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Survival of *Campylobacter jejuni* on beef and pork under vacuum packaged and retail storage conditions: Examination of the role of natural meat microflora on *C. jejuni* survival

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ABSTRACT

The ability of *Campylobacter jejuni* ATCC 11168 to survive on beef and pork stored under chilled, vacuum packaged and retail display conditions were examined. In addition, the effect of natural microflora on commercial beef and pork on the survival of *C. jejuni* under these storage conditions was examined. When sterile cores of beef and pork were inoculated with ~10^5 to 10^6 cfu cm^{-2} *C. jejuni*, and were stored under aerobic or vacuum packaged conditions at −1.5 or 4 °C, its numbers dropped significantly and *C. jejuni* could not be enumerated by direct plating after 21 d of the 6 wks study. In contrast, survival of *C. jejuni* on commercial vacuum packaged beef and pork was significantly enhanced, resulting in only 1 log cfu cm^{-2} reduction at the end of 6 wks. During 7 d of display in a retail case, numbers of *C. jejuni* dropped quickly, but could be enumerated by direct plating even after the 7 d. The presence of high numbers of inoculated *C. jejuni* on beef and pork had no significant effect on the natural microflora numbers compared to uninoculated controls when the meat was stored either in vacuum or in a retail display case. These results show that natural microflora on vacuum packaged meat afford enhanced survival of *C. jejuni* present on the surfaces of both beef and pork when stored at refrigeration temperatures. Hence, strict hygienic practices or the implementation of decontamination technologies are recommended to ensure safety of meat with respect to this pathogen.

1. Introduction

Campylobacter jejuni is one of the leading zoonotic causes of human gastroenteritis in Europe and North America (CDC, 2010; EFSA, 2005; PHAC, 2005). It has a very low infectious dose (Black et al., 1988; Robinson, 1981) and in addition to the seldom fatal but severe gastroenteritis caused by this organism, the serious immune-mediated Guillain-Barré syndrome, which is a chronic development in some previously infected individuals (van der Meche and van Door, 2000), make this organism a substantial public health concern. Based on collated epidemiological data from outbreaks, undercooked poultry is regarded as the major risk factor for foodborne campylobacteriosis (Fields and Swerdlow, 1999). In addition, outbreaks of infection due to *C. jejuni* have been linked to a number of sources including eggs (Finch and Blake, 1985), seafood (Feldhusen, 2000), unchlorinated water (Bopp et al., 2003; Jakopanec et al., 2008; Koenraad et al., 1997) and raw milk (Heuvelink et al., 2009; Peterson, 2003; Schildt et al., 2006). *C. jejuni* is often present in the gastrointestinal tracts (Gill and Harris, 1982a; Hudson et al., 1999; Inglis and Kalischuk, 2003, 2004; Inglis et al., 2005; Van Laack et al., 1993) and on carcasses (Gill and Harris, 1982a; Kotula and Stern, 1984; Stern, 1981) of meat animals. It has also been isolated from bulk packed red meats (Vanderlinde et al., 1998) and retail ready meats (Wong et al., 2007).

A large proportion of Canadian beef and pork is delivered to retail stores sealed in vacuum packages. The meat is subsequently opened at retail and cut into steaks, chops and roasts, which are typically repackaged in an oxygen permeable overwrapped film and placed in a retail display case. With the elimination of oxygen in vacuum packaged meat, the growth of aerobic spoilage organisms is significantly reduced, thereby extending product storage life (Lambert et al., 1991). The storage life of vacuum packaged fresh primals and subprimals is generally reported as approximately 35–45 d, while the expected shelf life of product packaged in oxygen permeable overwrap at retail is 3–7 d.

