Research Paper

Prevalence of *Campylobacter coli* and *Campylobacter jejuni* in Retail Chicken, Beef, Lamb, and Pork Products in Three Australian States

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ABSTRACT

The aim of this study was to investigate the prevalence and distribution of *Campylobacter* species in a variety of fresh and frozen meat and offal products collected from retail outlets in New South Wales (NSW), Queensland (Qld), and Victoria (Vic). A total of 1,490 chicken, beef, lamb, and pork samples were collected from Australian supermarkets and butcher shops over a 2-year sampling period (October 2016 to October 2018). *Campylobacter* spp. were detected in 90% of chicken meat and 73% of offal products (giblet and liver), with significantly lower prevalence in lamb (38%), pork (31%), and beef (14%) offal (kidney and liver). Although retail chicken meat was frequently contaminated with *Campylobacter*, the level of contamination was generally low. Where quantitative analysis was conducted, 98% of chicken meat samples, on average, had <1,000 CFU *Campylobacter* per carcass, with 10% <21 CFU per carcass. *Campylobacter coli* was the most frequently recovered species in chicken meat collected in NSW (53%) and Vic (56%) and in offal collected in NSW (77%), Qld (59%), and Vic (58%). In beef, lamb, and pork offal, *C. jejuni* was generally the most common species (50 to 86%), with the exception of pork offal collected in NSW, where *C. coli* was more prevalent (69%). *Campylobacter* prevalence was significantly higher in fresh lamb (46%) and pork (31%) offal than in frozen offal (17 and 11%, respectively). For chicken, beef, and pork offal, the prevalence of *Campylobacter* spp. was significantly higher on delicatessen products compared with prepackaged products. This study demonstrated that meat and offal products are frequently contaminated with *Campylobacter*. However, the prevalence is markedly different in different meats, and the level of chicken meat portion contamination is generally low. By identifying the types of meat and offal products types that pose the greatest risk of *Campylobacter* infection to consumers, targeted control strategies can be developed.

HIGHLIGHTS

- Retail chicken meat is frequently contaminated with low levels of *Campylobacter*
- *C. coli* was more commonly detected in chicken meat and offal than *C. jejuni*
- *C. jejuni* was more commonly detected in beef, lamb, and pork offal than *C. coli*
- In nonchicken offal, prevalence of *Campylobacter* was highest on lamb offal.
- Prevalence of *Campylobacter* was higher in fresh than in frozen offal.

Key words: Beef; *Campylobacter coli*; *Campylobacter jejuni*; Chicken; Lamb; Pork

Campylobacteriosis is the most common foodborne zoonosis worldwide, with Australian notification rates among the highest in industrialized countries (28). In 2018, 32,086 cases were notified in Australia, a crude incidence of 130 cases per 100,000 population (3). Globally, most foodborne human infections are caused by thermophilic *Campylobacter jejuni* (>80%) and, to a lesser extent, *Campylobacter coli* (43). The illness is typically acute and self-limiting, characterized by diarrhea, fever, and abdominal cramps that resolve within several days to 2 weeks (1). Postinfection campylobacteriosis complications can include immune-mediated reactive arthritis (7,000 cases per 100,000 cases of campylobacteriosis) and Guillain-Barré syndrome (30 cases per 100,000 cases of campylobacteriosis) (15, 37).
In developed countries, food production animals are the main reservoir for Campylobacter infections in humans (46). Many animal species may act as reservoir hosts or carriers of Campylobacter, making source tracing of the original source of human infection challenging (49). Campylobacter readily colonizes the gastrointestinal tract of poultry and wild birds and is found in livestock (cattle, dairy cows, sheep, and swine) (24). In poultry, Campylobacter is commonly believed to be a commensal inhabitant, although there are some suggestions it may have a negative effect on chicken health (25). In cattle, pigs, and sheep, Campylobacter infrequently causes clinical signs, but it can cause diarrhea in young stock and, occasionally, sporadic abortions (19). Chickens are common reservoir hosts, possibly due to their higher body temperature (42°C), which contributes to optimal conditions for Campylobacter growth in the cecum and colon (22). Feces, dust, and soil can spread Campylobacter onto feathers, fleece, hair, and animal skin preprocessing. During processing at the abattoir, poultry and livestock carcasses can become contaminated if intestinal contents are spilled and contact is made with fecal material or contaminated equipment (11, 12, 38). In Australia, a baseline retail survey conducted in 2005 in New South Wales and South Australia found 90% of fresh chicken meat positive for Campylobacter spp. (44). International surveys show beef, lamb, and pork products have lower levels of Campylobacter than poultry (7, 31, 33, 42, 45, 56, 61, 62).

