5-14 SURVIVAL OF CAMPYLOBACTER JEJUNI IN SELECTED GASEOUS ENVIRONMENTS

D. M. Kinsman[1], M. D. Greenberg[1], and N. J. Stern*, *Meat Science Research Laboratory, Beltsville Agricultural Research Center, Agricultural Research Service, USDA, Beltsville, Maryland, 20705 USA. [1]University of Connecticut, Department of Animal Industries, Storrs, Connecticut 06268

ABSTRACT

Campylobacter jejuni is known as a significant enteropathogenic bacterium for humans. Several foodborne outbreaks have implicated foods of animal origin as vehicles in the transmission of the pathogen to humans. The organism is a commensal for a wide range of livestock animals, and subsequently, has a high prevalence on meats purchased by the consumer. We instituted this study to determine whether the practice of vacuum-packaging or other selected modified atmostpheres would influence the survival of the organism in meat products. Vacuum-packaged and oxygen-permeable, polyvinyl chloride film wrapped broiler chickens were compared to determine the survival of the bacterium. No significant ($P<0.01$) difference in the survival of C. jejuni was observed, irrespective of the packaging system assessed by either surface rinse or drip sampling. Recovery of indigenous Campylobacter from red meat carcass samples was greater in the vacuum packaged samples (19/63) than in the oxygen permeable wrapped samples (7/63) after the samples were held at refrigeration temperatures for one week. Significantly ($P<0.01$) greater numbers of the organism were recovered by surface rinse sampling in both packaging systems when compared with the numbers obtained from purge sampling. The influence of modified atmospheres on the survival of inoculated C. jejuni in ground beef, held for 2 weeks at 4C, was also assessed. The atmospheres which were compared included (a) 100% nitrogen, (b) vacuum (atmosphere of -20 inches mercury), (c) 80% carbon dioxide and 20% nitrogen, and (d) 5% oxygen, 10% carbon dioxide and 85% nitrogen. Significantly ($P<0.01$) greater survival of C. jejuni was demonstrated in the 100% nitrogen atmosphere when compared with the other systems. These results indicate that the organism survives at variable rates in different atmospheres, but these differences were relatively small, and are unlikely to impact on the public health.

INTRODUCTION

Campylobacter jejuni is now established as an enteropathogen responsible for endemic and epidemic enteritis in humans. The epidemiologic evidence of Campylobacter induced disease indicate that both foods of animal origin and improperly treated waters serve as important vehicles in the transmission of the infectious agent to man (Brouwer et al., 1979; Oosterom, 1981; Tiehan and Vogt, 1978). The organism is present as a commensal in the intestinal tract of domestic livestock animals (Hanninen and Raevuori, 1981; Smibert, 1965) and typically does not manifest any pathological signs in these animals.

During slaughter and meat processing, intestinal contents may frequently contaminate carcass meats (Stern, 1981). Subsequently, if the meat is improperly processed or if post-processing contamination does occur, the final meat product may contain the human enteropathogen and cause disease in man. Several reports have implicated red meats and poultry as vehicles in the transmission. Raw hamburger (steak tarter), in the culinary traditions of Holland, was consumed in a Dutch military camp, with an explosive outbreak of campylobacteriosis resulting (Oosterom, 1981). The outbreak was epidemiologically traced to the consumption of this food product. Other cases have now implicated both red meats (Park et al, 1982; Peel and McIntosh, 1978) and poultry products (Brouwer et al., 1979) as sources for Campylobacter infection.

C. jejuni is a microaerophile, with optimum growth under an atmosphere containing 5% oxygen, 10% carbon dioxide and 85% nitrogen (Kiggins and Plastridge, 1956). Although this precise mixture of gases is not likely to be found in packaged meat systems, it is likely that atmospheres either more or less conducive to the survival of the organism could be encountered. After the use of modified atmospheres and selective packaging, subsequent metabolic activities of both indigenous microflora and meat catabolism occurs and alters the gas makeup (Ingram, 1962). The use of vacuum packaging and oxygen impermeable wraps to market meat products has been increasing (Christenson, 1979). Therefore, we wished to determine whether this technology might enhance survival of Campylobacter, and thus we studied the potential role of vacuum packaging and modified gas atmospheres on the viability of C. jejuni in meat products during product storage.

