A literature review for the project:

Development of accurate predictive models for the assessment of the survival of *Campylobacter jejuni* and *C. coli* under food-relevant conditions. Project code: FS241040

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Executive summary

Campylobacter are major zoonotic pathogens and control in the food chain is a public health necessity. One approach is to use potentially lethal processes like heat or freezing. *Campylobacter* is said to be sensitive to hostile environments, but survives well in foods and many infection vehicles had been heated before consumption or subject to refrigeration. Predictive models can inform processors about food treatments to eliminate pathogenic bacteria like *Campylobacter,* but data used in current models are inappropriate as techniques used did not:

- Recognise the population biology of *Campylobacter*
- Allow detection of injured cells, which may still be a health threat.
- Take account of interactions with foods and the environment.

We believe that *Campylobacter* survival is underestimated and its growth potential in foods is largely undetermined. The project is examining the growth/survival of *Campylobacter* under conditions relevant to those found in food, taking account of how this is affected by food characteristics. The work is using highly sensitive recovery techniques to detect live *Campylobacter* following treatment and will therefore provide accurate data to properly inform generic predictive models.

Part of the project is to critically review existing data on the heat resistance of *Campylobacter* spp. in environments and conditions relevant to the above project. The review found that there was a wide variety of techniques used in published studies and many different strains of *Campylobacter*. As such, it was not possible to undertake a meta-analysis. The variation between different experimental techniques and the many poor ones used highlights the need for the work in progress in this FSA-funded study.

1. Introduction

Predictive models of the growth and survival of micro-organisms are essential tools for the food industry and government/policy makers for studying the response of food-borne pathogens to changes in their biochemical and physical environment. However, there are few such models for the study of *Campylobacter* survival in the food-chain. Two models have been published quite recently. One (Yoon *et al*. 2004) examined the survival of a *C. jejuni* isolate in chicken meat at 4 and 30°C and is thus not relevant to work on in our study. The second (Sansalone *et al*. 2010) examined the impact of potassium sorbate on the survival of *C. jejuni* at -2 and 4°C. Thus this is also not relevant to our work. The absence of predictive models for the survival of *Campylobacter* at high temperature is a direct consequence of the lack of validated data. Furthermore, techniques used in studies to look at the behaviour and survival of *Campylobacter* under processing conditions, thus far has been used uncritically without wider consideration given, for example, to using the most sensitive recovery methods for sub-lethally injured cells.

In order to critically evaluate the data currently available, we have undertaken a review of existing published sources that have examined the effects of oxidative stress, pH, pre-chilling and high temperature on the survival of *Campylobacter* in foods. In addition, we also propose a Gold Standard in experimental design for conducting such studies that will provide a robust methodology for studying the response of *Campylobacter* to changes in its biochemical and physical environment. Such an approach will generate data that can be used to produce validated predictive models for the study of *Campylobacter* behaviour in foods.

2. Methods

Electronic searches were conducted to identify reports of published, peer-reviewed studies. Highly sensitive search strategies were designed, including combinations of appropriate subject headings and text word terms, interventions under consideration and specific study designs. No restrictions were placed on searches with regard to language or date. The National Centre for Biotechnology Information (NCBI) database, PubMed, was used to search for evidence of studies. Other databases were also explored but did not reveal any peer-reviewed publications that were not already in PubMed. A reference list of all studies potentially to be included was created in order to identify additional and potentially relevant publications. A panel comprising the PIs and Drs Jones and Close provided adjudication in the event of uncertainty as to the relevance of including a particular study. Advice was also sought from members of the expert steering group for this project.

2.1. Inclusion and exclusion criteria

2.1.1. Inclusion Criteria

Evidence was considered from all studies examining the survival of *Campylobacter* in poultry, other meat products and laboratory based culture media. Studies were included that examined the impacts of extremes of temperature, the effects of pH and oxidative stress on the survival of *Campylobacter*. The suspending substrate used during experiments was also used to select relevant studies; namely those that used poultry and meat products. It was agreed by the expert steering group that the most critical aspect of experimental design and approach centred on the enumeration and recovery method for *Campylobacter.* Thus, studies were selected according to how they conducted enumeration and recovery, including the incubation time and incubation temperature for enumeration, and whether they would have an impact on the recovery of sub-lethally injured *Campylobacter* cells and thus underestimate survival. The inclusion of antibiotic supplements to media (selective isolation) has been shown to greatly affect the recovery of sub-lethally injured *Campylobacter* and that recovery is best achieved using non-selective agar, especially with the inclusion of quenching agents to prevent oxidative stress (Humphrey, 1986, Smigic *et al.*, 2010).

2.1.2. Exclusion criteria

Studies not involving experimental simulation of exposure of *Campylobacter* to extreme conditions (temperature, pH and oxidative stress) in culture media, poultry or meat products were excluded. We recognise that other stresses such as survival in water, freezing and/or pressure might support information on variation in stress resistance between strains. However, this is beyond the scope of the current review.

2.2. Data extraction strategy

Two independent reviewers (Drs Close and Jones) screened titles and abstracts of all identified studies. Reviewers extracted the details of study design, methodological approach and outcomes. Where there was uncertainty in study design, experimental approach and outcome precluded immediate inclusion, the PIs adjudicated accordingly.

3. Data Analysis and Findings

The lack of conformity in experimental design precluded undertaking a formal meta-analysis of experimental outcome. Nevertheless, an indirect comparison of experimental approaches was undertaken, thus allowing the inclusion of non-standardised comparative data.

3.1. Number and type of studies Included

The outcome of unique combinations of key-word searches identified 336 potential data sources (Table 1.). Initial screening of studies identified a subset of 110 data sources appropriate to this study. In order to prevent duplication of published sources, cross-validation of data sources was undertaken by each reviewer. An additional four data sources were identified as citations from the within the screened studies obtained via the key-word searches. These studies were not identified using the main search criteria and are categorised as "Additional Studies" within Table 1. The cross-validation process retained 55 unique data sources (Appendix 1.). Further refinement of data sources was necessary in order to identify those studies that corresponded to the survival of *Campylobacter* in poultry meat, other meat products and laboratory based culture media explicitly (challenge conditions are presented in Appendix 2). In all 32 data sources were retained (Table 2.1.-2.4.).

3.2 Experimental design to determine *Campylobacter* survival under food relevant conditions

The pre-existing literature contains a wide range of experimental designs investigating the survival of *Campylobacter* spp. when the bacteria are subjected to physical stresses, such as extremes of temperature as a result of heat and chilling, and chemical stresses including changes in pH and oxidative conditions. The following summary details the extensive variation which has been found during the review of published literature.

Survival of pre-isolated strains of *Campylobacter* in culture conditions subjected to heat challenge

There is considerable variation in experimental design for *Campylobacter* undergoing heat challenge. In the work considered here, *Campylobacter* were grown in culture broth, or on appropriate agar media, re-suspended in a diluent and subjected to heat challenge by the introduction of a small proportion of broth to pre-heated media (instant heat challenge). In addition, the growth phase of the bacterial cells has been considered. Kelly *et al*. (2001), for example, investigated the heat resistance of early and late stationary phase *C. jejuni* and *C. coli* cultures, in addition to ones

representing those in exponential phase, at 50^{o}C for up to 75 minutes. The exponential cultures appearing to have increased resistance to such mild heat stress compared to stationery phase ones. Martinez-Rodgriguez *et al*. (2004) also examined growth phase and heat resistance in a single *C. jejuni* isolate at 50^{o}C, with up to 75 minutes exposure and additionally its response to oxidative stress (exposure to hydrogen peroxide and aeration). Sagarzazu *et al.* (2010) determined the effect of heat stress in exponential and stationary *C. jejuni* (55^{o}C for up to 6 minutes) and subsequently the effect of high hydrostatic pressure on these cells. Additional exposures to acid and cold stresses were also applied prior to high hydrostatic pressure and these experiments were performed on a single isolate of *C. jejuni*. Klancnik *et al.* (2006) tested a single *C. jejuni* isolate, grown in Preston broth, at 55^{o}C over a time range of 3-20 minutes, with additional experiments assessing the effect of oxidative stress and 'starving' of cells by centrifugation and resuspension in Ringer's solution. In a number of other studies, a single temperature challenge with one sampling time was investigated (Jasson *et al.*, 2007; Mihaljevic *et al.*, 2007 and Habib *et al.*, 2010). However, in these studies other stresses such as pH, chilling, freezing and oxidative stress (Jasson *et al.*, 2007 and Habib *et al.*, 2010) and atmospheric oxidation and cell 'starving' (Mihaljevic *et al.*, 2007) were also tested. Jasson *et al.* (2007) tested a number of isolates from different sources such as, poultry, water, bovine and human, whereas all the isolates used by Habib *et al.* (2010) were of poultry origin, with multi-locus sequence types (STs) assigned previously (10 strains of ST-45 and 9 strains ST-21). In the later study, members of clonal complex, CC-21 were shown to survive heat and chill stress challenge models significantly better than those isolates belonging to the CC-45 suggesting that such resistance maybe associated with certain lineages (Habib *et al.*, 2010). The experiments of Jasson *et al.* (2007) were carried out using Bolton broth, whereas those of Habib *et al.* (2010) used Mueller Hinton Broth, after initial growth in Bolton broth. Mihaljevic *et al.* (2007) examined a single poultry and human clinical isolate with the bacterial suspension prepared in Preston broth and incubated for 9 hours.

