Impact of different storage factors on the survivability of Campylobacter jejuni in turkey meat

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Abstract
Campylobacter jejuni is often prevalent in turkey and poultry, but the effects of storage temperatures and storage periods and the interruption of the cooling chain on its survival have not been evaluated so far. In this study, 700 samples of turkey meat were artificially contaminated by inoculating their surface with 10^3 CFU of C. jejuni per sample, wrapped in airtight cellophane bags, and stored under different chilling and freezing conditions for various storage periods; this was followed by analysis of the cultures. Subsequent to incubation at 25°C for 48 h, C. jejuni was reisolated in only 7% of the samples. When the samples were stored under refrigerator conditions at 4°C, the organism was reisolated in 42% of the samples after 1 week, and in 28% of the samples after 2 weeks. The recovery rates in the samples that had been stored frozen at −20°C without interruption of the cooling chain were 68% after 2 weeks and 24% after 4 weeks. Different storage conditions were simulated in order to examine the impact of an interruption of the cooling chain on the survival of Campylobacter.

Introduction
Campylobacter species are found in the intestinal tracts of many birds and mammals used for food production. They are among the most frequently reported causes of bacterial gastroenteritis in humans in many developed and developing countries around the world (Bhaduri & Cottrell, 2004). The disease is caused by Campylobacter jejuni or, less commonly, Campylobacter coli, and must be regarded as an emerging disease (Coker et al., 2002; Kist, 2002). The pathogen is widespread in the environment, but can multiply only in living organisms (e.g. fowl, cattle and pigs). Humans are normally infected indirectly through the consumption of undercooked (e.g. poultry meat, poultry liver) or raw (e.g. raw milk, fondue chinoise) food. Cross-contamination occurring during the preparation of fresh or frozen turkey meat, poultry products and pork, or as a result of contaminated drinking or surface water (e.g. from recreational waters), is of considerable importance in this context (Gillespie et al., 2005). The significance of C. jejuni and C. coli as a cause of gastrointestinal diseases is constantly increasing, with the number of Campylobacter infections already exceeding those caused by Salmonella and Shigella in many countries (Altekruse et al., 1999).

The disease occurs primarily in children under 5 years of age and in young adults aged 15–29 years. The annual incidence rate shows seasonal and regional variations. The German national average is 16 cases per 100,000 inhabitants (Robert, 2005). The minimum infectious dose in humans is thought to be as low as 500 organisms (Robinson, 1981). The clinical disease (campylobacteriosis) is indistinguishable from salmonellosis and yersiniosis. Therefore, diagnoses can only be confirmed by identification of the infectious agent (Park et al., 1983).

Materials and methods

Bacterial strains and growth conditions
Campylobacter jejuni ssp. jejuni DSM 4688 (Penner 23) was used during this study. The strain was maintained at −70°C in Mikrobank-System. Cultures were subcultured from frozen stocks in Casein peptone–Sojamehl peptone broth and were grown at 42°C in a multigas incubator under microaerobic conditions (5% O2, 10% CO2 and 85% N2).

Artificial contamination, temperatures and time
Seven hundred samples of turkey meat were artificially contaminated by inoculating their surface with 10^3 CFU of C. jejuni per sample. They were then wrapped in airtight...
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cellophane bags and stored under different chilling and freezing conditions for various storage periods, ranging from 2 days to 4 weeks. Subsequently, the cultures were analyzed. In addition, a short interruption of the cooling chain at 0 °C was performed.

Reisolation and identification of Campylobacter in artificially contaminated Turkey meat

Ten grams of the artificially contaminated turkey meat was incubated in 100 mL of Preston Enrichment Broth under microaerobic conditions (5% O₂, 10% CO₂ and 85% N₂) at 42 °C for 48 h. Subsequent to the enrichment process, CCDA and Karmali selective culture media were each covered with 0.1 mL of the broth and then incubated under the same conditions at 42 °C for another 48 h. Presumptive single Campylobacter colonies were identified as follows: motility under a phase contrast microscope, Gram’s stain, and oxidase and catalase tests. Most presumptive isolates were further identified biochemically by means of an analytical profile index using the API Campy System (bioMérieux) (Atanassova, 2002).

Results

As a control, 100 samples were immediately examined before artificial contamination. Campylobacter was not detected.

One hundred subsamples were stored at ambient temperature for 48 h. Campylobacter jejuni was reisolated from 7% of the samples.

To examine the reisolation process of Campylobacter under refrigerator conditions, a further 100 subsamples of turkey meat artificially contaminated with C. jejuni were stored at a temperature of 4 ± 1 °C and examined for cultures after 1 week. Campylobacter jejuni was reisolated from 42% of the samples.

A further 100 samples were likewise stored for 2 weeks at a temperature of 4 ± 1 °C. Campylobacter jejuni was reisolated from 28% of the samples.

A further 100 subsamples were stored at a temperature of −20 ± 1 °C for 1 week, thawed to ± 0 °C, and again frozen for 1 week at −20 ± 1 °C. Campylobacter jejuni was reisolated from 37% of the samples.