MATERIALS AND METHODS

Cultures, Inoculation, and Sample Storage:

The following strains of Campylobacter jejuni were employed in this study: USN 509 (human clinical isolate from J.C. Coolbaugh (Naval Medical Research Institute, Bethesda, Maryland); ATCC 29428 (American Type Culture Collection, Rockville, Maryland); MGC-2, MGC-4 and 7AC (chicken isolates) from the U.S.D.A. - Agricultural Research Service - Meat Science Research Laboratory stock culture collection. Inocula for the storage studies were prepared by transferring 1.0 ml of the stock cultures, maintained in Fluid Thioglycollate (Difco; Detroit, Michigan), to screw-capped test tubes containing 10 ml of Brucella broth (Oxoid; Columbia, Maryland) with 0.1% agar, which were incubated overnight at 42 C. A 2.0 ml portion from each of the five strains was combined to

945

formulate a mixed culture of 10 ml. The inoculum was added to 10 liters of 0.1% peptone (Difco) to obtain a final concentration of approximately 10^5 colony forming units per ml.

Fresh broiler carcasses were purchased from local retail outlets and returned to the laboratory on ice. Upon arrival to the laboratory, the chickens were split in half in a hygienic manner, and the halves were placed in the above prepared inoculating bath for three minutes. The halves were then removed and allowed to drip for an additional five minutes. Subsequently, one half-carcass was placed on a 2S plastic tray (A & E Plastics) and overlayed with an aerobic polyvinyl chloride (PVC) film. The other half-carcass was placed in a standard closure Cry-O-Vac bag and evacuated using a Multivac Model 3696/24, vacuum packager. The samples were held at 4 C and sampled at 48 and 96 hours. At each sampling interval, the accumulated drip from the carcasses and surface rinse were quantitatively assayed for C. jejuni, by spread plating onto Campy BAP medium (Blaser et al., 1979), and for the associated aerobic plate counts (APC).

Healthy pigs, sheep and cattle were slaughtered, skinned and eviscerated using conventional methods at the U.S.D.A., Agricultural Research Service, BARC Abattoir, Beltsville, Maryland. Flank samples of the animals were removed prior to carcass rinsing and were assayed for the presence of Campylobacter. Paired samples were obtained from the flank region from the point of insertion anterior to the stifle joint to a point approximately six inches from the proximal location. These regions were sampled because the flank region is thought to be relatively contaminated by feces (Gill and Harris, 1982) and to minimize carcass disfiguration. These flank samples were randomly assigned to vacuum or PVC packaging systems as described above. After one week storage at 2.5 C the samples were rinsed with 250 ml of Cary Blair medium without agar (CB), the rinsings filtered through a double layer of cheesecloth, centrifuged (10 min, 16,000 x g) and the pellet was resuspended in five ml of CB for both direct plating onto Campy-BAP plates and for enrichment cultures as described by Doyle and Roman (1982).

Bacterial Enumeration:

Surface Counts--The broiler chicken half-carcasses were rinsed with 250 ml of CB. The rinsings were then filtered through cheesecloth and centrifuged at 16,000 x g for 10 minutes at 4 C. The supernatant was decanted and the pellet containing the representative microflora was resuspended in 5 ml of CB. The cultures were serially diluted in CB and spread plated onto Campy-BAP medium (Blaser et al., 1979). Plates were incubated at 42 C in an atmosphere of 5% oxygen, 10% carbon dioxide and 85% nitrogen (microaerobic conditions) for 48 hours. Colonies typical of C. jejuni were counted and several representative colonies were prepared as wet mounts for examination using phase contrast microscopy to verify characteristic morphology and motion.

Drip Counts--The accumulated drip was aseptically collected from each package with a sterile pipette. The drip was filtered, centrifuged, resuspended and enumerated in the same manner as with the surface rinse determinations.

Aerobic Plate Counts--Counts were determined by serially diluting the above described bacterial pellet and plating onto Trypticase Soy agar (BBL; Cockeysville, Maryland) and incubating at 25 C for 48 hours.

Modified Gaseous Environments:

Frozen ground beef was obtained from the U.S.D.A., Agricultural Research Service-BARC Abattoir. Portions of the meat to be inoculatd were screened for C. jejuni by selective enrichment (Doyle and Roman, 1982). Prior to inoculating, the ground beef was allowed to thaw for 24 hours under refrigeration conditions. A mixture of the five previously listed strains was prepared as indicated above. The mixture was inoculated by using a Hobart Food Cutter Model 84141 to provide uniform level of approximately 10^5 colony forming units per gram. The inoculated 25-gram samples were dispensed into Whirlpak bags which were left open in an anaerobic jar (Oxoid; Columbia, Maryland). The ambient atmosphere was replaced by evacuation and replacement with one of the following: (a) 5% oxygen, 10% carbon dioxide and 85% nitrogen; (b) vacuum of approximately -0.6 bar atmospheres; (c) 100% nitrogen; or (d) 80% carbon dioxide and 20% nitrogen. The anaerobic jars were stored at 4 C for a two week period. At each sampling interval (4, 7 and 14 days), a Whirlpak bag was removed and the 25 gram sample was mixed with 225 ml of 0.1% peptone by stomaching for 30 seconds (Seward Lab-Blender 400; London, England). The homogenated slurries were serially diluted using 0.1% peptone and were spread plated in duplicate onto Campy-BAP agar plates to provide quantitative comparisons.