Sorqvist (1989), using one poultry *C. jejuni* and two pig *C. coli* isolates and a number of techniques to perform heat challenge experiments in pre-heated physiological saline. For the 'test tube' method, 0.1ml of suspension was added to 2.9ml heated saline (60^{o}C for 1-10 minutes), whereas in the 'glass cup' method, 2ml of suspension was added to a larger volume, 58ml heated saline (one *C. coli* isolate: 58, 60 and 62^{o}C/2.5, 1.0 and 0.75 minutes respectively; one *C. coli* and one *C. jejuni* isolate: 56, 58 and 60^{o}C/4.0, 2.5 and 1.0 minutes respectively).

The effect of pre-chilling of *C. jejuni* prior to heat challenge has been considered (Hughes *et al.*, 2009; 2010), where a proportion of culture broth inoculum was kept at 6^{o}C for 24 hours prior to heat exposure. The effect of this additional pre-heat challenge was compared to the corresponding result for culture broth which had not been chilled, with the former only showing a marginal effect on heat

resistance. In Hughes *et al.* (2010), 200µl of *C. jejuni* culture broth (Nutrient broth 2, with *Campylobacter* growth supplement) was inoculated into 20ml of pre-heated media and this study examined a wide range of isolates (19 from a number of sources) at a single temperature (52^{o}C) with a subset of strains also tested at 55^{o}C and 60^{o}C for up to 10 minutes. Hughes *et al.* (2009) tested two isolates at 56^{o}C only, for up to 30 minutes with 5 minute sampling intervals, by inoculating 10µl of culture into 1ml of pre-heated NB2.

Within the literature there is much variation in the periods of time used for temperature exposure experiments, with variation in the number of sampling points used, with some studies investigating survival at the end of the time period and others during treatment. This strongly supports the need for a comprehensive study.

Moore and Madden (2000) tested a single *C. coli* isolate over a temperature range of 48-63^{o}C, for up to 6 minutes, with culture inoculum introduced into pre-heated PBS. An additional heating substrate of sterile 'scald' water, containing particulate solids, was also used in these experiments as a heating medium. The effect of culture age on heat sensitivity was examined by heat-treating culture broths, at 62^{o}C for 7 minutes. This work used cultures ranging in age from 2-45 hours.

Birk *et al.* (2004) tested two *C. jejuni* isolates at a relatively low challenge temperature (48^{o}C) for sampling times up to 150 minutes, with sampling at 30 minute intervals, and introduced an additional substrate of 'chicken rinse' juice to act as a challenge media for comparison with brain-heart infusion broth. Viability of these *C. jejuni* strains during low temperature exposure (5 and 10^{o}C/up to 30 days) was also examined.

Murphy *et al.* (2005) tested seven *C. jejuni* isolates, including three of poultry origin, using a number of different media (Brucella broth, *Campylobacter* enrichment broth, Mueller Hinton broth, Brain Heart Infusion broth and Tryptone soya broth with yeast extract) over a temperature range of 48-55^{o}C, with up to 10 minutes heat exposure. In addition, the responses to acid stress (pH 4.5) were determined in mid-exponential and stationary growth phase. Aerotolerance levels were also considered by incubating for 5 hours aerobically at 42^{o}C prior to subsequent acid challenge at pH 4.5.

Two isolates each of *C. jejuni* and *C. coli* were tested by Nguyen *et al.* (2006), over a temperature range of 50-60^{o}C and exposure time up to 40 minutes, by adding 5ml of cell suspension to 45ml of pre-heated heart infusion broth (HIB) and removing 1ml for serial dilution at regular intervals. An additional viability study, using a single strain of each *C. jejuni* and *C. coli*, heated a smaller sealed volume (15µl) of cell suspension at a rate of 10^{o}C/minute up to a maximum of 70^{o}C, followed by a decrease in temperature of 200^{o}C/minute to 20^{o}C.

Gill and Harris (1982) performed heat tolerance experiments on 12 *C. jejuni* isolates (6x human and 6x bovine/ovine). Cells in a 0.2ml cell suspension (10^8 approximately) were challenged in 1.8ml peptone/yeast broth between 50-70^{o}C for up to 3 minutes with samples being taken at 20 second intervals before cooling. This was part of a wider study which examined the growth/survival of *C. jejuni* over an extended time range in meat (pH range 5.8-6.8) at temperatures (25/37^{o}C) elevated from those of normal meat storage conditions, freezing conditions (-1 and -18^{o}C) and tolerance to lactic acid at 37^{o}C.

Survival of strains of *Campylobacter* spp. in artificially contaminated meat subjected to heat challenge: The number of studies, which investigated the survival of *Campylobacter* that had been inoculated onto meat and then subjected to heat challenge, was low and those considered also performed other experiments within their studies.

Blankenship and Craven (1982) used a five human strain *C. jejuni* composite, along with a further isolate used separately, to test survival within poultry meat exposed to temperatures of 49-57^{o}C for up to 20 minutes. Experimental contamination of meat was achieved by introducing 4 ml of *C. jejuni* cell suspension into 40g of autoclaved ground chicken breast, followed by stomaching for 5 minutes and division into 2g samples for experimental challenge. In addition, the composite and single strain of *C. jejuni* was also subjected to the same range of heat challenge by introduction of cells into pre-heated peptone broth. Survival of *C. jejuni* after inoculation to the surface of autoclaved meat was examined over a range of temperatures (4-43^{o}C) under ambient conditions, along with survival on raw chicken by inoculating it with a nalidixic acid resistant *C. jejuni* isolate.

Bergsma *et al.* (2007) and Sampers *et al.* (2010) inoculated chicken meat and chicken burger meat respectively with culture inoculum of *C. jejuni* followed by an overnight incubation at 4^{o}C prior to heat challenge. Bergsma *et al.* (2007) used a five *C. jejuni* isolate cocktail, whereas Sampers *et al.* (2010) only used a single *C. jejuni* isolate of poultry origin, with both studies using frying as their challenge method. In addition, Sampers *et al.* (2010) also performed their heat challenge on naturally contaminated burgers. Including searing time, Bergsma *et al.* (2007) challenged for up to 15 minutes, recording a surface mean temperature of 127(+/-18)^{o}C for whole fillets, with Sampers *et al.* (2010) heating for up to 9.5 minutes, recording an internal maximum temperature of 80^{o}C. Following heat challenge, in both studies, the cooked meat was homogenised by stomaching prior to enumeration of *C. jejuni*. Bergsma *et al.* (2007) also included a consumer survey and Sampers *et al.* (2010) also investigated the effect of refrigeration, freezing and salt concentration on *C. jejuni* in naturally contaminated meat.

Survival of strains of *Campylobacter* spp. in culture and artificially contaminated meat subjected to other stress challenges

Other experimental work with non-heat challenge conditions has been considered due to the variation in recovery and enumeration of *Campylobacter* spp. and is listed in table format. The commonest classification was for the effect of chilling on meat artificially contaminated with *Campylobacter* spp. (9 papers), with seven of these also detailing the effect of freezing on recovery.

A further two papers reported experiments of the effect of chilling on *C. jejuni* under culture conditions. Chan *et al* (2001) investigated the effect of chilling and freezing on *C. jejuni* in culture broth and 'chicken rinse' juice, whereas Garenaux *et al.* (2008) examined the combined effect of oxidative stress and reduced temperature, in addition to oxidative stress caused by addition of paraquat to culture broth at 25 and 42^{o}C for up to 7 days. Both studies used a range of *C. jejuni* isolates with Chan *et al.* (2001) using a total of 19 *C. jejuni* strains and Garenaux *et al.* (2008) using 13.

Rajkovic *et al.* (2010) investigated the combined effect of acid decontamination of *C. jejuni* on meat with additional oxidative stress, as a result packaging conditions, using a single bovine *C. jejuni* isolate.

Chaveerach *et al.* (2003), using seven *C. jejuni* and three *C. coli* poultry isolates, tested for survival after the addition of culture broth to Mueller Hinton broth adjusted to pH 4 with formic acid, with a further incubation up to 4 hours. Smigic *et al.* (2009 and 2010) also investigated the effect of pH on *C. jejuni* survival in culture conditions with the earlier study using exposure to lactic acid and HCl as a control, both at pH 4, prior to recovery. Smigic *et al.* (2010) also reported the recovery of *C. jejuni* in culture after exposure to lactic acid under different atmospheres, including a further oxidative stress in the presence of air or a modified high oxygen atmosphere during recovery on agar.

