**Campylobacter And Salmonella In Raw Chicken**

**Full title**  
Baseline Rates of *Campylobacter And Salmonella* In Raw Chicken in Wales, U.K. in 2002

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Abstract

The Public Health Laboratory Service in Wales, in cooperation with Local Authorities and the Food Standards Agency Wales, carried out a survey to establish baseline figures for the contamination of raw retail chicken with *Salmonella* and *Campylobacter* available within Wales, a devolved part of the UK with a population of approximately three million. Seven hundred and thirty-nine samples were taken between November 2001 and December 2002. Overall, 71% of samples were contaminated with *Campylobacter* and 8% were contaminated with *Salmonella*. There were no significant differences between fresh and frozen carcasses and between samples taken from retailers or butchers. There was seasonal variation in the *Campylobacter* contamination of fresh chicken, with a peak in June and the lowest positive rate in February and March. There was no similar peak observed in frozen samples or for *Salmonella*. 
Salmonella and Campylobacter are two of the most prominent bacterial genera associated with food-borne disease. Campylobacter is the most common bacterial source of human gastrointestinal infections in industrialized nations. Thermophilic Campylobacter species, including C. jejuni, C. coli and C. lari, are of particular interest since they are most associated with human gastrointestinal disease (8). In the UK, it was estimated that during 2000 Campylobacter accounted for the largest number of cases of food borne disease and 86 deaths, while Salmonella was estimated to be responsible for the fourth largest number of food borne disease cases and 119 deaths (1).

Salmonella and Campylobacter have many natural reservoirs, including poultry, domestic pets and rodents (4, 10). Chicken, both raw and cooked, has long been associated with Campylobacter and Salmonella infections in humans (4, 9, 10). Recent raw chicken surveys in the U.K. have reported Campylobacter isolation rates of 68%, 73% and 87% and a Salmonella isolation rate of 29% (4, 5, 7).

Campylobacter species tend to be relatively sensitive to the intrinsic and extrinsic parameters used to control microbial growth in foods. They are sensitive to heat, water activity, pH, osmotic stress and oxygen (8, 10). Salmonella tend to be more robust and can survive and grow in a much wider range of pH, temperatures and salt concentrations (10).
A survey for *Salmonella* and *Campylobacter* in raw retail poultry was carried out under the auspices of the Welsh Food Microbiological Forum (a collaboration of environmental health departments, food testing laboratories of the NPHS for Wales and the Food Standards Agency, Wales) between November 2001 and December 2002 throughout Wales, a part of the UK with a population of approximately three million. Seven hundred and thirty nine samples were examined over fourteen months for *Salmonella* and *Campylobacter* using plate-based methods.
Materials and Methods

Sample preparation. Whole raw chickens (fresh and frozen) were randomly sampled by local authority environmental health departments and submitted to food laboratories for examination. Chickens were stored at <5°C before examination and frozen samples were allowed to defrost. Initially, the neck skin was removed and divided into two. The carcass was then placed into a sterile bag and manually rinsed for 2 minutes in 225ml of buffered peptone water (BPW), ensuring that all surfaces, internal and external, had contact with the rinse. The rinse was then poured into a sterile jar and a portion of neck skin added. 25ml of this rinse was then pipetted into 225ml of Campylobacter enrichment broth (CEB) and the remaining part of the neck skin added to the CEB. All media was supplied by Oxoid, Basingstoke, U.K.

Campylobacter method. The CEB was incubated under microaerophilic atmospheric conditions (Campygen™, Oxoid, Basingstoke, UK) for 24 hours (+/-6 hours) at 37°C (+/-1°C), followed by incubation at 41.5°C (+/-1°C) for 24 hours (+/-6 hours). The CEB was sub-cultured onto Charcoal Cefoperazone Desoxycholate Agar (CCDA) plates and incubated in a microaerophilic atmosphere (Campygen™, Oxoid, Basingstoke, UK) at 37°C (+/-1°C) for 48 hours (+/-6 hours). All media was supplied by Oxoid, Basingstoke, U.K. Presumptive positive colonies were confirmed by oxidase reaction, growth under microaerophilic conditions (Campygen™, Oxoid, Basingstoke, UK) and microscopic determination of cell morphology using carbol fuchsin stained preparations and examination for curved or spiral shaped cells using the oil immersion lens (x400 magnification).
A modified version of the direct plating method (6) was used to enumerate a limited number of randomly selected rinse samples. The modified method used CCDA plates (Oxoid, Basingstoke, U.K.) incubated at 37°C (+/- 1°C) for 48 hours (+/- 6 hours). Confirmation of presumptive positive colonies was as above.

**Salmonella method.** The BPW was incubated for 18-24 hours at 37°C (+/- 1°C), followed by selective enrichment of 0.1ml in 10ml of Rappaport-Vassiliadis (RV) and of 1ml of 9ml of Selenite Cystine broth (SC). The RV broth was incubated at 42°C (+/- 1°C) for 18-24 hours and the SC broth was incubated at 37°C (+/- 1°C) for 18-24 hours. The broths were then subcultured onto Brilliant Green agar and Xylose Lysine Desoxycholate agar and incubated at 37°C (+/- 1°C) for 18-24 hours. All media was supplied by Oxoid, Basingstoke, U.K. Presumptive positive colonies (non-lactose fermenting with suitable colony morphology) were then confirmed using serological (Polyvalent O and Polyvalent H antigens, Murex Biotech, Dartford, U.K.) and biochemical tests (API 20E, Biomerieux, Marcy L’Etoile, France).

