Prevalence of *Campylobacter* spp. in turkey meat from a slaughterhouse and in turkey meat retail products

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Received 19 January 2006; revised 4 October 2006; accepted 5 October 2006.
First published online January 2007.

DOI:10.1111/j.1574-695X.2006.00180.x

Editor: George Mendz

**Keywords**

*Campylobacter*; prevalence; turkey; processing; retail.

**Abstract**

One hundred and forty-four samples of chilled turkey meat from six flocks, taken directly from the slaughterhouse, and 100 samples of turkey meat retail products were examined. Over one-quarter (29.2%) of the tested samples from the slaughterhouse were *Campylobacter* positive, showing high variability in the flocks. The lowest percentage of *Campylobacter*-positive samples was found in flocks I and III (8.3%), whereas, in flock VI, 91.7% of the samples were *Campylobacter* positive. Turkey meat retail products showed a prevalence of 34% for *Campylobacter*. Heat-treated meat was negative for *Campylobacter*. Quantitative studies of the samples taken at the slaughterhouse revealed a mean log range of 1.9–2.5 CFU g⁻¹ *Campylobacter* spp. Results from the quantification of retail products gave a mean log value of 2.1 CFU g⁻¹.

**Introduction**

Food-borne diseases caused by bacteria have gained in importance worldwide. *Campylobacter* and *Salmonella* are the main causes of food-related gastroenteritis. In the USA, over two million cases of *Campylobacter*-related illness are reported annually. These infections are mostly transmitted through food, with *Campylobacter* infections being responsible for 5% of food-related deaths (Mead *et al*., 1999). In 2004, the incidence of *Campylobacter* infection in the European Union exceeded that of *Salmonella* infection for the first time (‘Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union in 2004, 2006). In 2005, the number of cases of *Campylobacter* enteritis in Germany was higher than the number of cases of *Salmonella* infection (Epidemiologisches Bulletin). Raw poultry meat is commonly contaminated with *Campylobacter*. In particular, chicken meat has been reported to be contaminated with rates up to 100%. Poultry meat is one of the major sources of infection of *C. jejuni* (Zhao *et al*., 2001; Borck & Pedersen, 2005).

During slaughter and processing, cross-contamination and further spread of *Campylobacter* can occur. Even after chilling and cutting of poultry products, high contamination rates with *Campylobacter* are possible. (Logue *et al*., 2003) showed that up to 34.9% of examined turkey carcasses were positive for *Campylobacter* after chilling. Therefore, a substantial proportion of processed poultry meat retail products can be contaminated with *Campylobacter*. When examining turkey breast meat retail products, (Zhao *et al*., 2001) detected 14.5% of *Campylobacter*-positive samples. The results for turkey products range from 2 to 64% of positive specimens (Bryan & Doyle, 1995). Turkey meat is increasingly being chosen by consumers in Europe because of the fear of BSE and the adherence to low-fat diets. The consumption of turkey meat in Germany has increased in recent times, and reached 6.5 kg per person in 2005. Consequently, it is of great importance to identify and assess the potential risks arising from turkey products.

The aim of this study was to examine turkey meat from the slaughterhouse and turkey meat retail products for the prevalence of *Campylobacter* by qualitative and quantitative methods.

**Materials and methods**

**Turkey meat from the processing plant**

Immediately after slaughter and cutting, 144 samples of turkey meat were taken from a turkey processing plant in
northern Germany. The samples were taken from six different flocks slaughtered every other Monday within a period of 6 weeks. After cutting the parts (breasts, thighs and drumsticks, wings, hearts, livers and gizzards), the samples were chilled and transported to the laboratory for microbiological testing.

**Turkey meat and turkey meat products from retail outlets**

One hundred samples taken from a retail network of two supermarkets supplied by the slaughterhouse above were examined: fresh turkey products [turkey breast meat, turkey cutlets, turkey thighs and drumsticks, offal (turkey hearts, gizzards and livers)]; marinated products (steaks in various cutlets, turkey thighs and drumsticks, offal (turkey hearts, gizzards, livers)); heat-treated products (smoked turkey breasts, smoked turkey sausages, turkey meat balls, turkey meat ham).

**Bacterial isolation**

Turkey meat samples were qualitatively and quantitatively examined for *Campylobacter* according to the International Organization for Standardization (ISO) method ISO-10272-1,2 : 2002. The methods are described briefly below.