Table 1: Combinations of search terms used to identify relevant articles for *Campylobacter*

Unique Combinations			Number Screened	Number Retained	Cross Validation
Campylobacter	Poultry	Survival	142	28	28
Campylobacter	Temperature	Survival	142	35	14
Campylobacter	Survival	Stress	54	12	1
Campylobacter	Resistance	Heat	44	11	4
Campylobacter	Temperature	Stress	42	14	3
Campylobacter	Recovery		336	6	1
Additional Studies			4	4	4

Table 2.1. *Campylobacter* exposed to oxidative stress and recovery parameters

Author	**Year**	**Enumeration Media Type**	**Time Min. (H)**	**Time Max. (H)**	**Temp °C**	**Selective**	**Non-selective**
Rajkovic *et al.*	2010	mCCDA	48	48	42	Yes	No
Garenaux *et al.*	2008	CAB	48	48	42	No	Yes

Table 2.2. *Campylobacter* exposed to high temperature and recovery parameters

Author	Year	Enumeration Type	Time Min. (H)	Time Max. (H)	Temp °C	Selective	Non-selective
Bergsma *et al.*	2007	KA	72	72	37	Yes	No
Bergsma *et al.*	2007	PB/KA	72	72	37	Yes	No
Birk *et al.*	2004	B2A	48	96	37	No	Yes
Blankenship and Craven	1982	BA/FBP	48	72	37	No	Yes
Blankenship and Craven	1982	BA/FBP(+nal acid)	48	72	37	Yes	No
Gill and Harris	1982	CSAB	Not indicated	Not indicated	43	Yes	No
Habib *et al.*	2010	mCCDA/MHA	Not indicated	Not indicated	Not indicated	Yes	Yes
Hughes *et al.*	2009	CAB	48	48	48	No	Yes
Hughes *et al.*	2010	CAB	48	48	37	No	Yes
Jasson *et al.*	2007	mCCDA/CAB	48	48	37	Yes	Yes
Kelly *et al.*	2001	MHA/FBP	48	48	39	No	Yes
Klancnik *et al.*	2006	CCDA	24	48	42	Yes	No
Martinez-Rodriguez *et al.*	2004	MHA/FBP	48	48	39	No	Yes
Mihaljevic *et al.*	2007	KA	24	48	42	Yes	No
Moore and Madden	2000	TSAYE	Not indicated	Not indicated	Not indicated	No	Yes
Murphy *et al.*	2005	CSAB	48	48	42	Yes	No
Nguyen *et*	2006	mCCDA	48	48	42	Yes	No

al.							
Sagarzazu *et al.*	2010	TSAYE	96	96	37	No	Yes
Sampers *et al.*	2010	mCCDA	48	48	41.5	Yes	No
Sorqvist	1989	BB2A	48	48	42	No	Yes

Table 2.3. Exposure of *Campylobacter* to pre-chilling and/or freezing and recovery parameters.

Author	**Year**	**Enumeration Type**	**Time Min. (Hours)**	**Time Max. (Hours)**	**Temp ^{o}C**	**Selective**	**Non-selective**
Bhaduri and Cottrell	2004	TSA/mCCDA	48	48	42	Yes	Yes
Chan *et al.*	2001	MHA	40	40	42	No	Yes
Davis and Connor	2007	CC	48	48	42	Yes	No
Duffy and Dykes	2006	mCCDA	Not indicated	Not indicated	37	Yes	No
Eideh and Al-Qadiri	2011	PA	48	48	42	No	Yes
El-Shibiny *et al.*	2009	mCCDA	24	48	42	Yes	No
Garenaux *et al.*	2008	CAB	48	48	42	No	Yes
Gonzalez *et al.*	2009	mCCDA	48	48	37	Yes	No
Hanel and Atanassova	2007	CCDA/KA	48	48	42	Yes	No
Oyarzabal *et al.*	2010	mCCDA	48	48	42	Yes	No

Table 2.4. Exposure of *Campylobacter* to changes in pH and temperature and recovery parameters

Author	**Year**	**Enumeration Type**	**Time Min. (Hours)**	**Time Max. Hours)**	**Temp ^{o}C**	**Selective**	**Non-selective**
Curtis *et al.*	1995	TSA	72	72	30	No	Yes
Curtis *et al.*	1995	CCDA	72	72	37	Yes	No
Curtis *et al.*	1995	CCDA(+strep and nal acid)	72	120	37	Yes	No
Smigic *et al.*	2010	CAB/mCCDA	48	48	37	Yes	Yes
Smigic *et al.*	2009	CAB	48	48	37	No	Yes
Chaveerach *et al.*	2003	CAB	72	72	37	No	Yes
Chaveerach *et al.*	2003	CCDA	48	48	37	Yes	No
Rajkovic *et al.*	2010	CAB	48	48	37	No	Yes

Table 3.1. Description and key of enumeration/recovery protocols used during all experimental approaches.

Description	Acronym
Blood Base II Agar	BB2A
Brucella Agar-FBP	BA/FBP
Columbia Agar Base (5% defibrinated blood)	CAB
Campylobacter-Cefex (with Cefoperazone)	CC
Charcoal Cefoperazone Deoxycholate Agar	CCDA
Campylobacter Enrichment Broth	CEB
Campylobacter Selective Agar Base	CSAB
Ferrous Sulphate, Sodium Meta-bisulphite, Sodium Pyruvate (Campylobacter Growth Supplement)	FBP(CGS)
Kalmali Agar	KA
Modified Charcoal Cefoperazone Deoxycholate Agar (Cefoperazone/Amphotericin)	mCCDA
Mueller Hinton Agar	MHA
Preston Agar	PA
Preston Broth	PB
Tryptone Soya Agar	TSA

3.2. Enumeration Media

The variability in enumeration media for all experimental types is synthesised in Table 3. In all, 25 different types of enumeration media have been used to explore aspects of *Campylobacter* survival under differing bio-physical challenges.

Two unique combinations of enumeration media were found in studies examining the survival of *Campylobacter* following oxidative stress (Table 2.1). However, 13 different combinations of enumeration media were used to determine the resistance of *Campylobacter* to extremes of temperature (Table 2.2). Similar degrees of variability in the use of enumeration media was found in each remaining experimental types; studies examining the survival of *Campylobacter* to high temperature following Pre-chilling (Table 2.3) were found to have used eight combinations of enumeration media while studies examining the effects of pH (Table 2.4) used four combinations.

3.3. Incubation temperature for enumeration plates

A high degree of variability was also found in temperature profiles for recovering *Campylobacter* following challenges to extremes of temperature. In all studies, five unique temperature profiles were used. There was greater commonality between studies examining the effect of oxidative stress on *Campylobacter*. Each study used an identical incubation temperature of 42^0C (Table 2.1). By contrast, studies examining the effect of extremes of temperature on survival of *Campylobacter* used six different temperatures for the incubation of enumeration plates; 30, 37, 39, 41.5, 42 and 43^0C (Table 2.2). In addition, Moore and Madden (2000) and Habib *et al.* (2010) failed to clarify the temperature ranges used during their studies. Studies that examined the effects of pre-chilling were seen to use two different temperature profiles, namely 37 and 42^0C (Table 2.3). Similarly, studies examining the effect of pH used two incubation temperatures of 30 and 37^0C (Table2.4).

3.4. Enumeration and Recovery Time

Studies used a unique combination of nine temporal profiles while examining impacts of oxidative stress, temperature, pH, and pre-chilling on the survival of *Campylobacter*. As in the case of enumeration temperature, studies examining the effect of oxidative stress were found to use a uniform temporal range of 48 hours (Table 2.1) following the challenge/treatment. Studies that examined *Campylobacter* survival in extremes of temperature were seen to be more variable. Here, six unique temporal profiles were found, while one study failed to report the duration of incubation of samples to specific temperatures for recovery of cultures (Gill and Harris, 1982) (Table 2.2). In most instances, temporal profiles were held constant between 48-96 hours. However, several studies used variable incubation times in their experimental designs; Birk *et al.* (2004) 48-96 hours, Blankenship and Craven (1982) 48-72 hours and Klancnik *et al.* (2006) and Mihaljevic *et al.* (2007) 24-48 hours. A similar pattern for incubation time was found for studies where *Campylobacter* were subjected to pre-chilling; three different temporal profiles were found, while one study failed to note the duration of incubation of samples (Duffy and Dykes, 2006) (Table 2.3). Only one study varied the temporal profile, where El-Shibiny *et al.* (2009) incubated cultures from 24-48 hours. The remaining studies incubated plates for 40 or 48 hours. Studies designed to determine the effects of pH on the survival of *Campylobacter* showed similar degrees of variability where four different temporal profiles were used for recovery. One study varied incubation time to increase the likelihood of recovery; Curtis *et al.* (1995) 72-96 hours and 72-120 hours. The remaining studies used the same time period for incubation of plates for recovery (Table 2.4.).

3.5. Selection for Sub-lethal Effects

Studies also included both selective and non-selective media to allow for the recovery of sub-lethally damaged *Campylobacter* cells. However, not all studies were comprehensive in examining the effects of both selective and non-selective media on the survival of *Campylobacter*. One study determined whether they could recover sub-lethally damaged cells when determining the impacts of oxidative stress on survival of *Campylobacter* (Garenaux *et al.,* 2008). Seven studies (Blankenship and Craven, 1982; Sorqvist 1989; Kelly *et al.,* 2001; Birk *et al.,* 2004; Martinez-Rodriguez *et al.,* 2004; Jasson *et al.,* 2007 and Hughes *et al.,* 2009) examining the effects of extremes of temperature on the survival of *Campylobacter* also used non-selective media to recover sub-lethally injured cells (Table 2.2.). Similarly, five studies incorporated non-selective media while examining the effects of pre-chilling on the survival of *Campylobacter* (Chan *et al.,* 2001; Bhaduri and Cottrell, 2004; Duffy and Dykes, 2006; Garenaux *et al.,* 2008 and Eideh and Al-Qadiri, 2011) (Table 2.3.). Finally, four studies used non-selective media while examining the effects of pH on the survival of *Campylobacter* (Curtis *et al.,* 1995; Chaveerach *et al.,* 2003; Rajkovic *et al.,* 2009; Smigic *et al.,* 2009 and Smigic *et al.,* 2010) (Table 2.4.).