Campylobacter infections in humans are usually sporadic in nature. Outbreaks are infrequent and have been attributed to consumption of unpasteurized milk, undercooked poultry liver pâté, and untreated drinking water (28, 48). Source attribution studies from England and New Zealand attributed 57 and 76% of Campylobacter infections in humans to chickens (40, 60). Additionally, a European Food Safety Authority assessment estimated the handling, preparation, and consumption of chicken meat to account for 20 to 30% of human cases of campylobacteriosis, with 50 to 80% attributable to the chicken reservoir as a whole (broilers as well as laying hens) (10). Consequently, the handling and ingestion of raw or undercooked poultry meat, and/or cross-contamination between raw and ready-to-eat food during food preparation, are important risk factors for human infection (36).

The CampySource project aims to apply genomics, epidemiology, and source attribution modeling to identify locally relevant risk factors and sources to reduce human illness from Campylobacter in Australia (58). This article reports (i) the prevalence and species distribution of C. coli and C. jejuni (referred to collectively as Campylobacter spp.) in raw chicken meat and chicken, beef, lamb, and pork offal, (ii) the level of Campylobacter contamination on chicken meat, and (iii) the association between Campylobacter spp. contamination with product type, storage condition, packaging type, and state.

MATERIALS AND METHODS

Study protocol. Samples were collected weekly (6 to 10 samples per week) on a successive rotating schedule to include a variety of portions and offal types from three Australian states, New South Wales (NSW), Queensland (Qld), and Victoria (Vic), between October 2016 and October 2018. In Vic, six samples (four chicken meat and two chicken offal) were collected each week, with half of the samples collected in Melbourne and half in the regional city of Bendigo. Samples from NSW were taken from the Hunter regional area and metropolitan suburbs around Sydney. In Qld, samples were taken from the regional cities of Toowoomba, Rockhampton, Townsville, and Cairns, as well as southern metropolitan Brisbane. All three states are on the east coast of Australia, with Qld the northernmost and Vic the southernmost state. Climatic conditions vary among these states, with equatorial and tropical regions further north moving to subtropical then temperate regions further south.

Sample collection. Packages of fresh and frozen chicken meat and chicken offal (giblet and liver) were collected from retail food outlets in NSW, Qld, and Vic. Fresh and frozen beef, lamb, and pork offal (kidney and liver) were also collected in NSW and Qld. Giblet, liver, and kidney samples are referred to collectively as offal hereafter. Both delicatessen samples collected by hand through an inverted plastic bag, and prepackaged samples (plastic wrapped, modified atmosphere or vacuum packaged) taken directly from refrigerated or freezer cabinets, were collected from supermarkets and butcher shops. Chicken product was identified as either conventionally farmed or free range. Chicken meat portions sampled were breast, drumstick, Maryland (thigh and drumstick), thigh, wing, and whole bird. Because Campylobacter is infrequently found on beef, lamb, and pork muscle meat, we elected to sample from organ meats (offal) to obtain suitable numbers of isolates for subsequent whole genome sequencing and source attribution modeling.

Campylobacter selective enrichment. After collection, samples were stored in coolers at refrigeration temperature (1 to 8°C) and transported to the laboratory. All samples were tested in their respective jurisdiction within 48 to 72 h of collection.

Isolation of Campylobacter spp. from chicken meat was performed as described in ISO 10272-1:2017 (26) and AS 5013.6.2015 (51) using the rinse method with modifications (Supplemental Table S1). Briefly, samples were placed in a sterile plastic bag containing buffered peptone water or enrichment broth and were agitated manually; subsequently, a subsample of the rinse solution (Vic, NSW) was combined with enrichment broth, or the entire rinse solution was incubated (Qld). After homogenization, samples were incubated at 37°C for 2 to 4 h to resuscitate injured cells, followed by microaerobic incubation (85% N2, 5% O2, and 10% CO2) at 41.5°C for 44 ± 4 h.