In the meat industry, vacuum packaging and storage at strictly controlled temperatures of −1.5 °C are widely used to store and export raw meat. A number of studies have raised concerns of the potential of these packaging conditions to increase the risk of campylobacteriosis by allowing the growth of *C. jejuni* (Gill and Harris,

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1982b; Hänninen et al., 1984; Van Laack et al., 1993). However, studies investigating the effect of vacuum and modified atmosphere packaging on the survival of *C. jejuni* on meat (Gill and Harris, 1982b; Hänninen et al., 1984; Van Laack et al., 1993) have collectively indicated that chill storage under vacuum or modified atmosphere is likely to increase the safety of meat. More recently in 2001, a study investigating the effect of these preservative packing of meat at a strictly controlled −1.5 °C on survival of *C. jejuni* showed no significant changes in numbers of this pathogen (Dykes and Moorhead, 2001), raising causes for concern. *C. jejuni* is fastidious with respect to growth requirements, is sensitive to oxygen (Stern et al., 1992) and only grows in the temperature range of 30–44 °C. Therefore, survival rather than growth on meat is the concern. While growth of *C. jejuni* will not occur at chill temperatures, it is necessary to understand the nature and extent of the effect of different meats, the natural bacterial populations on these meats and modern commercial meat packaging and storage practices on the survival of this pathogen. Studies to date have focused on the effect of storage parameters rather than the influence of the natural bacterial populations on beef and pork on the survival of *C. jejuni* in vacuum packaged and retail display conditions. Hilbert et al. (2010) noted the importance of the interaction between spoilage bacteria and *C. jejuni*. They demonstrated *in vitro* that coincubation of *C. jejuni* with *Pseudomonas* spp. enhanced the ability of *C. jejuni* to survive aerobic incubation at 35 °C. Coincubation did not affect the growth of *Pseudomonas* spp. The aim of the present study was to examine the survival of *C. jejuni* on beef and pork under vacuum packaged and retail display conditions at normal meat storage temperatures. In addition, the effect of natural microflora on survival of *C. jejuni* on beef and pork was examined.

2. Materials and methods

2.1. Bacteria and culture media

C. jejuni NCTC 11168 (ATCC 700819) was used in this study. The *C. jejuni* culture was maintained at −80 °C and resuscitated by streaking on tryptic soy agar plates (TSA, Oxoid, Nepean, ON Canada) at 42 °C for 24 h under microaerophilic conditions generated using CampyGen™ (Oxoid) gas generation sachets. An isolated colony was transferred to 75 ml tryptic soy broth (TSB, Oxoid) containing Campylobacter Growth Supplement (Oxoid, SR0117) and incubated under microaerophilic conditions for 48 h at 42 °C. Subsequently, 750 μl of this culture was transferred to 75 ml of TSB with Campylobacter Growth Supplement and incubated under microaerophilic conditions for 24 h at 42 °C. Cells were harvested by centrifugation and resuspended in 0.1% sterile peptone water (same volume as supernatant discarded). This culture was used to inoculate meat in all our studies.

2.2. C. jejuni inoculation and survival on sterile beef and pork

Fresh boneless beef and pork loins (*Longissimus dorsi*) of normal muscle quality were obtained from the Lacombe Research Centre abattoir. Sterile beef and pork muscle tissue discs (10 cm^2) were aseptically excised from the meat as previously described (Greer and Dilts, 1995). The pH values of muscle tissue discs were determined. Surface pH was measured using an Accumet AP61 portable pH meter (Fisher Scientific, Ottawa, ON Canada) equipped with a flat surface electrode (Accumet 13-620-289, Fisher Scientific). Each core was placed individually on a styrofoam tray (Scott National Food Service, Edmonton, AB Canada) and was inoculated with 100 μl volume of the *C. jejuni* suspension (prepared as described in section 2.1) to a final level of ~ 10^5 to 10^6 cfu cm^{-2}. The bacteria were allowed to adhere for 5 min. The trays with the inoculated or uninoculated control cores were then transferred to 8″ × 6″ commercial barrier bags (oxygen transmission rate of 40–50 cc m^{-2} 24 h^{-1}; Winpak Ltd., Winnipeg, MB Canada) for vacuum packing (Multivac AGI, Knud Simonsen Industries Ltd, Rexdale, ON Canada) The vacuum packaged cores were incubated at −1.5 or 4.0 °C and *C. jejuni* numbers enumerated or recovered using enrichment over a 6 wks storage time. To enumerate *C. jejuni*, cores were removed aseptically from the vacuum packs, transferred to 90 ml 0.1% sterile peptone water and mixed in a Seward stomacher (Fisher Scientific) at medium setting for 2 min. Appropriate dilutions were plated on Campylobacter Blood-Free Selective Agar (mCCDA; Oxoid) plates and the plates were incubated under microaerophilic condition for 48 h at 42 °C. Another core was transferred to Brucella broth containing Campylobacter Growth Supplement (Oxoid; SR0232) and enriched for *Campylobacter* spp. under microaerophilic conditions at 42 °C for 48 h followed by swabbing onto mCCDA plates to detect presence or absence of *Campylobacter* spp. The mCCDA plates were incubated under microaerophilic conditions for 48 h at 42 °C.