3.6. Decimal Reduction Time (D-Values) and Thermal Death Time

Where appropriate, Decimal Reduction Times (D-Values) were extracted from studies. In circumstances where the D-Values are not explicitly calculated, values were estimated from information provided within the text. Nevertheless, in some studies, data were not available and values could not be calculated. D-values were obtained for 11 of the 17 (Tables 3.2a – 3.2l) studies examining the effects of high temperature on the survival of *Campylobacter* (Table 2.2). Studies varied according to the strains used and only two used an identical stain (Birk *et al.,* 2004 and Hughes *et al.,* 2009). However, the ranges of temperatures used during these studies differed, and as such, direct comparison of the survival of this particular strain is not possible. Nevertheless, we are able interpret the variation in the survival of different strains of *Campylobacter* from a qualitative perspective. Tables 3.2a – 3.2l present D-values obtained from 11 studies examining the effects of high temperature on *Campylobacter*. In all, D-values are provided for 19 different strains. Multiple D-values are provided for certain strains examined over a range of temperatures. However, this was observed in five studies; namely Blankenship and Craven (1982) (Table 3.2c); Gill and Harris (1982) (Table 3.2d); Moore and Madden (2000) (Table 3.2i); Nguyen *et al.* (2006) (Table 3.2j) and Sorqvist (1989) (Table 3.2l). The remaining studies provided D-values for only single temperatures. In addition, Hughes *et al.,* (2009, 2010) compared pre-chilled and non-chilled cells. By contrast, Sagarzazu *et al.* (2010) used pre-adapted cells in the range 40-45C prior to 55C challenge, while also

comparing stationary and exponential cells. Kelly *et al.,* (2001) also compared the effect of growth phase on D-value. Variability was also encountered in the substrate used during experimental simulations. Bergsma *et al.* (2007) (Table 3.2a) conducted simulations whereby the survival of a composite of *Campylobacter* strains was determined using two substrates (chicken fillet and diced chicken) at 127^{O}C. In each case D-values were found to be different. Birk *et al.* (2004) (Table 3.2b) used a similar approach and also found differences in D-value as a function of substrate.

Table 3.2a. Decimal Reduction Times for Bergsma *et al.* (2007).

Substrate	**Strain**	**Temperature (^{o}C)**	**D-value (minutes)**
Chicken Fillet	Composite 5 Strains	127.0	1.95
Diced Chicken	Composite 5 Strains	127.0	0.59

Table 3.2b. Decimal Reduction Times for Birk *et al.* (2004).

Substrate	**Strain**	**Temperature (^{o}C)**	**D-value (minutes)**
Chicken Juice	NCTC11168 [ST43-CC21]	48.0	120
BHI Medium	NCTC11168 [ST43-CC21]	48.0	115
Chicken Juice	81-176	48.0	80
BHI Medium	81-176	48.0	70

Table 3.2c. Decimal Reduction Times for Blankenship and Craven (1982).

Substrate	Strain	Temperature (°C)	D-value (minutes)
Chicken	H-840	49.0	20.5
Chicken	H-841	51.0	8.77
Chicken	H-842	53.0	4.85
Chicken	H-843	55.0	2.12
Chicken	H-844	57.0	0.79
Chicken	Composite	49.0	NA
Chicken	Composite	51.0	9.27
Chicken	Composite	53.0	4.89
Chicken	Composite	55.0	2.25
Chicken	Composite	57.0	0.98
Peptone	H-840	49.0	15.2
Peptone	H-841	51.0	4.9
Peptone	H-842	53.0	1.71
Peptone	H-843	55.0	0.64
Peptone	H-844	57.0	0.25
Peptone	Composite	49.0	14.9
Peptone	Composite	51.0	7.02
Peptone	Composite	53.0	2.7
Peptone	Composite	55.0	1.09
Peptone	Composite	57.0	NA
Chicken	H-840	49.0	20.5

Table 3.2d. Decimal Reduction Times for Gill and Harris[1] (1982).

Substrate	Strain	Temperature (°C)	D-Value (minutes)
Peptone yeast extract	Animal Strain 6	50.0	2.2
Peptone yeast extract	Animal Strain 6	55.0	0.695
Peptone yeast extract	Animal Strain 6	60.0	0.345
Peptone yeast extract	Animal Strain 6	65.0	0.22
Peptone yeast extract	Animal Strain 6	70.0	0.185

Table 3.2e. Decimal Reduction Times for Gill and Harris[2] (1982).

Substrate	Strain	Temperature (°C)	D-Value (minutes)
Peptone yeast extract	Animal Strain 1	60.0	0.362
Peptone yeast extract	Animal Strain 2	60.0	0.385
Peptone yeast extract	Animal Strain 3	60.0	0.322
Peptone yeast extract	Animal Strain 4	60.0	0.263
Peptone yeast extract	Animal Strain 5	60.0	0.71
Peptone yeast extract	Animal Strain 6	60.0	0.345
Peptone yeast extract	Human Strain 1	60.0	0.35
Peptone yeast extract	Human Strain 2	60.0	0.952
Peptone yeast extract	Human Strain 3	60.0	0.477
Peptone yeast extract	Human Strain 4	60.0	0.455
Peptone yeast extract	Human Strain 5	60.0	0.645
Peptone yeast extract	Human Strain 6	60.0	0.4

Table 3.2f. Decimal Reduction Times for Hughes *et al.* (2009).

Substrate	Strain	Temperature (°C)	D-Value (minutes)
NB2(inc. CGS)	2097E48 [ST21-CC21]	56.0	4.50
NB2(inc. CGS)	11168 [ST43-CC21]	56.0	4.00

Table 3.2g. Decimal reduction times for Hughes et al (2010)

Substrate	Strain	Temperature (°C)	D-Value (minutes)
NB2(inc. CGS)	2293	52.0	5.20
NB2(inc. CGS)	2344D/3 [ST45-CC45]	52.0	1.20
NB2(inc. CGS)	M1 [ST137-CC45]	52.0	1.12

Table 3.2h. Decimal Reduction Times for Kelly *et al.* (2001)

Substrate	Strain	Temperature (°C)	D-Value (minutes)
Brucella broth(FBP)	NCTC 11351 (Exponential)	50.0	50.0
Brucella broth(FBP)	NCTC 11352 (Early Stationary)	50.0	50.0
Brucella broth(FBP)	NCTC 11353 (Late Stationary)	50.0	34.0

Table 3.2i. Decimal Reduction Times for Moore and Madden (2000).

Substrate	Strain	Temperature (°C)	D-Value (minutes)
PBS	*C.coli* N139	49.9	6.35
PBS	*C.coli* N139	53.8	3.42
PBS	*C.coli* N139	55.4	1.483
PBS	*C.coli* N139	56.6	0.702
PBS	*C.coli* N139	56.7	0.79
PBS	*C.coli* N139	57.2	0.53
PBS	*C.coli* N139	59.7	0.26
PBS	*C.coli* N139	60.0	0.365
PBS	*C.coli* N139	62.1	0.173
PBS	*C.coli* N139	62.5	0.095

Table 3.2j. Decimal Reduction Times for Nguyen *et al.* (2006).

Substrate	Strain	Temperature (°C)	D-Value (minutes)
Heart infusion broth (FBP)	*C. jejuni* AR6	50.0	36.00
Heart infusion broth (FBP)	*C. jejuni* AR6	55.0	5.30
Heart infusion broth (FBP)	*C. jejuni* AR6	60.0	0.70
Heart infusion broth (FBP)	*C. jejuni* L51	50.	39.00
Heart infusion broth (FBP)	*C. jejuni* L51	55.0	4.60
Heart infusion broth (FBP)	*C. jejuni* L51	60.0	0.80
Heart infusion broth (FBP)	*C. coli* DR4	50.0	60.00
Heart infusion broth (FBP)	*C. coli* DR4	55.0	6.60
Heart infusion broth (FBP)	*C. coli* DR4	60.0	0.90
Heart infusion broth (FBP)	*C. coli* L6	50.0	51.00
Heart infusion broth (FBP)	*C. coli* L6	55.0	6.60
Heart infusion broth (FBP)	*C. coli* L6	60.0	1.40

Table 3.2k. Decimal Reduction Times for Sagarzazu *et al.* (2010)

Substrate	Strain	Temperature (°C)	D-Value (minutes)
Tryptone soya broth	NCTC 11351 (adapted 40°C)	55.0	3.30
Tryptone soya broth	NCTC 11351 (adapted 42°C)	55.0	4.00
Tryptone soya broth	NCTC 11351 (adapted 45°C)	55.0	5.00

Table 3.2l. Decimal Reduction Times for Sorqvist (1989)

Substrate	Strain	Temperature (°C)	D-Value (minutes)
Physiological saline	*C. coli* C59	58.0	0.42
Physiological saline	*C. coli* C59	60.0	0.13
Physiological saline	*C. coli* C59	62.0	0.07

Z-values were calculated for each of the three studies that generated multiple D-values for strains used during experimental simulation. These are shown in Table 3.2m.

Table 3.2m. Z-values calculated from three studies where multiple D-values were provided.