Campylobacter spp. were isolated from chicken, beef, lamb, and pork offal as above with some modifications (Table S2). Briefly, a 25-g subsample of offal was transferred to a sterile bag containing Preston or Bolton broth and agitated. Homogenized samples were incubated as described above.

Campylobacter spp. isolation and identification. Isolates collected in Qld and Vic were identified to genus level based on their ability to form colonies on Campylobacter-selective media after microaerobic incubation at 41.5 ± 1°C for 44 ± 4 h. In Qld, Campylobacter spp. were isolated on Preston agar (Oxoid, Hampshire, UK) and modified charcoal cefoperazone deoxycholate agar (CCDA; Oxoid); whereas, in Vic, Campylobacter spp. were isolated on Preston agar and Skirrow agar (Oxoid). In NSW, the SinglePath Campylobacter GLISA-Rapid test (gold-labeled immunosorbent assay, Merck, Darmstadt, Germany) was used for qualitative detection of Campylobacter spp. in food, following
manufacturer’s instructions. In NSW, *Campylobacter* spp. were confirmed by (i) streaking a subsample onto RAPID*Campylobacter* agar (Bio-Rad Laboratories Inc., Hercules, CA) and incubation at 41.5°C for 24 to 48 h and (ii) performing a *Campylobacter* latex agglutination test (Oxoid). Further *Campylobacter* confirmation was performed by traditional culture-based techniques, including Gram stain and oxidase test.

NSW *Campylobacter* spp. isolates were identified to species level at the Microbiological Diagnostic Unit Public Health Laboratory. In Qld, identification to species level was performed by a rapid hirpurate hydrolysis test and PCR as described by Klena et al. (30) and Linton et al. (34) or by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS). The PCR protocol by Linton et al. (34) was modified as follows: denaturation at 94°C for 7 min, followed by 38 PCR cycles (94°C for 1 min, 58°C for 1 min, and 72°C for 1 min), and a final extension at 72°C for 7 min, using Qiaqen multiplex mastermix (Qiagen GmbH, Hilden, Germany) as the enzyme mix. In Vic, identification to species level was performed by MALDI-TOF MS. Isolates confirmed as *Campylobacter* were transferred to a charcoal swab (BD, Franklin Lakes, NJ) and sent to the

**RESULTS**

In total, 785 samples of chicken (meat and offal) were tested for *Campylobacter* spp., along with 216 samples of beef, 208 of lamb, and 281 of pork offal. The prevalence of

<table>
<thead>
<tr>
<th>Type</th>
<th>NSW (n)</th>
<th>Qld (n)</th>
<th>Vic (n)</th>
<th>Total (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chicken meat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>19 (84)</td>
<td>75 (93)</td>
<td>23 (100)</td>
<td>117 (93)</td>
</tr>
<tr>
<td>Drumstick</td>
<td>18 (78)</td>
<td>61 (90)</td>
<td>23 (94)</td>
<td>102 (89)</td>
</tr>
<tr>
<td>Maryland&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15 (80)</td>
<td>30 (80)</td>
<td>9 (89)</td>
<td>54 (81)</td>
</tr>
<tr>
<td>Thigh</td>
<td>19 (95)</td>
<td>64 (94)</td>
<td>23 (96)</td>
<td>106 (94)</td>
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<tr>
<td>Wing</td>
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<td>Whole</td>
<td>22 (73)</td>
<td>53 (81)</td>
<td>14 (100)</td>
<td>89 (82)</td>
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<td>330 (90)</td>
<td>107 (96)</td>
<td>552 (90)</td>
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<tr>
<td><strong>Chicken offal</strong></td>
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<td></td>
<td></td>
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<tr>
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<td>30 (93)</td>
<td>11 (91)</td>
<td>41 (93)</td>
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<tr>
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<td>105 (57)</td>
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<td>192 (69)</td>
</tr>
<tr>
<td>Total</td>
<td>64 (83)</td>
<td>135 (65)</td>
<td>34 (88)</td>
<td>233 (73)</td>
</tr>
<tr>
<td>Total chicken</td>
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<td>465 (83)</td>
<td>141 (94)</td>
<td>785 (85)</td>
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<td></td>
<td></td>
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<tr>
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<tr>
<td>Liver</td>
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</tr>
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<td>74 (54)</td>
<td>134 (30)</td>
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<tr>
<td><strong>Pork offal</strong></td>
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</tr>
<tr>
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<td>11 (36)</td>
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<tr>
<td>Liver</td>
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<td>Total pork</td>
<td>142 (48)</td>
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</tbody>
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<sup>a</sup> Maryland, thigh and drumstick.