To examine the effect of oxygen permeability through the packaging material on *C. jejuni* survival, the above study was repeated with inoculated and uninoculated cores that were overwrapped with oxygen permeable, polyvinyl chloride film (oxygen transmission rate of 8000 cc m^{-2} 24 h^{-2}; Vitafilm Choice Wrap, Goodyear Canada Inc., Toronto, ON) or vacuum packaged in oxygen impermeable barrier bags that had a very low oxygen transmission rate of 0.4 cc m^{-2} 24 h^{-1} (Winpak).

These studies were repeated 3 times and final bacterial counts are means of these 3 replications.

2.3. C. jejuni inoculation and survival on commercial vacuum packaged beef and pork

Fresh vacuum packed beef and pork loins were obtained from a commercial meat plant. They were transported to the Lacombe Research Centre under refrigerated conditions and were stored at −1.5 °C overnight. These loins were cut into pieces weighing ~500 g (~750 cm^2) each using sterile knives and placed in commercial barrier bags. The pH values of muscle tissue were determined before inoculation for each incubation temperature. A 7.5 ml volume of the *C. jejuni* suspension (prepared as described in Section 2.1) was inoculated onto each of the pieces to a final level of ~ 10^5–10^6 cfu cm^{-2}. The inoculum was massaged onto the pieces before vacuum packing. The vacuum packed bags were incubated at −1.5 or 4.0 °C for up to 6 wks. Uninoculated control pieces were similarly processed by adding 7.5 ml of sterile water. Enumeration of *Campylobacter* spp. and background bacterial counts was performed on both the inoculated and uninoculated samples over the 6 wks storage period. To enumerate *Campylobacter* spp. and background bacterial counts, two 10 cm^2 cores were removed aseptically from the meat at times 0, 2, 5, 7, 9, 12, 14, 21, 28, 35 and 42 d. They were transferred to 90 ml 0.1% sterile peptone water and mixed in a stomacher at medium settings for 2 min. Appropriate dilutions were plated on selective and non-selective media. *Campylobacter* spp. was enumerated using mCCDA, incubated under microaerophilic conditions at 42 °C for 48 h. Selective media described by Baird et al. (1987) were used to enumerate *Brochothrix* spp., *Pseudomonas* spp., lactic acid bacteria and Enterobacteriaceae. Total aerobes were enumerated using TSA plates incubated for 48 h at 25 °C. Streptomycin thallous acetate agar (STAA; Oxoid) and cephaloridine fucidin cetrimide agar (CFC; Oxoid) was used to enumerate *Brochothrix* spp. and *Pseudomonas* spp., respectively, by incubating the plates at 25 °C for 48 h. Enumeration of presumptive lactic acid bacteria (LAB) was done on de Man, Rogosa and Sharpe agar (MRSA; Difco) with a 72–96 h anaerobic incubation at 25 °C. Enterobacteriaceae were enumerated by plating on Violet Red Bile Glucose agar (VRBGA; BD-Difco, BD Canada, Mississauga, ON Canada) with 18–24 h anaerobic incubation at 35 °C. Anaerobic conditions were