Author	Substrate	Strain	Z-value (^{O}C)
Blankenship and Craven	Chicken	H-840	5.8057
Blankenship and Craven	Chicken	Composite	6.1262
Blankenship and Craven	Peptone	H-840	4.4925
Blankenship and Craven	Peptone	Composite	5.2325
Gill and Harris	Peptone	Animal Strain 6	18.8675
Moore and Madden	PBS	*C. coli* N139	6.9601

Analysis of D-values

D-values and characteristics associated with individual studies (Table 3.2a – 3.2l) were analysed in order to explore the likelihood that D-values for *Campylobacter* may be sensitive to the underlying experimental design. Data were unbalanced insofar as D-values were not recorded for an identical range of temperature profiles for each study. Following the methodology of Pinheiro and Bates (2000), we used Generalized Least Squares (GLS) modelling approach to determine the sensitivity D-values to temperature while controlling for differences between studies according to the type of laboratory media used. Generalised Least Squares is a robust regression technique that is known to produce reliable estimates from unbalanced data (Pinheiro and Bates, 2000).

Results

Findings shown in Table 3.2n illustrate D-values decrease significantly with an increase in temperature (-21.073, P-value = 0.000). In addition, differences in D-values between laboratory medium were assessed by comparing Blood Base II Agar (B2A) (reference level) to types other media. Higher D-values for studies using laboratory media BA/FBP/NaCl, CSAB, mCCDA, MHA/FBP and TSAYE were found to be statistically significant (Table 3.2n). By contrast, studies using laboratory media BA/FBP and CAB recorded lower d-values. However, only those studies using BA/FBP were considered to statistically significant (-0.607, P-value = 0.000). Furthermore, studies using selective media were found to generate lower D-values than those studies that did not select against sub-lethally damaged cells (-0.614, P-value = 0.000). The statistical model was also assessed for its goodness of fit. Here, we used the method described by Naglekerk (1991) to obtain an $R^2 = 0.96$. This

indicates that the model has an exceptionally high goodness of fit. However, these findings should be interpreted with caution. Experimental characteristics such as *Campylobacter* species and isolates employed and food substrate were found to be linearly related to type of laboratory media. These variables produced a confounding effect and could not be included as explanatory variables in a formal statistical analysis.

Conclusion

In summary, and taking in to consideration that constraints described above, this analysis indicates that decimal reduction times for Campylobacter may vary significantly between laboratory media.

Table 3.2n. Table results from statistical analysis using generalised least-squares illustrating significance of temperature and differences in D-values between laboratory media types.

Coefficients:	**Value**	**Standard Error**	**T-value**	**P-value**
(Intercept)	37.276	0.852	43.728	0.000
Log Temperature	-21.073	0.493	-42.733	0.000
BA/FBP	-0.607	0.087	-6.954	0.000
BA/FBP/nacl	0.313	0.112	2.794	0.007
CAB	-0.423	0.287	-1.476	0.145
CSAB	2.219	0.149	14.915	0.000
mCCDA	0.780	0.113	6.887	0.000
MHA/FBP	0.170	0.091	1.866	0.067
TSAYE	1.364	0.085	16.015	0.000
Selective Media: Yes	-0.614	0.132	-4.655	0.000

3.7. Multivariate Analysis

In a recent review of the use of multivariate techniques, Ramette (2007) shows that multivariate data may be represented in a variety of forms; namely binary, ordinal and qualitative information, and also quantitative data such as a counts, measurements and frequencies. Data sets may also contain a mixture of different forms of these data (Legendre and Legendre 1998). For instance, Olapade *et al.* (2005) examined the microbial communities of streams using data that comprised qualitative descriptors of location and environment and quantitative measurements of water quality. A similar approach was adopted by Yannarell & Triplett (2005) to describe bacterial community composition in lakes and Edwards *et al.* (2006) also used quantitative and frequency data to describe variation in microbial community in soils. Kuramae *et al.* (2011, 2012) used a combined approach of Ward's method of Hierarchical cluster analysis and Canonical

Correspondence Analysis (CCA) to analyse variability in soil communities according to land-use types and environmental explanatory variables. Here, we adopt the analytical approach of Kuramae *et al.* in order to examine potential areas of similarity between experimental studies.

3.7.1. Hierarchical Cluster analysis

We used hierarchical cluster analysis utilizing Ward's method of minimum variance (Ward, 1963) in an attempt to categorize studies according to their experimental design; namely Media used, Time, Temperature (Minimum and Maximum) and Selective and Non-selective Media. All analyses were undertaken using the R-Package for Statistical Computing 2.14.1 (R Development Core Team, 2012). Findings are illustrated in the combined format of a dendrogram and adjacent data table. Each dendrogram illustrates the degree of clustering according to the characteristics of each study design (Figure 1-3). The dendrogram groups those studies that are similar in design.

The adjacent data table illustrates which characteristics of experimental design best describe similarity between studies. The data table is interpreted by cross-referencing characteristics associated with experimental design with each study, or groups of studies. The type of enumeration media used and whether the media were selective or non-selective is described categorically, where 1 or 0 determines the presence or absence of that characteristic, respectively. Categorical values are represented within data tables black and white squares, where black squares indicate the presence of that characteristic and the white squares the absence of that characteristic. For instance, we can determine that the study by Gill and Harris (1982) used the enumeration media type CSAB (Figure 1). This is signified by a large black square. By contrast, Habib *et al.* (2010) used the enumeration media type mCCDA/MHA. Furthermore, it is also determined that each study used selective media, as denoted by black squares. In addition, it can also be seen that Habib *et al.* (2010) used non-selective.

The representation of continuous variables within the data table differs in contrast to that of categorical data. Here, time of exposure and minimum and maximum temperature were recorded as continuous variables. The size and colour of each square is determined by the range of values observed for that particular variable. For instance, the minimum time of exposure (TimeMin) used during studies was observed to vary between 24 – 96 hours (Table 2.2). Thus, studies that used minimum values at the lower end of temporal spectrum are represented by large white squares, whereas studies with minimum values towards the higher end of temporal range are represented by larger black squares. In this way it is possible to observe similarities and differences between studies according to minimum and maximum time of exposure. For example, studies by Birk *et al.* (2004) Blankenship and Craven (1982) Bergesma *et al.* (2007) and used an identical minimum time of exposure (48 hours). By contrast, the maximum time of exposure varied between 48 – 96 hours

(Table 2.2) Birk *et al.* (2004) and Sagarzazu *et al.* (2010) used a maximum time of 96 hours whereas Blankenship and Craven and Bergesma *et al.* used a maximum time of 72 hours. All remaining studies applied minimum and maximum times between 24 and 48 hours. The correspondence between these studies can judged according to the similarity in the size of the squares.

From a general perspective, findings illustrated by each dendrogram suggest a lack of standardization between studies in terms of the enumeration media used (Figure 1 – 3). As such, there is little evidence in support of generality in approach between studies. However, fine-scale similarities can be seen between studies according to the temperature profiles, time of exposure and the use of selective and non-selective media used. For example, studies examining the effect of pH on the survival of *Campylobacter* can be grouped according to the range minimum and maximum time of exposure (Figure 2); Curtis *et al.* (1995, 1995, 1995) and Chaveerach *et al.* (2003) set minimum time of exposure from 72 – 120 hours (Table 2.4) whereas Chaveerach *et al.* (2003), Smigic *et al.* (2009, 2010) and Rajkovic *et al.* (2010) used an absolute time scale of 48 hours (Table 2.4). Studies examining the effects of pre-chilling can also be grouped according similarities in time and temperature. Duffy and Dykes (2006) and Gonzalez *et al.* (2008) each examined survival of *Campylobacter* at 37^{O}C (indicated by the white squares) (Figure 3). By contrast, remaining studies all examined survival of *Campylobacter* at 42^{O}C (Table 2.3).

Cluster analysis was not undertaken for studies examining the survival of *Campylobacter* following oxidative stress due to the small number of data sources available (Table 2.1).

3.7.2. Canonical Correspondence Analysis:

We used a constrained ordination technique to test the relationship between recovery media used in different studies and other characteristics associated with the experimental design of studies. Consequently, constrained ordination acts as a multiple regression (Borcard *et al.,* 2011) between two data matrices in order to explore potential areas of similarity between studies. Canonical Correspondence Analysis (CCA) (ter Braak, 1986, 1987; ter Braak and Prentice, 1988) was chosen as the means to investigate these relationships as the technique is able to incorporate data of mixed form, such as the presence/absence of a characteristic (use of a particular media), and relate this information to a suite of quantitative (Year, Time, Temperature) and binary explanatory variables (use of selective or non-selective media). The relationship between response and explanatory variables are determined graphically and numerically. Graphical interpretation is undertaken by means of a bi-plot. A bi-plot presents the response variable (media type) the explanatory variable (Year, Time, Temperature, Selective/Non-selective) within the same plotting region. The position of each response variable is interpreted relative to the position of each explanatory variable. Thus, it

may be possible to associate a media type with a particular experimental characteristic. Furthermore, it may also be possible to establish which explanatory variables are responsible for determining the structure among experimental studies. Numerical interpretation is facilitated by examining the variability explained. In the case of CCA, the proportion of variability explained by the explanatory variables is expressed as the Inertia (or the mean of Pearson's Contingency Co-efficient X^2) and is calculated as the sum cumulative proportion of inertia explained (Borcard *et al.,* 2011). This test statistic is analogous to the correlation coefficient (R^2) (Gauch, 1982). The proportion of inertia explained by the first two canonical axes for each constrained ordination is illustrated in Table 4.

