By introducing the estimated prevalence as one of the terms in the likelihood equation, a prevalence estimate is also produced with the MLE method. The estimated prevalence $p_{est}$ will be higher than the observed prevalence ($p_{obs}$) since a higher number of samples are considered to be positive due to the addition of a part of the results below the LoD. A similar method has previously been used in the EFSA Scientific Opinion on Campylobacter in broiler meat production (EFSA Panel on Biological Hazards (BIOHAZ), 2011). The MLE method was performed with the Solver Add-in for Excel 2010.

Evaluation of the relative risk in total from broiler meat available for sale in Denmark could not be done directly from the MLE fitted data as the model was not converging for some of the data; resulting in some missing data points. Therefore, the estimation of the total risk was based on the observed data.

The QMRA model used is described by Nauta et al. (2012). The model combines a consumer phase model and a dose response model (Teunis and Havelaar, 2000) as described by Nauta and Christensen (2011) and Nauta et al. (2012), and has also been applied by EFSA (EFSA Panel on Biological Hazards (BIOHAZ), 2011). The output of the risk assessment model is the mean probability of illness from consumption of a random sample of broiler meat. Relative risks of different broiler meat categories are calculated using Danish chilled meat in 2007 as baseline with relative risk 1. The year 2007 was chosen as baseline as this was the year before the implementation of the second Danish action plan (2008–2012).

3. Results

3.1. Campylobacter occurrence

Generally, the observed Campylobacter prevalence was higher in chilled meat compared to frozen meat for both Danish produced and imported meat (Fig. 1). The Campylobacter prevalence for Danish chilled meat remained fairly unchanged within the study period whereas the prevalence for the frozen meat was increasing. For the imported meat, the prevalence decreased for both chilled and frozen meat from 2005.

The concentrations of Campylobacter on the chilled meat were noticeably higher than on the frozen meat for both Danish produced and imported meat. No seasonality was observed for concentrations in the four different quarters ($P > 0.05$). For the semi-quantitative data, log-normal distributions were fitted using MLE to the extent possible. The available data did not converge for all quarters; hindering calculation of risk estimates for the implicated product/year combination. From the MLE an estimated prevalence, mean concentration and standard deviation of concentrations were obtained. Fig. 2 illustrates the fluctuation of mean Campylobacter concentration including the standard deviation of concentrations ($\sigma$). These estimates were used as input data in the risk assessment model.

3.2. Human risk

The output of the risk assessment was relative risk estimates for Danish produced and imported broiler meat. Three main outputs...
were generated; 1) the relative risk from each product category; for direct comparison of the risk from a random sample of Danish produced broiler meat or imported meat, (Fig. 3), 2) the relative risk stratified by the proportion of broiler meat available for sale, for comparison of the overall risk from Danish produced broiler meat and imported meat (Fig. 4), and 3) the relative risk in total from broiler meat available for sale in Denmark (Fig. 5). From Fig. 3, it is seen that the risk from imported meat (chilled and frozen) in the major part of the study period was higher compared to Danish produced meat. Fig. 4 illustrates how the overall risk from Danish produced meat increases, resulting in very similar risk estimates for Danish and imported meat, as a consequence of including the proportion of meat available for the consumer. As seen from Figs. 3 and 4, the risk fluctuated in the study period for all the meat categories. Note the decrease in risk from imported chilled meat since 2005 and the decrease 2005–2008 followed by an increase in risk 2008–2010 from Danish produced chilled meat. The risk from Danish produced meat surpassed the risk from imported meat between 2008 and 2009. Furthermore, the risk from Danish frozen meat increased during the study period while the risk from the imported frozen meat remained fairly steady. The risk from frozen meat, however, was lower than the risk from chilled meat.

The relative risk in total from broiler meat available for sale in Denmark showed a tendency to increase from 2001 to 2005 after which it decreased until 2009 (Fig. 5). The peak in 2005 was due to remarkably high mean concentrations for chilled meat, which is not readily explained. The high concentrations in Danish chilled meat in 2005 were confirmed by data from another Danish surveillance programme sampling chilled broiler meat at slaughter houses (data not shown).

Fig. 6 illustrates the relationship between human risk and observed prevalence. From this figure it is evident that the observed prevalence was associated with human risk; however, a strong correlation was not evident. QMRA clearly provided another risk ranking than the observed prevalence.

4. Discussion

In this study, QMRA modelling was introduced as a tool to evaluate changes over time in the risk of human disease from a specific food source. The risk estimates are based on information on prevalence and concentration on the meat included in the evaluation. The distributions of concentrations and in particular the high value tail are important in relation to human risk from Campylobacter, as high concentrations lead to high probability of illness (Rosenquist et al., 2003; Nauta et al., 2009). The tails are determined by the standard deviations of the concentrations, which are obtained from fitting lognormal distributions using a maximum likelihood estimation (MLE) approach for censored data, that can be applied to the
available semi-quantitative data (Busschaert et al., 2010). High standard deviations might reflect a ‘true’ large variation of the concentrations, but could also be a consequence of imprecise estimates obtained from the MLE when data were insufficient.

