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THE SURVIVAL OF *CAMPYLOBACTER JEJUNI* ON HOT VS. COLD BONED PORK

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SUMMARY

In the last decade, *C. jejuni* has gained recognition as a major human pathogen, transmissible through foods. *C. jejuni* is very susceptible to drying and chilling and usually the organism does not survive the carcass chilling process. After hot boning pork primals are packaged immediately to prevent surface dehydration. Hence, *C. jejuni* might prevail on hot boned but not on cold boned meat.

In a 'model' experiment we investigated the effect of time of boning and of vacuum packaging on the survival of *C. jejuni*.

Numbers of *C. jejuni* decreased during storage, regardless of packaging treatment. On cold unpackaged pork this decrease was slightly faster than on cold packaged pork. After packaging *C. jejuni* decreased at the same rate on hot and cold boned packaged pork. This indicates that the combined action of chilling and drying is probably crucial for reduction of *C. jejuni*. Hot packaging might therefore slightly enhance the risk of *C. jejuni* survival by eliminating dessication of the meat surface. Future research is neccesary to determine if accelerated processing constitutes a realistic hazard with regard to the risk of transmission of *C. jejuni* and other pathogens.

INTRODUCTION

The microbial spoilage and colonization of meat by pathogenic microorganisms has a specific character. Only part of the contaminating microflora possesses the physiological attributes necessary for survival and proliferation under the meat storage conditions encountered.

Hot boning and/or processing differs from cold boning in many respects, some of which may have an impact on the microflora of the end product. A major consideration is the difference in chilling and dessication rates. The initial flora on hot boned meat, packaged immediately after boning, is not subjected to cold shock until after several hours of refrigeration. Therefore, selection by dessication does not occur at all. Hot boned meat thus provides a warm and moist environment, an ideal medium for microbial growth. This may be expected to affect the keeping quality of hot boned meat by providing ample opportunity for multiplication of mesophilic bacteria (spoilage organisms and pathogens). Most microbiological studies on accelerated processed meat have been primarily concerned with assessment of the storage life. These reports indicate that accelerated processing is a microbiologically safe alternative to conventional processing if strict measures of Good Manufacturing Practices are adopted (van Laack, 1989). However, before one may feel certain about the wholesomeness of accelerated processed meat it is necessary not only to assess the number of organisms on accelerated processed meat but also to conduct an ecological survey of the bacterial population. Accelerated processing may select for mesophilic bacteria, possibly including the pathogen *Campylobacter jejuni*.

Only in the last decade has *C. jejuni* gained recognition as a major human pathogen, transmissible through foods. Oosterom et al., (1983) established that *C. jejuni* on pork carcass surfaces is very susceptible to drying. Although healthy pigs may be intestinal carriers of large numbers of *C. jejuni*, pork at the retail level is usually *Campylobacter*-free because the

organism does not survive the chilling process (Oosterom et al., 1983., Stern et al., 1985).
In the hot boning procedure, pork primals are packaged immediately, thereby preventing surface dehydration (weight loss!). Hence *C. jejuni* may survive on hot boned pork but not on cold boned meat.
The Dutch meat industry has not yet adopted the practice of packaging (cold boned) pork primals. Although small quantities of pork are wrapped before distribution, generally no packaging is used. Therefore, in examining the market potential of hot boning, hot boned packaged pork should be compared to cold boned unpackaged pork. Obviously, such a comparison does not allow the assessment of the separate and combined effects of time of boning and vacuum packaging.
Hence, in the present (pilot-) study we compared the survival of *C. jejuni* on hot boned/packaged, cold boned/packaged and cold boned/unpackaged pork, during chilled storage.