Figure 4 shows the relationship between enumeration media and experimental characteristics used in studies exploring heat resistance in *Campylobacter*. The proportion of inertia explained is shown in Table 4. (Pearson's Contingency Co-efficient X^2 = 0.135). The explanatory variables Time (Minimum and Maximum) and Temperature are highly correlated with the first canonical axis (CCA1) and are associated with the study using media type TSAYE. By contrast, studies using selective and non-selective media are highly correlated with the second canonical axis (CCA2). It is also possible to differentiate between studies and their respective media types according to whether they have used selective or non-selective media. For instance, media types TSAYE, PB, BA/FBP/ncl and mCCDA are associated with studies where selective media have been used whereas studies using BA/FBP, MHA/FBP and CAB media types are associated with non-selective media. The majority of studies (excluding TSAYE and mCCDA/MHA) are centred on the origin of both canonical axes and this indicative of similarity between studies in terms of temperature profiles and time (minimum and maximum) of exposure used in experimental design. However, with the exception of media types CAB and KA, all others were used in studies on only one occasion; as such it is not possible to draw conclusions as to the generality of a particular study design.

A bi-plot illustrating the variability in experimental design found within studies exploring the impacts of pre exposure of *Campylobacter* to pH is shown in Figure 5. In contrast to studies focused solely on heat resistance, it is evident that time and temperature are negatively correlated to one another (CCA1) and where studies use a higher temperature it is at a reduced time scale. Similarly, during studies were the time of exposure is higher, temperatures are lower. The major drivers of variability within the second canonical axis (CCA2) are the use of selective and non-selective media where studies employing TSA and CAB media types are associated with non-selective media and CCDA/ncl and CCDA were used with selective media. The proportion of inertia explained is X^2 = 0.471 (Table 4). The higher inertia calculated for this analysis is, in part, due to media types being used repeatedly during the course of different studies; namely CAB and CCDA. Figure 6 shows the

relationship between enumeration media and experimental characteristics used in studies exploring resistance in *Campylobacter* due to pre-chilling. The cumulative proportion of inertia explained by the first two canonical axes is X^2 = 0.261 (Table 4). The major source of variability associated with both canonical axes (CCA1and CCA2) is that of selective and non-selective media. This slightly unusual finding is attributed to the fact that all studies, irrespective of media used, use virtually identical time and temperature profiles. As such, the use of selective media was the only means with which to analyse trends of variability between these studies. In summary, each multivariate bi-plot summarises the variability found between studies according to the pre-challenge and type of media used. Heterogeneity in experimental design is such that combinations of media type, time, temperature and selective/non-selective media are often found to be unique. Thus, a synthesis of experimental design with regard to identifying commonalities between studies is not possible. In order to minimise the potential impacts of heterogeneity, a degree of standardization with regard to the use of different enumeration media, temporal and temperature profiles must first be attained.

Table 4. Inertia (variability explained) by the first two canonical axes of each constrained ordination.

	Heat Resistance		pH		Pre-chill	
	CCA1	CCA2	CCA1	CCA2	CCA1	CCA2
Proportion Explained	0.070	0.064	0.235	0.235	0.167	0.094
Cumulative Proportion	0.070	0.135	0.235	0.470	0.167	0.261

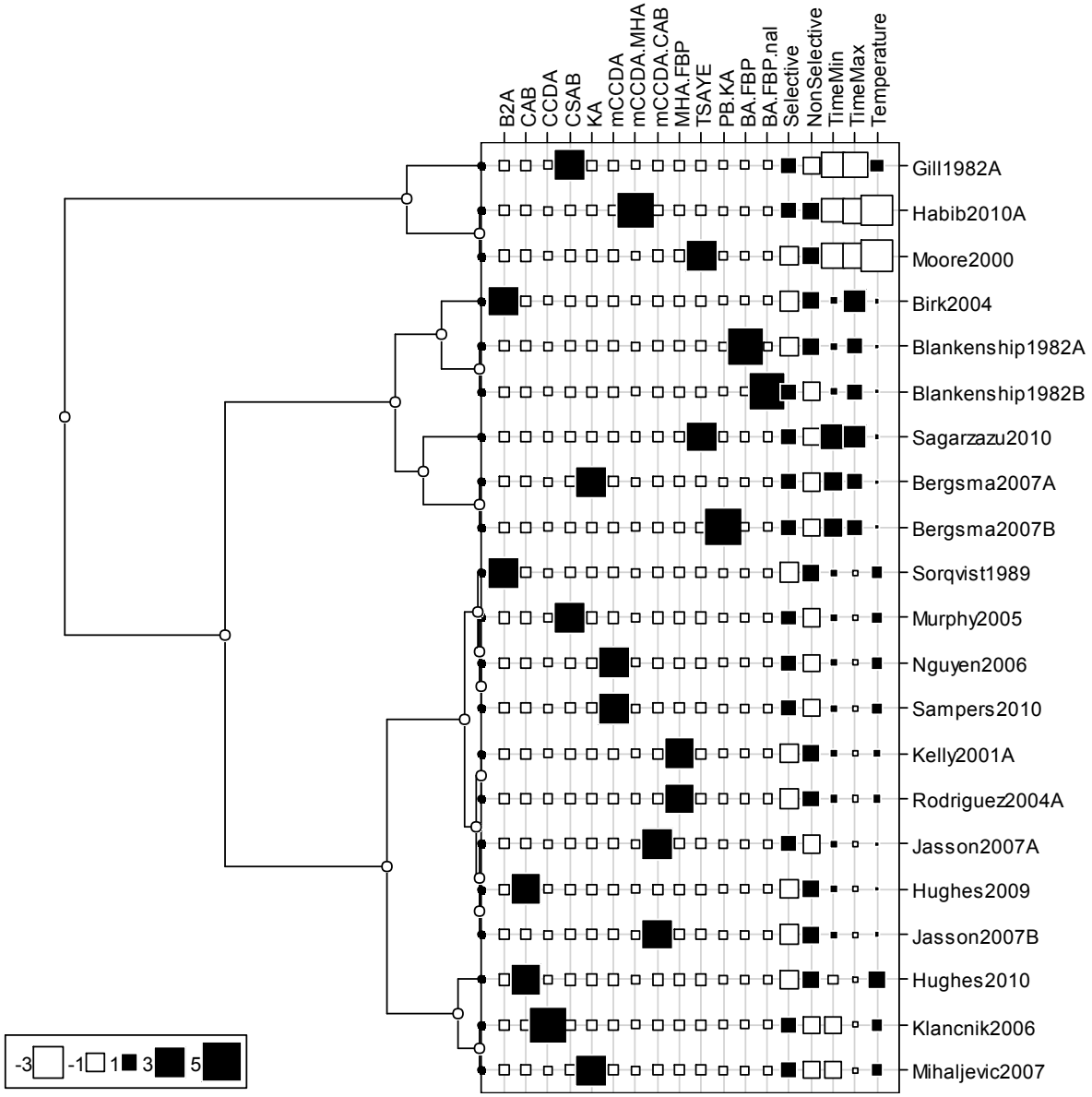

Figure 1. Hierarchical cluster analysis experimental design (for recovery/enumeration) in studies determining the resistance of *Campylobacter* to extremes of temperature.