**Qualitative detection**

Samples for qualitative examination were suspended 1 : 10 in Preston-Broth (Oxoid, Basingstoke, UK). Incubation of the enrichment culture was performed at 42 °C for 48 h in a micro-aerobic atmosphere (O₂ 5%, CO₂ 10%, N₂ 85%) in an aerated incubator (CO₂-Incubator, Binder, Tuttlingen, Germany). An aliquot of the enrichment culture was then streaked on to two selective media, charcoal cefoperazone desoxycholate agar (CCDA) (Oxoid) and Karmali (Oxoid), with subsequent incubation at 42 °C for 48 h in micro-aerobic conditions.

Colonies presumed to be *Campylobacter* were taken for confirmation by motility testing with phase contrast microscopy, Gram-staining, catalase and oxidase reaction. Differentiation was carried out by biochemical testing (apiCampy, BioMérieux, Nürtingen, Germany).

**Quantitative detection**

A representative portion of the sample was suspended 1 : 10 in Preston-Broth, followed by 10-fold dilutions in 0.9% NaCl–peptone–water. Aliquots (0.1 mL) of these dilutions were spread plated in duplicate onto CCDA and Karmali agar plates. This was followed by incubation at 42 °C for 48 h under micro-aerobic conditions.

In a variation to the ISO-10272-1,2 : 2002 method, presumed colonies of *Campylobacter* were inoculated on selective media until single pure colonies were obtained, followed by incubation at 42 °C for 24 h on Mueller–Hinton agar (Oxoid) containing 5% sheep’s blood (Oxoid), instead of Columbia blood agar.

**Pulsed field gel electrophoresis (PFGE)**

The preparation of DNA-containing agarose blocks for PFGE was adapted from the ‘CAMPYNET’ Prototype Standard Protocol for PFGE (http://campynet.vetinst.dk/PFGE.html). In brief, cells were grown for 24 h on Mueller–Hinton agar with 5% sheep’s blood at 37 °C under micro-aerobic conditions and suspended in Pett IV buffer at an OD of McFarland 6–7 (Densimat, BioMérieux). Five hundred microlitres were mixed with 500 μL ofCert agarose (Biozym Scientific GmbH, Hess Oldendorf, Germany). The mixture was cast into moulds and solidified for 20–30 min at 4 °C. Plugs were incubated in 3.0 mL of EDTA-Sarkosyl-Protease (ESP) lysis solution at 56 °C for at least 24 h. The plugs were washed four times for 30 min each time in 20 mL of Tris-EDTA (TE) buffer. DNA was cut for 18 h at 25 °C with Smal and at 37 °C with KpnI in 100 μL of restriction buffer according to the manufacturer’s instructions (New England Biolabs, Frankfurt, Germany).

Digested DNA plugs were loaded on to a 1% SeaKem genetic technology grade agarose gel and separated on a contour-clamped homogeneous electric field DR-II apparatus (BioRad Laboratories, München, Germany) in 0.5 × Tris-Borate-EDTA (TBE) buffer for 22.5 or 23 h (Smal or KpnI, respectively) at 10 °C. The electrophoresis conditions were 6 V/cm, the included angle was 120° and the ramp times for Smal and KpnI fragments were 0.5–40 s and 4–20 s, respectively. After electrophoresis, gels were stained in 0.0003% ethidium bromide solution for 7 min and destained in distilled water for 30 min. The bands were visualized under UV light, photographed using a digital camera (Intas, Science Imaging Instruments GmbH, Göttingen, Germany) and saved as TIFF files for use with BioNumerics (Applied Maths, Sint-Martens-Latem, Belgium).

Normalization was performed according to strain CNET 068, as recommended in the ‘CAMPYNET’ Prototype Standard Protocol for PFGE, on each gel, with one standard used for every four samples. A molecular weight standard (λ-Ladder, New England Biolabs) was also used as electrophoresis control. The construction of similarity matrices was carried out using BIONUMERICS software (Applied Maths) and the band-based Dice coefficient. The unweighted pair group method using average linkages (UPGMA) was employed to cluster patterns. Bands for analysis with the Dice coefficient were assigned manually, according to densitometric curves and the accompanying hard-copy photograph. All macro-restriction profiles were evaluated and assigned to arbitrarily defined ‘profile groups’ using a cut-off at 90%.
Results

Turkey meat in the processing plant showed a prevalence of 29.2% (42 of 144 samples) Campylobacter-positive samples. The number of positive samples taken from the various flocks slaughtered varied from 8.3% in the first and third flocks processed to 91.7% in the last flock slaughtered in the sixth week. The results of the Campylobacter-positive samples taken at the slaughterhouse are shown in Table 1.

The occurrence of Campylobacter in the various parts of the turkey carcass and offal in the processing plant varied in the range 4.2–9.7% (Table 2).