MATERIALS AND METHODS

Pig faeces were collected from the rectum of pigs and examined for presence of *C. jejuni*. At a slaughterhouse, the 16 loins of 8 pigs were hot boned within 1 h post mortem. After hot boning and trimming of visible fat, each of these loins was inoculated with *C. jejuni* by smearing the faeces over the entire ventral side of the muscle. Subsequently, each of 6 loins was cut into 6 chops of about 300 g, vacuum packaged and immediately chilled at 2 ± 2°C ('hot packaged'). The remaining 10 loins were chilled overnight at 2 ± 2°C, under conditions of mechanical ventilation. At 1 day post mortem, 6 of these chilled loins were cut into chops and vacuum packaged as described above ('cold packaged'). The other 4 loins remained unpackaged throughout the experiment ('cold unpackaged').
Meat was packaged in polyamide/polyethylene vacuum bags with an oxygen permeability of 25-30 ml/m^2, 24 h, measured at 23°C and 75% relative humidity (Wolff, Walsrode, F.R. Germany).
Pork chops from all treatment groups were stored in a cooling incubator at 0-2°C. After 0, 1, 2 and 5 days of refrigerated storage, one chop from each loin was sampled for *C. jejuni* by a destructive method (Snijders et al., 1984). Numbers of *C. jejuni* were assessed using spread plates of *Campylobacter* agar (Oxoid CM 689) containing lysed horse blood (Oxoid SR 117) and Preston's *Campylobacter* Selective Supplement [Oxoid SR 117, Bolton and Robertson (1982)]. Plates were incubated for 2 days at 42°C, under microaerobic conditions (BBL^R Gas-pack PWS envelopes without catalyst). For determination of the 'Most Probable Number' 1.0 ml of decimal dilutions of the macerate was added to 9.0 ml of Preston's enrichment medium (Nutrient Broth No. 2 CM67, Lysed Horse Blood, Preston's *Campylobacter* Selective Supplement and *Campylobacter* growth supplement Oxoid SR 84) and incubated at 42°C for 24 h under microaerobic conditions (Bolton and Robertson, 1982). Of the plates used for enumeration, $\sqrt{n}$ colonies were confirmed by Gram-stain, katalase and motility.
The experiment concerned a relatively small number of samples. As a result of this statistical analysis was considered to be of little value and was not done.

RESULTS AND DISCUSSION

Results are included in Table 1.
Number of *C. jejuni* decreased during storage, regardless of packaging treatment. This reduction appeared to be slightly faster on cold unpackaged pork than on cold packaged pork; at day 2 this was reflected in a Most Probable Number of *C. jejuni* on the cold packaged samples of 1.15 $\log_{10}$ CFU/cm^2 and .86 $\log_{10}$ CFU/cm^2 on the cold unpackaged pork.

Table 1: *The survival of* Campylobacter jejuni *(log_{10} CFU/cm^2) on inoculated pork loin muscle, after various periods of refrigerated storage at 2±2°C.*

Packaging procedure	Enumeration procedure	n	Storage period (days) 0	1	2	5
Hot packaged (at day 0) (HB/IN/VP/C)*	Direct plating**	6	4.12	3.98	2.84 (66%)	4.16 (50%)
	Enrichment***	6			>1.48	>0.90
Cold packaged at day 1) (HB/IN/C/VP)	Direct plating	6	3.86	3.34 (33%)	3.26 (16%)	3.29 (16%)
	Enrichment	6		>1.31	>1.15	>0.65
Un-packaged cold (HB/IN/C)	Direct plating	4	3.95	3.03 (25%)	<2.32	<2.32
	Enrichment	4		>1.48	>0.86	>0.36

* *Hot boned (HB) and inoculated (IN) with* C. jejuni*-containing pig faeces as affected by vacuum packaging (VP) and chilling (C)*

** *Average number of organisms on positive samples (i.e. samples containing more than 7 colonies on the 10^{-1} dilution plate; detection limit 2.32 log/cm^2). Figures in parentheses indicate % of positive samples (unless 100%)*

*** *'Average' Most Probable Number (e.g. if* C. jejuni *is present in the 10^{-1} dilution of m samples, and in the 10^{-2} dilution of n samples the 'average' Most Probable Number is expressed as m x 0.48 + n x 1.48/m+n)*

One day after inoculation and chilling, numbers of *C. jejuni* were greater on hot packaged pork than on unpackaged pork. However, after packaging *C. jejuni* numbers decreased at the same rate on hot and cold packaged pork. This indicates that the combined action of chilling and drying is probably crucial for reduction of *C. jejuni* from the surface of a muscle. Hot packaging might therefore slightly enhance the risk of *C. jejuni* survival by eliminating dessication of the meat surface.

The results clearly show that *C. jejuni* was still present on the unpackaged muscle surface after 1 day of chilling with ventilation. In studying the *C. jejuni* contamination of pig carcass surfaces, Oosterom et al., (1983) observed a rapid reduction from 3.51 to 1.00 log_{10} CFU/cm^2 *C. jejuni* during 24 h chilling in moving air at 0-4°C. We observed only a reduction from 3.95 to approximately 2.60 log_{10}/cm^2 (i.e. 33% of the samples having an average contamination of 3.34 log units and 67% of the samples containing < 2.32 log_{10} CFU/cm^2). The most likely cause of this discrepancy between Oosterom's study and ours is the difference in relative humidity (95% in our study, and only 60-70% in Oosterom's (1983) experiment).

A relative humidity of 60-70% is not often found in meat industry practice as the relative humidity in chilling rooms is generally kept high to limit evaporative weight losses. Under such conditions *C. jejuni* will not be eliminated as readily as Oosterom's results would indicate.

Before reaching any conclusions, the challenge-experiment nature of our study must be considered. Firstly the pork was contaminated with substantial amounts of faeces, spread directly onto the muscle surface. Should contamination with *C. jejuni*-containing gut contents occur during

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