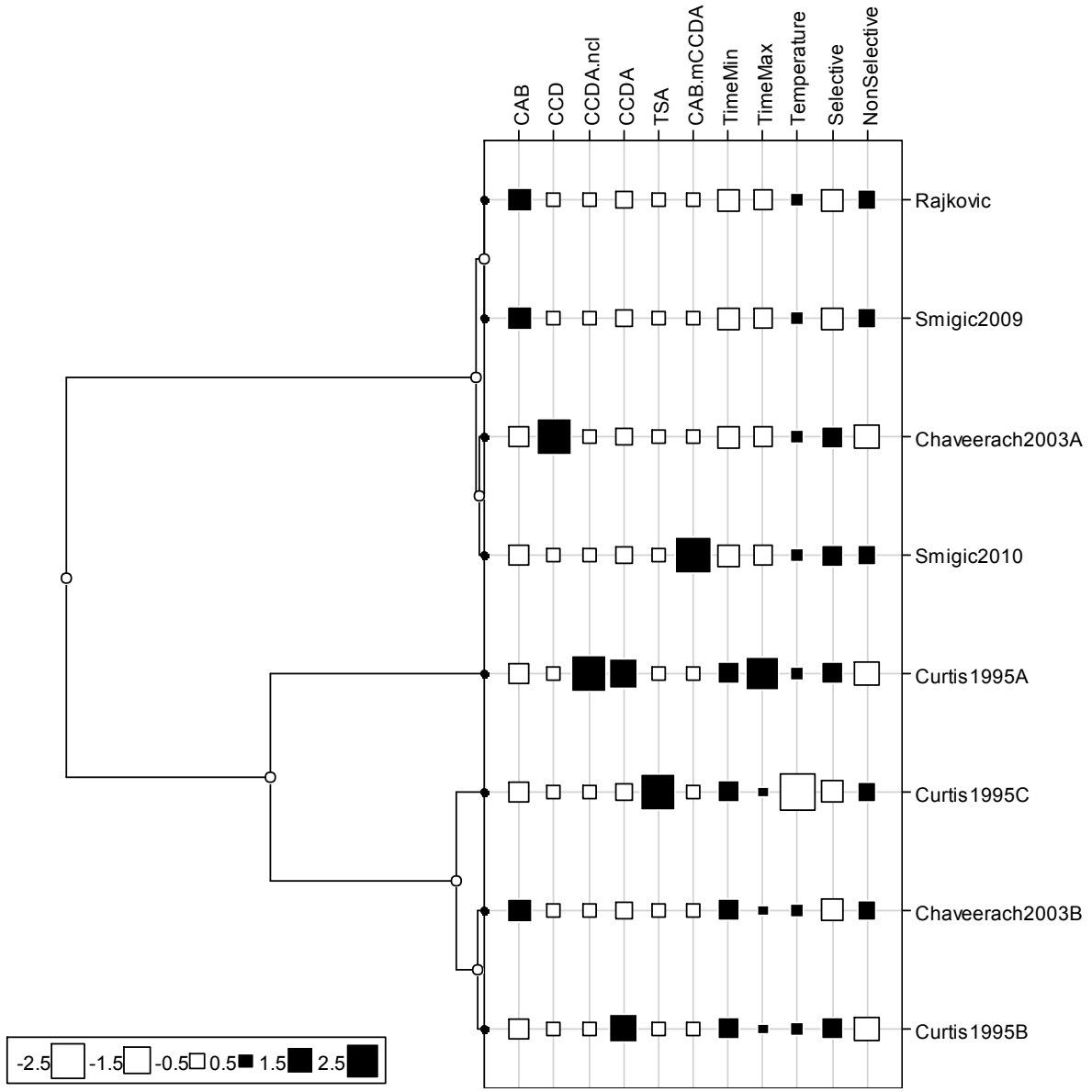

Figure 2. Hierarchical cluster analysis experimental design in studies (for recovery/enumeration) determining the resistance of *Campylobacter* to extremes of temperature following exposure to changes in pH.

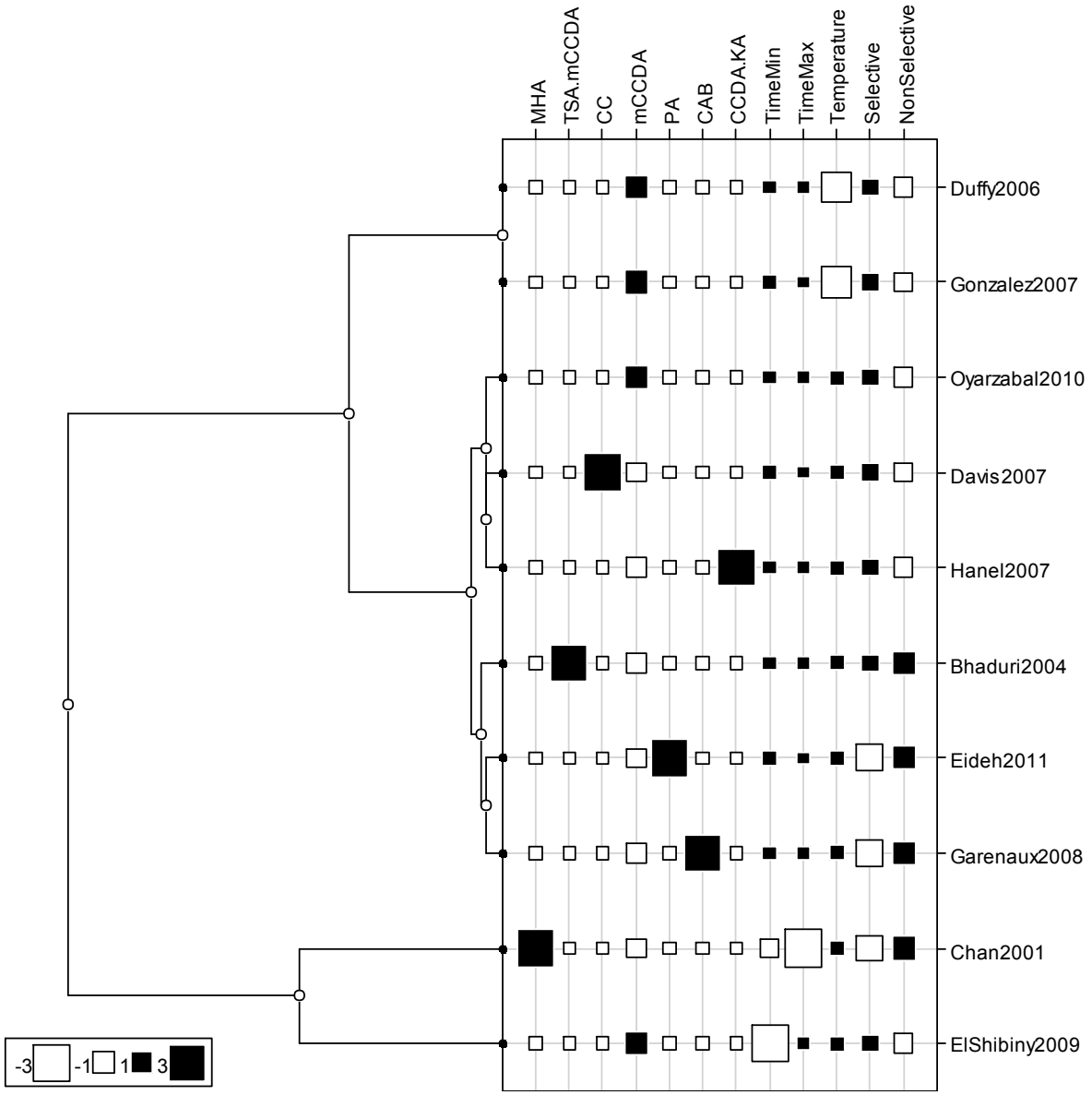

Figure 3. Hierarchical cluster analysis experimental design (for recovery/enumeration) in studies determining the resistance of *Campylobacter* to extremes of temperature following pre-chilling.

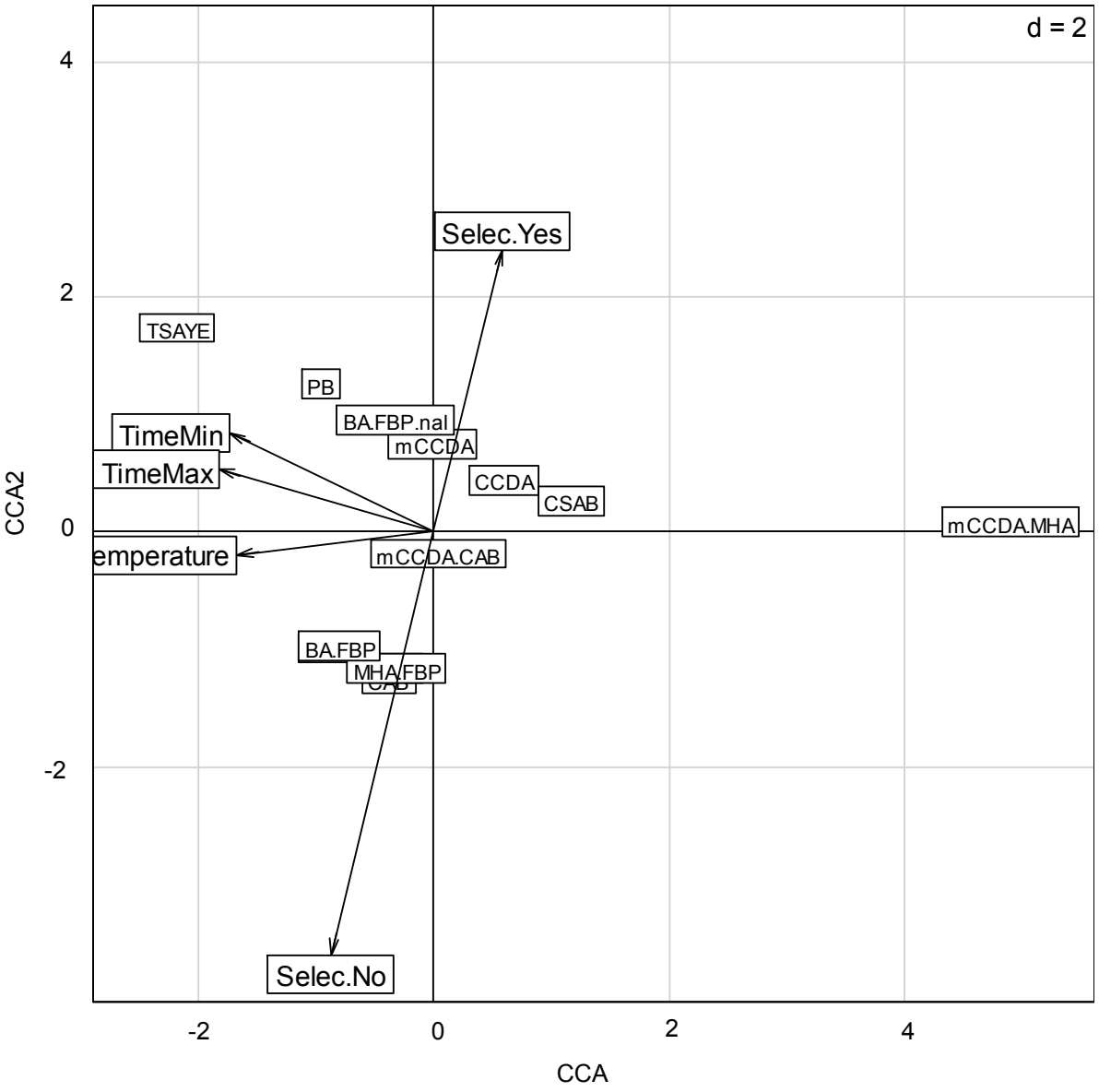

Figure 4. CCA scatter-plot illustrating the variability in experimental design (for recovery/enumeration) found within studies exploring heat resistance in *Campylobacter*. The proportion of the variation explained by the first and second canonical axes is expressed as Pearson's Contingency Co-efficient (X^2 = 0.135) (Table 4).