<sup>b</sup> NA, not applicable.

*Campylobacter* on chicken meat was 84% in NSW, 90% in Qld, and 96% in Vic (Table 1). The prevalence of *Campylobacter* on chicken offal was slightly lower, 83% in NSW, 65% in Qld, and 88% in Vic. The proportion of *Campylobacter*-positive chicken samples differed significantly among the three jurisdictions for both meat (*P* < 0.012) and offal (*P* = 0.003). Individual chicken meat portions ranged in prevalence from 73 to 100%. Whole chicken carcasses had a lower prevalence of *Campylobacter* than most meat cuts across the three jurisdictions, whereas thighs and wings had the highest prevalence (Table 1).

The proportion of *Campylobacter*-positive samples was low for beef offal in Qld (10%) and NSW (21%), whereas pork offal in Qld and NSW was 13 and 48% and lamb offal in Qld and NSW was 30 and 54%, respectively (Table 1). *Campylobacter* prevalence in samples collected in Qld was significantly lower in beef (*P* = 0.034), lamb (*P* = 0.001), and pork (*P* < 0.001) offal than in samples in NSW.

On average, <10,000 CFU per carcass was detected on 98% of the samples positive for *Campylobacter*. Higher levels of contamination were observed on whole bird samples, where 11% of samples positive for *Campylobacter* spp. had >10,000 CFU per carcass detected. All (100%) of

### Table 1. Summary of total samples collected from retail outlets in New South Wales, Queensland, and Victoria and proportion positive for *Campylobacter*, 2016 to 2018

<table>
<thead>
<tr>
<th>Type</th>
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<sup>a</sup> Maryland, thigh and drumstick.

<sup>b</sup> NA, not applicable.
the drumstick, Maryland, and wing samples positive for *Campylobacter* spp. had <10,000 CFU per carcass detected (Fig. 1).

*C. coli* was the dominant species on chicken meat and offal in NSW (52 and 77%; *P* = 0.025). *C. jejuni* was the most common species on chicken meat in Qld (59%), which was significantly higher than Qld chicken offal (33%) (*P* < 0.001) (Fig. 2). Dual contamination with both species ranged from 2 to 9%. *C. jejuni* was the most frequently isolated species on beef and lamb offal. *Campylobacter* species isolated from pork offal collected in NSW (69%) were predominantly *C. coli*, whereas in Qld, *C. jejuni* (50%) was slightly more common than *C. coli* (45%) (Fig. 3). Dual contamination was only observed in Qld pork offal (6%).

Conventionally farmed fresh prepacked chicken product (300 of 361, 83.1%) had a lower prevalence of *Campylobacter* compared with fresh prepacked chicken product farmed by free range (144 of 159, 90.6%) (*P* = 0.026). No difference in prevalence was observed for fresh prepacked skinless chicken meat (64 of 67, 96%) compared with skin on (94 of 106, 89%). Season and within-state geographic retail location (central Vic vs. southern Vic, North Qld vs. South Qld) had no statistically significant impact on *Campylobacter* prevalence (data not shown).

Fresh and frozen nonchicken offal samples were collected in NSW and Qld. Of samples, 75% were fresh, 18% were frozen, and 7% were not classified. Frozen beef, pork, and lamb offal had 1, 20, and 29% lower prevalence of *Campylobacter*, respectively, than did the respective fresh samples (Fig. 4). The difference in *Campylobacter* was statistically significant for lamb (*P* = 0.004) and pork (*P* = 0.004) offal (Fig. 4).