RESULTS AND DISCUSSIONS

Packaging Methods and Survival of Inoculated C. jejuni:

No clear differences were observed in the survival of Campylobacter as influenced by PVC or vacuum packaging procedures (Figure 1). The quantitative influence of sampling procedures are also illustrated in Figure 1 and indicate that declines in the numbers of the organism were parallel in both packaging systems. However, differences were observed in the numbers obtained by the surface rinse method as compared to the drip sampling method. The surface rinse method yielded significantly higher numbers ($P<0.01$) when compared with the drip sample method. On a per carcass-half basis, the drip vacuum packaged samples manifested a reduction of C. jejuni numbers of Log 0.91 while the surface rinse, vacuum packaged samples showed a decrease of Log 0.52. In the PVC wrapped carcass halves, numbers of the organism in the drip samples were reduced by Log 1.01 while the surface rinse samples had reductions of Log 0.62.

Sampling Method:

Further correlations were developed after comparing recovery rates of C. jejuni by surface rinse and drip sampling methods. Figure 2 illustrates comparisons of the Log number of C. jejuni recovered from surface and drip samples of the vacuum packaged chicken. The positive correlation coefficient of 0.73 indicates that the drip may be used to furnish a good index of the relative presence of the organism. A similar correlation of 0.66 was observed using the PVC wrapped product (data not shown). Within the limits of experimental error, and, at the levels of

C. jejuni typically associated with chickens, the drip sampling technique provides a good, non-destructive sampling for assessing the relative presence of C. jejuni. Previous work has shown that the bacteriological status of ready to eat poultry can be estimated by drip sample analysis (Mercuri and Kotula, 1964).

Packaging and Recovery of Indigenous C. jejuni and C. coli:

Table 1 represents the recovery of C. jejuni and C. coli from pork, lamb, and beef carcasses made through cultural enrichment procedures. The recovery rates of Campylobacter from surfaces of freshly slaughtered animals were very similar to previous observations (Stern, 1981). Incidence of Campylobacter recovery was fairly consistent among the initially positive samples and those held under vacuum packaging at refrigeration temperatures for one week. The corresponding samples held under PVC packaging at refrigeration temperatures for one week yielded fewer isolations of the organism. These differences attributed to the packaging methods were not consistent with the data previously noted, as no significant difference was observed in the inoculated chicken samples held under the different packaging conditions. Water activities relative to variability in transmission rates of packaging materials might explain the differences in survival of Campylobacter. Also, the longer period of storage (7 days compared with 4 days) might account for some of these differences noted.

Modified Atmospheres:

Comparison of the survival responses of C. jejuni inoculated into ground beef held under various atmospheric conditions were made in relationship to the control atmosphere of 5% oxygen, 10% carbon dioxide and 85% nitrogen. Results of the survival response of C. jejuni is represented in Figure 3. Statistical analysis indicated that the survival of the organism in ground beef was signficantly better ($P<0.01$) in an atmosphere of 100% nitrogen. The rates of inactivation, in terms of D-value, were 21.9 days for the 100% nitrogen, 14.4 days for the control, 13.2 days for vacuum and 14.0 days for the 80% carbon dioxide and 20% nitrogen atmospheres.

The enhanced survival of C. jejuni observed in the 100% nitrogen atmosphere corroborates the work of Koidis and Doyle (1983). Hanninen and colleagues (1984) reported no significant difference in survival of the organism inoculated onto fresh beef and held under (a) vacuum, (b) 20% carbon dioxide and 80% nitrogen, and an atmosphere of 5% oxygen, 10% carbon dioxide and 85% nitrogen. One explanation for the enhanced survival of C. jejuni in the 100% nitrogen atmosphere would be the lack of carbon dioxide contributing to carbonic acid formation, which would lower the pH of the system. It is recognized that Campylobacters are sensitive to low pH environments (Doyle and Roman, 1981), and subsequently the carbon dioxide in modified atmospheres could contribute to the die-off of the organism, and potentially could be used to control this pathogen.

ACKNOWLEDGEMENT

This work was supported by funds supplied by the Cooperative State Research Service, U.S. Department of Agriculture, grant 83-CRSR-2-2308.

REFERENCES

Blaser, M.J., Berkowitz, I.D., LaForce, F.M., Cravens, J., Reller, L. B. Wang, W-L. L. 1979. Ann. Intern. Med. 91:179-185.