Biochemical differentiation of the Campylobacter isolates revealed C. jejuni (61.9%), C. coli (14.3%) and presumptive C. fetus (23.8%). The isolates from the first five flocks were classified as C. jejuni (38.1%) and C. coli (9.5%). Presumptive C. fetus was isolated only in the sixth flock examined (23.8%); C. jejuni was found in 23.8% of the samples in the same flock and C. coli in 4.8%.

The results from the samples of fresh cooled turkey meat and turkey meat products obtained from the retail outlets are given in Table 3. Of the 100 samples examined, 34.0% were Campylobacter positive. The highest percentage of positive samples was observed in fresh cooled turkey meat (28.0%). Products for the barbecue, such as marinated steaks and fillets, showed 6.0% positive samples. All products heat treated during production were negative for Campylobacter. The most common isolated species from the retail products was C. jejuni (82.4%), followed by C. coli (11.8%) and two isolates of presumptive C. fetus.

Quantitative examination of parts of the turkey meat from the slaughterhouse resulted in counts of Campylobacter in a mean log range of 1.9–2.5 CFU g⁻¹ (Table 4).

Pulsed field gel electrophoresis results

Genotyping of Campylobacter ssp. isolates from turkey meat in the processing plant by PFGE revealed 13 different Smal and KpnI patterns (Fig. 1).

In flocks I–V, one or two different Smal/KpnI profile groups per flock were detected (Table 1). In flock VI, five different macrorestriction profiles were discovered. Campylobacter jejuni isolates with the Smal/KpnI profile group F/F were detected in two different flocks: flocks IV and V.

Discussion

As poultry is a well-known source of Campylobacter, handling of raw poultry meat by the consumer at home bears the risk of cross-contamination, especially in the case of inadequate kitchen hygiene. Therefore, it is important to determine the prevalence of Campylobacter in products at the end

Table 1. Campylobacter-positive samples in different flocks from the slaughterhouse and the Smal/KpnI profiles

<table>
<thead>
<tr>
<th>Flock number</th>
<th>Number of samples (n)</th>
<th>Campylobacter positive (%)</th>
<th>Smal/KpnI profile groups (number of strains)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>24</td>
<td>8.3</td>
<td>I/I (2) C/C (2)</td>
</tr>
<tr>
<td>II</td>
<td>24</td>
<td>33.3</td>
<td>D/D (2) E/E (2) K/K (2)</td>
</tr>
<tr>
<td>III</td>
<td>24</td>
<td>8.3</td>
<td>A/A (2) F/F (2)</td>
</tr>
<tr>
<td>IV</td>
<td>24</td>
<td>16.7</td>
<td>B/B (6) H/H (2)</td>
</tr>
<tr>
<td>V</td>
<td>24</td>
<td>16.7</td>
<td>I/I (2) L/L (10) MM (2)</td>
</tr>
<tr>
<td>VI</td>
<td>24</td>
<td>91.7</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>144</td>
<td>29.2</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Occurrence of Campylobacter in different parts of turkey meat from the slaughterhouse

<table>
<thead>
<tr>
<th>Turkey part, cooled</th>
<th>Number of samples tested (n)</th>
<th>Campylobacter-positive samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turkey breasts</td>
<td>22</td>
<td>6 (4.2)</td>
</tr>
<tr>
<td>Wings</td>
<td>22</td>
<td>8 (5.6)</td>
</tr>
<tr>
<td>Thighs + drumsticks</td>
<td>28</td>
<td>10 (6.9)</td>
</tr>
<tr>
<td>Steak</td>
<td>20</td>
<td>4 (2.8)</td>
</tr>
<tr>
<td>Offal (liver, heart, gizzard)</td>
<td>52</td>
<td>14 (9.7)</td>
</tr>
<tr>
<td>Total</td>
<td>144</td>
<td>42 (29.2)</td>
</tr>
</tbody>
</table>

Table 3. Campylobacter-positive turkey products from retail outlets

<table>
<thead>
<tr>
<th>Type of sample tested</th>
<th>Number of tested samples (n)</th>
<th>Campylobacter positive samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh meat (breast fillet, cutlets)</td>
<td>48</td>
<td>28 (28.0)</td>
</tr>
<tr>
<td>Marinated parts for grilling</td>
<td>16</td>
<td>6 (6.0)</td>
</tr>
<tr>
<td>Heat-treated meat</td>
<td>36</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>34 (34.0)</td>
</tr>
</tbody>
</table>

Table 4. Quantification of Campylobacter in parts of turkey meat from the slaughterhouse