The available surveillance data was used as a representative base for fitting a distribution to infer the variation for the whole population (all meat available for sale). Here we chose to use the MLE for estimation of $\sigma$, $\mu$ and $\sigma$ by fitting a zero-inflated lognormal distribution. Zero-inflation is needed because many batches of broiler meat are not found to be contaminated. The lognormal distribution is frequently used to describe the variation of concentrations of foodborne pathogens (Kilsby and Pugh, 1981; Crépet et al., 2007; Busschaert et al., 2010).

It would be straightforward if evaluation of the effectiveness of intervention measures of specific food sources could be measured directly on the registered number of human cases. However, broiler meat only represents one of many sources for human campylobacteriosis, and as the specific proportion of cases attributable to broiler meat is unknown, the number of human cases is an imprecise measure for evaluating intervention effectiveness.

We chose to use QMRA for the evaluation of the effect of the implemented action plans, using the estimate of relative risk as a comparative measure as opposed to only considering changes in prevalence. Prevalence is frequently used to evaluate changes in contamination status of food products, however, this may not be a good indicator of the human health risk associated with the food considered (Nauta and Havelaar, 2008; Nauta et al., 2008), as confirmed in this study.

The Campylobacter prevalence for Danish chilled meat sampled at retail remained fairly unchanged within the study period, 2001–2010, with a slight decrease from 2005 to 2008. This is not in complete agreement with results from another Danish surveillance programme, where the proportion of Campylobacter positive samples of chilled meat at the large Danish slaughterhouses decreased within the period 2004–2006 (Rosenquist et al., 2009; Anonymous, 2012). According to the sampling scheme, retail samples should represent the products available for sale. This sampling might lead to an overrepresentation of meat from the smaller producers when compared to the actual sale. The sale of meat from smaller producers accounts for approximately 2% of the total sale, while products from the smaller producers may account for more than 2% in the display counter. Meat from the smaller producers often consists of special productions, i.e. meat from older birds with a higher probability of being positive for Campylobacter, organic production, etc. Furthermore, the smaller slaughterhouses do not have the possibility of implementing for example scheduling. Being aware of the prevalence being higher from these productions (data not shown) the modelled risk from Danish meat might be somewhat higher than the actual risk. However, the way of sampling was consistent over years, so a bias would be consistent.

The model predicts that the risk of human campylobacteriosis from a random sample of Danish produced broiler meat decreased from 2005 to 2008 and increased in the period 2008–2010. The slight decrease in human risk and prevalence for Danish chilled broiler meat from 2005 to 2008 coincides with an increase for Danish frozen meat. This might be an effect of scheduling of meat from negative broiler meat from 2005 to 2008 coincides with an increase for Danish frozen products seems to be a consequence of scheduling of meat from positive flocks to frozen production.

The risk from a random sample of Danish produced and imported meat differed. However, the inclusion of the amount of meat available for sale increased the overall risk from Danish meat. This was a direct result of the fact that the proportion of Danish meat available for sale was higher compared with import. Though, the proportion was diminished over years.

Changes in the risk estimates were observed within the different categories of meat. Overall, an increasing tendency in the relative risk in total from broiler meat available for sale in Denmark was turned around after 2005 and kept on a stationary level with minor fluctuations. It would be attractive to study whether the observed trends in the relative risks are significant. However, this would require an uncertainty analysis for the risk estimates, and at present no method is available to us to perform this type of analysis. Furthermore, it should be stressed that this kind of study, which is based on register data, does not allow a comparison with a situation without interventions.

The present evaluation shows that the Danish initiatives in the first action plan (2003–2007) against Campylobacter seem to have had an effect. The scheduling directed meat from positive flocks to production of lower risk products. The case-by-case risk assessments provided an incentive for retailers to heighten standards for their suppliers. For live broilers the prevalence was reduced by intensified biosecurity (Rosenquist et al., 2009). Half way through the second action plan (2008–2010), no additional risk reductions were observed for Danish meat. Efficacy of consumer campaigns was not evaluated; it has previously been concluded that the effect of consumer information in terms of risk reduction will usually be very small (Nauta et al., 2008).

A national control strategy only affects the proportion of meat that originates from the domestic production. Therefore, the effect of interventions will not target all broiler meat available for consumption and accordingly, the direct impact on human cases will be less distinct than if all meat was domestically produced. The case-by-case initiative targeting also imported meat was therefore essential in trying to cover all meat available for consumption in Denmark. It should be kept in mind that the action plans initiated in the Danish broiler production are on a voluntary basis so consistency in complying with interventions cannot be strictly assumed.

In conclusion, QMRA modelling seemed to be a valuable tool for evaluating changes over time in risk of human campylobacteriosis from broiler meat and thereby evaluating effects of action plans in this production. The QMRA allows a more detailed evaluation of the Campylobacter status in relation to human risk as oppose to prevalence data alone.

The most important intervention adopted for Danish produced broiler meat seemed to be the scheduling of meat from Campylobacter positive broiler flocks to freezing to the extent possible. Even though the risk from frozen broiler meat has been increasing it still remained lower than the risk from chilled meat. With regard to imported meat, the case-by-case risk assessments are believed to have influenced the decreasing human risk from this meat category.

Acknowledgements

The Danish Regional Veterinary and Food Authorities are acknowledged for collecting and analysing samples, the Danish Agriculture
and Food Council for providing data on sales and the Danish Veterinary- and Food Administration for funding the study.

References