Brouwer, R., M.J.A. Mertens, T.H. Siem, and J. Katchaki. 1979. Antonie va Leeuwenhoek 45:517-519.

Christenson, S. Kent. 1979. Meat Processing. July 28.

Doyle, M.P. and Roman, D.J. 1981. J. Food Prot. 44:596-601.

Doyle, M.P. and Roman, D.J. 1982. Appl. Environ. Microbiol. 43:1343-1353.

Koidis, P. and Doyle, M.P. 1983. European J. Clin. Microbiol. 2:384-388.

Gill, C.D. and Harris, L.M. 1982. Appl. Environ. Microbiol. 43:977-980.

Hanninen, M.L. and Raevuori, M. 1981. Nordisk Veterinar Medicin 33:441-445.

Hanninen, M.L., Korkeala, H., and Pakkala, P. 1984. J. Appl. Bacteriol. 57:89-94.

Ingram, M. 1962. J. Appl. Bacteriol. 25:259.

Kiggins, E.M. and Plastridge, W.N. 1956. J. Bacteriol. 72:397-400.

Mercuri, A.J. and Kotula, A. W. 1964. J. Food Sci. 29:854-858.

Oosterom, J. 1981. Abstracts of the Public Health Laboratory Service International Workshop on Campylobacter infection. Univ. of Reading. p. 37.

Park, C.E., Lior, H., Pauker, P., and Purvis, U. 1982. Ontario, Can. Dis. Wkly. Rep. 8:177-178.

Peel, R.N. and McIntosh, A.W. 1978. Lancet. 3:1212.

Smibert, R.M. 1965. Am. J. Vet. Res. 26:320-327.

Stern, N.J. 1981. J. Food Sci. 48:1291, 1293.

Tiehan, W., and Vogt, R. L. 1978. Morb. Mort. Wkly Rpt 27:207.

Table 1. Isolation of C. jejuni from red meat carcass samples, and from vacuum packaged or Polyvinyl chloride wrapped meats at 2.5°C for one week.

Livestock Species	No. of isolations/ carcasses sampled	No. of isolations/meat samples held at 2.5°C for one week	
		Vacuum	PVC
Porcine	10/27 (37%)	10/27 (37%)	4/27 (15%)
Ovine	10/36 (28%)	9/36 (25%)	3/36 (8%)
Bovine	0/26 (0)%	not done	not done

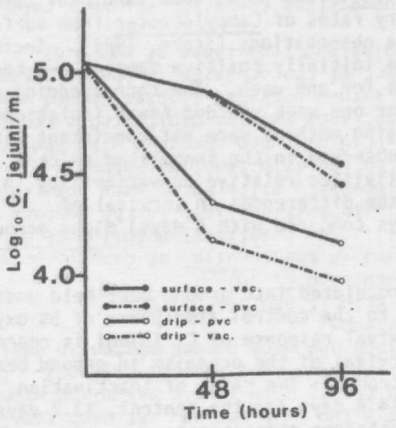

Figure 1. Recovery rate of inoculated C. jejuni from vacuum packaged and oxygen permeable packaged chickens.

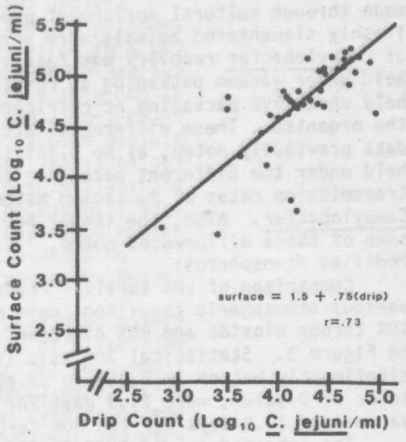

Figure 2. Recovery rates of inoculated C. jejuni by a surface rinse and drip sampling of vacuum packaged chickens stored at 4°C for 96 hours.

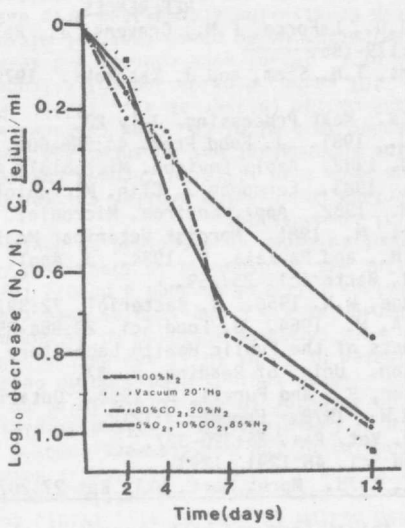

Figure 3. Influence of selected gaseous environments on the survival of a mixed culture of C. jejuni inoculated into ground beef and stored at 4°C.