Higher *Campylobacter* prevalence was found on fresh delicatessen samples than on fresh prepackaged chicken offal samples (*P* = 0.017), beef offal (*P* = 0.001), and pork offal (*P* < 0.001) (Table 2). *Campylobacter* prevalence did not vary significantly with presence or absence of liquid within fresh prepackaged meat and offal sample packages (data not shown).

**DISCUSSION**

Chicken and livestock animals can harbor *Campylobacter* and represent sources for human campylobacteriosis. We found that most chicken meat and chicken offal samples tested were positive for *Campylobacter* spp., whereas the percentages of *Campylobacter*-positive samples were lower in lamb, pork, and beef offal. It was more common to isolate *C. jejuni* on lamb offal and beef offal, whereas *C. coli* was
more common on chicken offal and pork offal. Chicken products from poultry raised using conventional farming methods were found to have a lower prevalence of *Campylobacter* spp. compared with poultry that were reared free range. On lamb and pork offal, *Campylobacter* spp. were more frequently recovered from fresh samples compared with samples that were purchased frozen. Prepackaged chicken offal, beef offal, and pork offal had a lower positive proportion of *Campylobacter* spp. than delicatessen samples.

Although *Campylobacter* spp. were frequently recovered from retail chicken meat samples, the contamination level was relatively low. Where quantitative analysis was conducted, over 98% of chicken meat portion samples (breast, drumstick, Maryland, thigh, and wing), on average, had <10,000 CFU of *Campylobacter* detected per carcass. This is in line with recent surveys conducted in NSW (data not shown) and is relatively consistent with recent findings by Habib et al. (17), although they found that 18.7% of samples had >20,000 CFU per carcass compared with 2% at >10,000 CFU per carcass in our study. Food Standards Australia New Zealand guidelines for poultry meat set a microbiological target for *Campylobacter* at the end of processing at <10,000 CFU per whole chicken carcass to verify suitable control (13). The *Campylobacter* dose required to cause illness in humans has been reported at between 360 and 800 CFU (5, 20). Our findings suggest that even though raw chicken meat commonly harbors *Campylobacter* spp. at retail, the level of contamination in most products is likely below the national guideline used to reduce risk of campylobacteriosis associated with retail chicken meat.

The prevalence of *Campylobacter* spp. on chicken meat in our study was comparable with other Australian reports (83 to 95.8%) (9, 14, 29, 44) but was higher than that in surveys from Canada, the United Kingdom, and the United States (75, 73.3, and 46.6%, respectively) (6, 27, 57). The Australian chicken meat industry typically finds an approximate proportion of 70% *C. jejuni* and 30% *C. coli* (2), although this may vary by company and jurisdiction (55). We found, on average, that a third of our chicken product was slaughtered outside of the state where collected, while a subanalysis showed that species distribution was not statistically significantly different within individual abattoirs sampled by different health departments (data not shown). The latter suggests that abattoir, or even preprocessing differences in species carriage between flocks, may have influenced the distribution of *Campylobacter* spp. found in retail products.

Chicken offal in our study had a lower proportion positive for *Campylobacter* spp. than did chicken meat. Although we hypothesized that poultry product with the skin on would have a higher prevalence of *Campylobacter* (17, 18, 52), we found no statistical difference between the

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**FIGURE 3.** Distribution of *Campylobacter coli* and *Campylobacter jejuni* in retail beef, lamb, and pork offal collected in New South Wales (NSW) and Queensland (Qld), October 2016 to October 2018. Error bars indicate 95% confidence intervals.

**FIGURE 4.** Percentage of *Campylobacter*-positive fresh and frozen retail beef, lamb, and pork offal samples collected in New South Wales and Queensland, October 2016 to October 2018. Error bars indicate 95% confidence intervals.
prevalence of Campylobacter on chicken meat with the skin on compared with skinless chicken meat, in line with another Australian study (44). However, we did observe higher levels of contamination on whole birds and higher prevalence of Campylobacter in chicken products from free-range farms in comparison with conventionally farmed chickens. In Australia, antibiotics are not used in poultry processing, and there are no differences between processing hygiene interventions in free-range and conventional chickens; however, free-range birds have access to a range and, thus, may be at an increased risk of disease in comparison to conventionally farmed birds. This may explain the differences in Campylobacter contamination observed at retail; however, research undertaken by the Queensland Department of Agriculture and Fisheries indicated that there was no statistically significant difference in Campylobacter contamination between free-range and conventional flocks at farm level (23).

