A Survey of *Campylobacter* in Beef Cuts at Retail

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**Executive Summary**

**Background**

*Campylobacter jejuni* is one of the most common causes of bacterial diarrheal illness in the United States (CDC). According to the U.S. Food and Drug Administration, research has shown that 20 to 100% of retail chickens are contaminated with the organism (FDA). It is assumed these high numbers are because many healthy chickens carry *Campylobacter* in their intestinal tract (Franco, Williams, 2001). However, *Campylobacter* is often carried by healthy cattle as well; little research is currently available to determine the prevalence of *Campylobacter* in beef products that are available in U.S. supermarkets. Therefore, it is important for us to establish a baseline for *Campylobacter jejuni* prevalence within the United States in order to better understand the prevalence of this organism and ultimately prevent food-borne illness from beef products available at supermarkets.

**Objectives**

1. Determine through microbiological analyses the prevalence of *Campylobacter* in U.S. fresh ground beef and whole muscle cuts at the supermarket level.
2. Determine if the prevalence of *Campylobacter* differs among package types, using the four most common forms of packaging available in U.S. supermarkets (modified atmosphere packaging (MAP), traditional polyvinylchloride overwrap (PVC), chub, and laminate vacuum packaging).

**Methods**

Ground beef (n = 1,109) and whole muscle (n = 293) cuts were collected from retail stores across the United States. These samples were collected by Texas Tech graduate students and staff who purchased samples from a wide array of supermarket types from different regions (East, South, Midwest, and West) across the United States.

**Sample collection**

Ground beef samples were purchased (n = approximately 20 per store) from differing lean/fat composition (96/4, 93/7, 92/8, 90/10, 85/15, 80/20, 73/27) to insure that all samples did not originate from the same grind; and to obtain equal representation of the three different packaging types (MAP, PVC, Chub, Laminate). Whole muscle cut samples consisted of round (top, bottom, and inside) and strip loin steaks (n = 3 per store). Sample collection occurred during the months of February through May. All samples were individually labeled and shipped to Texas Tech University, Lubbock, TX in insulated coolers. Samples were weighed upon arrival. Each sample was aseptically opened using a knife sanitized in 95% ethanol and flamed prior to sample collection using a sterile metal spatula. Aliquots of ground beef were taken randomly from five locations in the package (all four corners and from the middle) to adequately represent the package. Approximately 25 g of total sample was placed into Whirl pack bags (Nasco Ft. Atkinson, WI) which were sealed. Samples were then transported to the Experimental Science Building (Texas Tech University, Lubbock, TX).
and stored at 4 °C in a laboratory cooler. Whole muscle samples remained inside the original packaging and were stored under the same conditions.

Sample preparation

The 25 g ground beef samples were taken from the Whirl pack bags (Nasco Ft. Atkinson, WI) and placed into stomacher bags. A 200 mL portion of Bolton selective broth (a selective media designed for *Campylobacter*) was added to the bag and samples were stomached for 2 min at 230 RPM using a stomacher. Whole muscle cuts were placed into Ziploc® bags with 90 mL of Buffered Peptone Water (BPW). A whole muscle rinse was preformed while samples were manually shaken for 2 min. The whole muscle cut was removed with sterile utensils and 100 mL of double strength Bolton selective broth was added to the bag. The content of the bags (approximately 160 mL) for ground beef and whole muscle cut samples was then placed into screw-top bottles (PYREX® 160mL milk dilution bottles, Corning Incorporated Life Sciences Lowell MA) to provide the microaerophilic environment that is needed to grow *Campylobacter* (Nachamkin, 2007). The bottles were placed in racks and incubated for 4 h at 37ºC and then for another 20 h at 42ºC.