A further 100 subsamples were frozen for 2 weeks at a temperature of −20 ± 1 °C. Campylobacter jejuni was reisolated from 68% of the samples.

A further 200 subsamples were subjected to the same procedure for 4 weeks. Campylobacter jejuni was isolated from 24% of the 100 subsamples that were stored frozen at a temperature of −20 ± 1 °C for 4 weeks without interruption of the cooling chain. For the 100 subsamples that were thawed to ± 0 °C after 2 weeks and again frozen for a further 2 weeks, the positive reisolation rate was 9%. The results are summarized in Table 1.

Discussion

The turkey meat samples tested were confirmed to be Campylobacter-free. At temperatures below 30 °C, organisms of the species Campylobacter are normally no longer able to multiply; although some authors have described multiplication of C. jejuni at ambient temperatures and at +4 °C (Lee et al., 1998). Therefore, Campylobacter is not able to multiply when foods are handled or stored at room temperature (Hazeleger et al., 1998; Jacobs-Reitsma, 2000). After incubation at 22–25 °C for 48 h, C. jejuni was reisolated in only 7% of the samples. Other studies were able to detect Campylobacter in foods for up to 3 days and in milk for up to 14 days (Schäfer, 1992). Some studies have reported a discrepancy between the occurrence and the spread of infectious diseases and the negative isolation and cultivation of the organism from the food involved in the outbreak. When stored at 4 °C, the organism was reisolated in 42% of the samples after 1 week and in 28% of the samples after 2 weeks. It was possible to reisolate the organism over a significantly longer period of time than described in the literature so far. Some authors have reported survival periods of up to 7 days. Only in milk was Campylobacter detected after as long a period as 168 days (Beutling, 1998; Hazeleger et al., 1998; Atanassova, 2002; Solow et al., 2003). However, this study shows that even after 2 weeks of storage at refrigerator temperatures, turkey meat can still be contaminated with C. jejuni.

Some authors have demonstrated that the risk of infection associated with frozen poultry meat is similar to that associated with freshly slaughtered poultry (Beutling, 1998; Ring & Atanassova, 1998; Kullman & Häger, 2002; Bhaduri & Cottrell, 2004). The recovery rates in the samples that had been stored at −20 ± 1 °C without interruption of the cooling chain were 68% after 2 weeks and 24% after 4 weeks.

Table 1. Campylobacter recovery rates for different storage temperatures, storage periods and artificial interruptions of the cooling chain

<table>
<thead>
<tr>
<th>Storage temperature (°C)</th>
<th>Period</th>
<th>Recovery rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22–25</td>
<td>48 h</td>
<td>7</td>
</tr>
<tr>
<td>4 ± 1</td>
<td>1 week</td>
<td>42</td>
</tr>
<tr>
<td>4 ± 1</td>
<td>2 weeks</td>
<td>28</td>
</tr>
<tr>
<td>−20 ± 1</td>
<td>2 weeks</td>
<td>68</td>
</tr>
<tr>
<td>−20 ± 1</td>
<td>2 weeks, thawed to ±0 °C after 1 week</td>
<td>37</td>
</tr>
<tr>
<td>−20 ± 1</td>
<td>4 weeks</td>
<td>24</td>
</tr>
<tr>
<td>−20 ± 1</td>
<td>4 weeks, thawed to ±0 °C after 2 weeks</td>
<td>9</td>
</tr>
</tbody>
</table>
Different storage conditions were studied in order to examine the impact of an interruption of the cooling chain on the survival of Campylobacter. The recovery rates found were significantly lower than those in the samples that had not been subjected to an interruption of the cooling chain. The rates were 37% after 2 weeks and 9% after 4 weeks. The temporary thawing of the samples at 0°C and the consequent release of water can result in reactivation of the organisms on the surface of the meat. The subsequent freezing process may then lead to additional dehydration of the surface of the meat to a depth of c. 0.5 cm and thus to a further decline in the bacterial count. This may account for the reduced recovery rate in the temporarily thawed samples in comparison to those constantly maintained in frozen condition.

In the German armed forces, the refreezing of food is forbidden under the JSP 46/28 (Food Hygiene) regulations (Lebensmittelhygiene (JSP), (1991)). Therefore, the interruption of the cooling chain represents a worst case scenario. This is also confirmed by studies conducted by (Bhaduri & Cottrell, 2004).

**Conclusions**

Diseases caused by Campylobacter are increasing in significance worldwide. At the same time, cross-contamination through mishandling of turkey meat in private households and the failure to maintain adequate storage conditions is increasing. At present, little information is available on the impact of storage conditions on the survival of C. jejuni in turkey meat. The objective of this study was to establish the survival of C. jejuni in artificially contaminated turkey meat with respect to storage temperatures and storage periods, and to study the interruption of the cooling chain. The results show that raw and raw frozen turkey products must be considered to have an increased C. jejuni contamination rate even after 4 weeks of storage. Therefore, there is a considerable risk of C. jejuni infection for the consumer, either through direct contamination or through cross-contamination during the preparation of ready-to-eat meals during the handling or processing of raw, chilled or frozen turkey meat. Thus, it is essential for turkey meat to be thoroughly cooked before it is served.

**References**