**Chi-square (χ²) hypothesis test.** The chi-square test for percentage positive rates was calculated manually using a published formula (3).
Results and Discussion

This survey provided new and original information on the rates of *Campylobacter* and *Salmonella* contamination in raw chicken in the U.K. It provided updated current rates for retail chickens in Wales, following on from the last known survey that was carried out by the U.K. Food Standards Agency (FSA) in Wales in 2000. It also provides, as far as the authors are aware, it was the first set of data that includes the seasonal variation in *Campylobacter* and *Salmonella* rates over fourteen months. Previous surveys have provided snapshot surveys over a limited time period and did not appear to take potential seasonal variation into account.

One of the current goals of the FSA is to decrease food borne disease by 20% by April 2006. *Campylobacter* and *Salmonella* are obviously major foci in achieving this target. The FSA is currently considering ways to reduce the levels of both organisms in chicken and has recently produced a draft strategy document looking at ways to specifically reduce *Campylobacter* levels. Evaluation of contamination rates within retail chicken is considered by the FSA to be a major part of this process, in order to assess the control methods implemented at broiler production level. Based upon the work presented in this paper, a rolling program of surveying retail chicken has now been recommended in the draft strategy document, which will provide trends and seasonal differences in *Campylobacter* and *Salmonella* over a substantial period (2).

An overview of the results is shown in Table 1. The mean number of samples examined per month was 52.8 (95% CI +/- 9.5). The *Campylobacter* isolation rate of 70.8% is similar to those of 68% and 73.2% reported in recent UK studies (4, 5). The *Salmonella* isolation rate of 8% was significantly lower than the 29% previously
reported from similar work in Wales (4), but this difference may reflect differences in sample type and/or examination method or may be a true reflection of the biosecurity measures implemented in the U.K. broiler production industry in recent years.

There was no statistically significant difference (using the $\chi^2$ test) in Campylobacter isolation rate between fresh and frozen chickens or between samples taken from retailers or butchers. Variation in Salmonella isolation rate showed no significant differences (using the $\chi^2$ test) between fresh and frozen and between samples taken from retailers and butchers. Quantification of Campylobacter in randomly selected rinse samples (n=44, 6%) gave a mean value of 160 cfu/ml of rinse (95% CI +/- 64 cfu/ml). A mean of 80 cfu/ml of rinse has been previously reported (6).

When focusing on the seasonal variation in isolation rates, frozen chickens were not considered because the slaughter date of these samples was not necessarily related to sampling date. For fresh chickens (n=553), slaughter occurred a maximum of 7-10 days before sampling and the contamination status of a fresh bird was therefore a reflection of the seasonal contamination rate. Monthly variation in isolation rates in fresh chickens is shown in Figure 1. Campylobacter isolation showed variation throughout the year, with a peak in June and the lowest rates in January, March and December. Salmonella isolation rate showed a slight upward trend throughout, but with no obvious seasonal peaks.

It can be concluded that Salmonella continues to contaminate raw chickens, albeit at lower levels than in the past, while Campylobacter are present at high levels. Although thermophilic Campylobacter on chicken carcasses are incapable of growth
under normal storage conditions it is obvious from the results presented here that they can survive and remain viable for long periods at low temperatures. It is also obvious that, given the frequency of \textit{Campylobacter} found, there is a need to emphasize the importance of handling chickens and raw poultry products carefully, both in the home and in catering establishments, and on the importance of implementing effective and useful HACCP plans within commercial environments.

The observation of the distinct seasonal variation in \textit{Campylobacter} contamination stresses the importance of carrying out long-term surveys on chickens, rather than ‘snap-shot’ surveys, which may give an artificially high or low result, dependent on season sampled.

This survey provides updated Welsh baseline figures for 2002 and sets the benchmark to monitor improvements in the quality of raw chicken available to consumers in Wales.
Acknowledgments

The authors would like to acknowledge the role of the Welsh Food Microbiological Forum, Food Standards Agency Wales, the NPHS for Wales and Welsh Local Authorities in the survey, with special thanks to the sampling officers and laboratory staff who sampled and examined the chickens.
References


**Figure 1**    Monthly variation in fresh chicken isolation rates

(Diamonds-*Salmonella*; Squares-*Campylobacter*)
Table 1  Summary of percentage positive rates for *Campylobacter* and *Salmonella*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>% Positive <em>Campylobacter</em></th>
<th>% Positive <em>Salmonella</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>All chickens</td>
<td>70.8</td>
<td>8.4</td>
</tr>
<tr>
<td>(n=739)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>70.2</td>
<td>8.0</td>
</tr>
<tr>
<td>(n=553, 75%)</td>
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<td></td>
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<tr>
<td>Frozen</td>
<td>72.6</td>
<td>9.7</td>
</tr>
<tr>
<td>(n=186, 25%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butchers</td>
<td>70.4</td>
<td>6.8</td>
</tr>
<tr>
<td>(n=220, 30%)</td>
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<td></td>
</tr>
<tr>
<td>Retailers</td>
<td>70.9</td>
<td>9.1</td>
</tr>
<tr>
<td>(n=519, 70%)</td>
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