Our findings are comparable with those of a recent study by the NSW Food Authority, which found Campylobacter prevalences on lamb, pork, and beef offal of 55.9, 26.8, and 11.0%, respectively (41). A United Kingdom retail study by Little et al. (35) also found a comparable prevalence pattern, with Campylobacter most frequently isolated from lamb offal (36.6%), followed by pork offal (18.3%) and beef offal (12.2%). Although we did not quantify the level of Campylobacter on offal, a recent Australian report found >100 CFU/g in 3.6% of lamb, 2.8% of pork, and 0% of beef offal (41). We isolated C. jejuni more frequently than C. coli from lamb and beef offal, which agrees with a number of international studies (8, 16, 32, 35, 39, 47, 59). C. coli was more common on pork offal. However, the species distribution varied between NSW and Qld. Laboratory test methods, Campylobacter status preprocessing, or the dominant species at the abattoir are all potential influencing factors that have an impact on retail offal species distribution.

On lamb and pork offal, the prevalence of Campylobacter spp. was 20 to 29% higher on fresh samples than frozen samples, in agreement with other studies (53, 54). Harrison et al. (21) reported that storing chicken livers at –25°C can significantly reduce, but not eliminate, Campylobacter. They showed that multiple freeze-thaw cycles, compared with freezing liver and thawing overnight in a refrigerator, resulted in greater reduction of Campylobacter spp. (up to 3 log).

In Australia, national (AS 5013.6:2015) and international (ISO 10272-1:2017) standard test methods are used for detection and enumeration of Campylobacter spp. from meat and poultry. Although these are widely accepted laboratory methods for the detection of Campylobacter spp. from food, particularly poultry products, variations occur within these standard protocols. A limitation of our study was that samples collected in NSW, Qld, and Vic were processed independently by their respective National Association of Testing Authorities accredited laboratories. Each laboratory followed preestablished individual procedures for their jurisdiction based on ISO 10272-1:2017 (26) or AS 5013.6:2015 (51) in conjunction with AS 5013.20:2004 (50) for the enrichment, isolation, and speciation of Campylobacter. This led to some differences in procedure by state. For example, NSW used Bolton broth, whereas Qld and Vic laboratories used Preston broth to recover Campylobacter spp. The lower recovery rates of Campylobacter spp. from chicken meat in NSW (84.3%), compared with Qld (89.2%) and Vic (96.0%), may be due to partially to the enrichment broth used to recover Campylobacter spp. For example, in addition to Campylobacter, other bacteria such as Escherichia coli and Pseudomonas spp. are frequently recovered from meat samples enriched with Bolton broth (4). These bacteria may outcompete the microorganism of interest and result in an underestimation of the prevalence of Campylobacter on the product. Methodological differences, such as the type and volume of the rinse medium and enrichment broth as well as the selective agar used to isolate Campylobacter spp., may affect the results, particularly the recovery rate and species distribution. As a result, direct comparisons with other surveys that have investigated the prevalence of Campylobacter on retail meat should be made with caution.

Poultry and livestock naturally harbor Campylobacter in their intestinal tracts. We found a high prevalence of Campylobacter spp. in chicken products and a relatively lower prevalence in lamb, pork, and beef offal. When we quantified the level of Campylobacter contamination on chicken meat, generally low numbers of the bacteria were recovered. However, a small proportion of chicken product, particularly whole bird (10%), thigh (5%), and breast (3%), had levels of Campylobacter that exceeded the current Food Standards Australia New Zealand microbiological target (<10,000 CFU per carcass) for raw chicken meat before distribution. Reducing bacterial load below this target will limit the risk of campylobacteriosis to consumers. However, consumers should continue to practice good food safety, including adequately cooking meat products and avoiding cross-contamination of raw meat with fresh ready-to-eat foods.

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SUPPLEMENTAL MATERIAL

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