<table>
<thead>
<tr>
<th>Turkey part, cooled</th>
<th>Number of samples tested (n)</th>
<th>Campylobacter, mean log CFU g⁻¹ (range, log CFU g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turkey breasts</td>
<td>22</td>
<td>1.9 (1.4–2.6)</td>
</tr>
<tr>
<td>Wings</td>
<td>22</td>
<td>2.3 (1.6–2.9)</td>
</tr>
<tr>
<td>Thighs, drumsticks</td>
<td>28</td>
<td>2.0 (1.6–2.9)</td>
</tr>
<tr>
<td>Steak</td>
<td>20</td>
<td>2.3 (2.1–2.7)</td>
</tr>
<tr>
<td>Liver, heart, gizzard</td>
<td>52</td>
<td>2.5 (1.6–4.0)</td>
</tr>
<tr>
<td>Total</td>
<td>144</td>
<td></td>
</tr>
</tbody>
</table>
of the processing chain and to obtain a better insight into the contamination of retail products in order to assess these risks.

The results of this study showed that 29.2% of cooled turkey meat samples from the slaughterhouse were positive for Campylobacter. The prevalence of Campylobacter in meat samples obtained from individual flocks ranged from 8.3% in two flocks to 91.7% in the sixth flock tested. In comparison, examination of turkey samples at a processing plant in the USA showed that 34.9% of samples were Campylobacter positive (Logue et al., 2003). With regard to the range of Campylobacter-positive samples in individual turkey flocks at processing plants, the results of Borck & Pedersen (2005) demonstrated a high variation, ranging from 4 to 96%, similar to the results obtained in this investigation.

In this study, quantitative examination for Campylobacter in turkey meat from the slaughterhouse after cutting showed a mean log range of 1.9–2.5 CFU g⁻¹. Rosenquist et al. (2006) found Campylobacter in the neck skin of chicken carcasses after chilling at levels of log 1.43–3.24 CFU g⁻¹.

For samples taken at the retail outlets, the prevalence was 34.0% in this study. The Food Standards Agency in the UK found that an average of 50% of retail carcasses were contaminated with Campylobacter (Anon. 2001). Whyte et al. (2004) found a considerably lower rate (37.5%) of positive samples in turkey retail products.

With regard to species variety in the turkey samples from the slaughterhouse, C. jejuni was found most often (61.9%), followed by presumptive C. fetus (23.8%) and C. coli (14.3%). The species identification was performed using the apiCampy system; therefore, C. fetus isolates should be confirmed with molecular probes. However, in previous studies, the identification proved to be very reliable and PFGE also indicated correct identification (Fig. 1). In samples taken at the retail outlets, C. jejuni was found in 82.2% and C. coli in 11.8% of isolates. Other authors have described similar levels for C. jejuni and C. coli, which were also most commonly isolated from turkey samples at processing plants (Logue et al., 2003) and chicken carcasses at retail outlets (Jorgenson et al., 2002).

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*Fig. 1. Dendrogram of the pulsed field gel electrophoresis (PFGE) DNA fragments (SmaI and KpnI DNA patterns) and profile groups (PGs) of 26 Campylobacter jejuni, six C. coli and 10 presumptive C. fetus strains isolated from turkey meat in a processing plant.*

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The results for flock VI showed a high rate of Campylobacter-positive samples (91.7%). Isolates of presumptive C. fetus were detected only in this flock. Using PFGE, one to five different SmaI/KpnI profile groups were detected per flock. The finding of different profile groups in the flocks showed that cross-contamination occurred during processing or that the turkeys from these flocks carried a number of different genotypes. The examination of Campylobacter strains by PFGE showed 13 different patterns. Although several different patterns were found for strains of C. jejuni (nine) and C. coli (three), all 10 isolates of C. fetus showed the same pattern, which differed significantly from the profiles of the other strains.

The results from the retail outlets revealed that 28.0% of samples from fresh turkey meat products, such as breast fillets, thighs and drumsticks, steaks and medallions, were positive for Campylobacter. Some types of marinated turkey products, in which various kinds of spices were used in the marinade, were also positive (6.0%). As new products with different kinds of marinades and seasonings are coming on to the market, and as, so far, little knowledge is available on the influence of these preparations with regard to the prevalence of Campylobacter, further research is necessary in this field.

All heat-treated products were negative for Campylobacter. The D-value for Campylobacter in meat is 1 min at a temperature of 60 °C. Another D-value determined for C. jejuni in ground chicken is 45 s at 57 °C (Jacobs-Reitsma, 2000). This leads to the conclusion that heat-treated products, such as those tested, are unlikely to be contaminated with Campylobacter.

References