Plating and agglutination

After the 24 h incubation period the screw-top bottles were removed from the incubator and prepared for plating. The bottles were shaken well and 100 µl was pipetted onto *Campylobacter* selective agar called blood-free campylobacter plates more commonly known as MCCDA. Spread plating techniques were used to cover the plates. Blood-free Campylobacter selective agar was also used to make the plates for the plating of samples. Plates were placed into anaerobic chambers with Campylobacter gas packs (Oxoid Ltd. Company or BD E2) and were originally incubated at 37ºC, however, after initial samples were incubated it was apparent that optimal growth was not being achieved, therefore incubation temperature was increased to 42 ºC for 48 h. After incubation the plates were checked for growth, and plates with growth were agglutinated. A Campylobacter latex agglutination kit (L-CAM01T, Scimedx Corporation Denville, NJ) was used to determine positive colonies. Campylobacter antibodies are bound to the latex in the kit and form a ring on the kit plate, this visual agglutination is used to determine if the colonies are Campylobacter. A total of ten samples were tested at a time with a positive and negative control. The negative control contained all of the reagents but does not contain a bacterial specimen and the positive control contains only the positive reagent and the latex. All data is presented as the percentage of positive samples.

Important Results

A total of 1,402 samples were analyzed for *Campylobacter*, of that total 1,109 were ground beef samples and 293 were whole muscle cuts. Of the 1,402 samples analyzed, 7.13% tested positive for *Campylobacter* (Figure 1). A comparison was made between ground beef and whole muscle cuts (Figure 2). The percentage of ground beef samples that tested positive were 6.31%. The percentage of whole muscle beef cuts that tested positive was 10.24%. Of the 1,109 ground beef samples (n = 355, 446, 302, and 6 total samples for modified atmosphere packaging
In order to evaluate the presence of *Campylobacter* in ground beef samples of varied package type, two relationships were derived: the percentage of positive samples among all package types and the percentage of positive samples within each package type. Among the 1,109 ground beef samples testing positive (n = 70) for *Campylobacter*, 1.89, 1.98, 2.34, 0.09% were attributed to MAP, chub, PVC and laminate packaging (Figure 3). Within each packaging category, 5.92, 7.28, 5.83 and 16.66% of MAP, chub, PVC and laminate samples, respectively, were positive for *Campylobacter* (Figure 4). It should be noted that the sample size for laminate packaging was small (n = 6) due to limited retail availability.

There are few published studies that report the prevalence of *Campylobacter* in beef products. However, among the few studies available, the prevalence reported is relatively similar. Food Microbiology Fundamentals and Frontiers reports that *Campylobacter* isolation in beef cattle averages 6% with the range being as low as 1% to as high as 54% (Nachamkin, 2007). Altekruse et al. (1999) reported that the prevalence of *Campylobacter* in ground beef is 5%. The prevalence’s that were found in this study range as low as 6% to as high as 10% and appear to validate the reports that have been cited.

**Impact on the Beef Industry**

Although there is still much work to be done in order to have a more complete understanding of *Campylobacter* and its role as a food-borne pathogen, based on the results from this study *Campylobacter* is present in ground and whole muscle beef products as well as all packaging types available at the supermarket level. Further research would allow for a greater understanding of where and why contamination is occurring.
Lay Summary

The objectives of this project were to determine the prevalence of *Campylobacter jejuni* in U.S. ground beef and whole muscle cuts at retail and to determine the prevalence of *Campylobacter jejuni* among ground beef package types. Ground beef (n = 1,109) and whole muscle (n = 293) samples were collected from retail outlets across the United States. Ground beef package types were characterized as: modified atmosphere packaging (MAP), chub, traditional polyvinyl chloride film overwrap (PVC), and laminate packaging. Microbial analysis was conducted on all samples to determine those positive for *Campylobacter jejuni*. Of the 1,402 samples analyzed, 7.13% tested positive for *Campylobacter*. The percentage of ground beef samples that tested positive *Campylobacter* were 6.31%. The percentage of whole muscle beef cuts that tested positive for *Campylobacter* was 10.24%. To characterize the data further, two comparisons were made using packaging type: the percentage of positive ground beef samples among all package types and the percentage of positive ground beef samples within each package type. Among ground beef samples testing positive for *Campylobacter*, 1.89, 1.98, 2.34, and 0.09% were attributed to MAP, chub, PVC and laminate packaging. Within each packaging category, 5.92, 7.28, 5.83 and 16.66% of MAP, chub, PVC and laminate samples, respectively, were positive for *Campylobacter*.

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