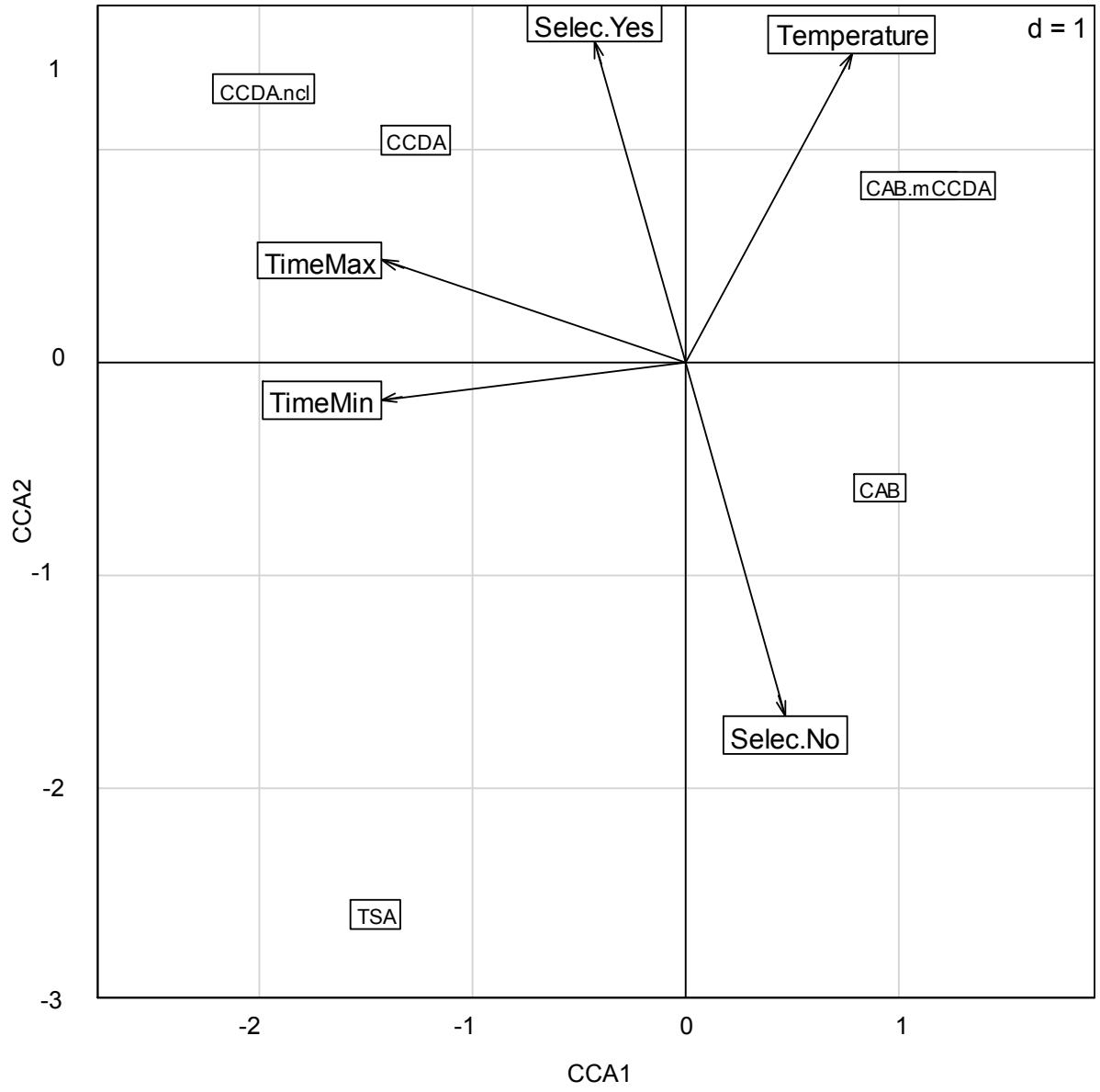

Figure 5. CCA scatter-plot illustrating the variability in experimental design (for recovery/enumeration) found within studies exploring the impacts of pre exposure of *Campylobacter* to pH. The proportion of the variation explained by the first and second canonical axes is expressed as Pearson's Contingency Co-efficient (X^2 = 0.47) (Table 4).

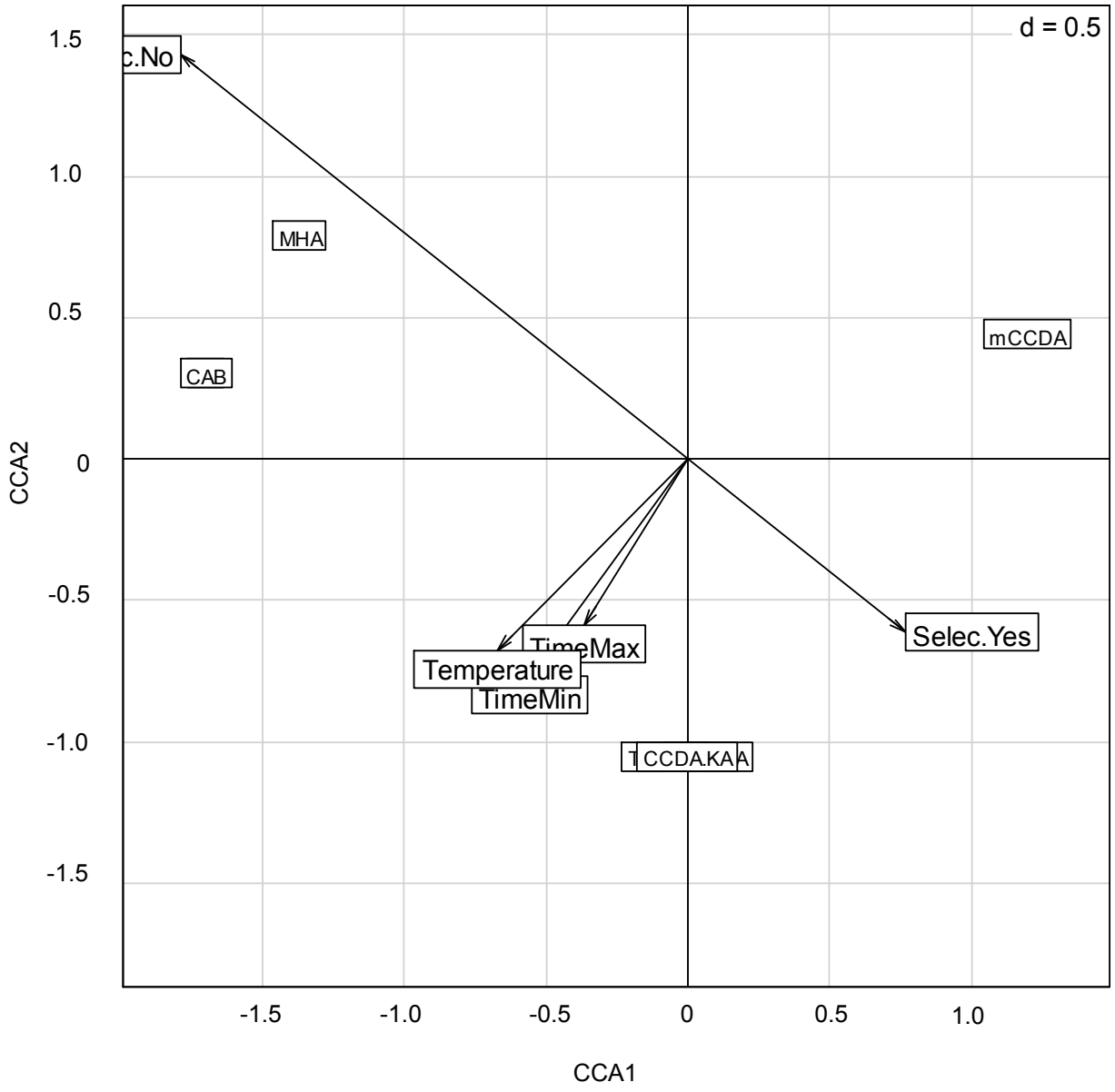

Figure 6. CCA scatter-plot illustrating the variability in experimental design (for recovery/enumeration) found within studies exploring the impact of pre-chilling on the survival of *Campylobacter*. The proportion of the variation explained by the first and second canonical axes is expressed as Pearson's Contingency Co-efficient (X^2 = 0.261) (Table 4).

Conclusions:

C. jejuni and *C. coli* are major zoonotic pathogens and there is a very strong public health need to control these bacteria in the food chain. One approach is to use potentially lethal processes like heat or freezing or chemical treatments to kill them. *Campylobacter* is believed to be sensitive to hostile environments, but clearly survives well in foods and many infection vehicles had been heated before consumption or subject to refrigeration. Industry requires tools and an evidence-based guide to best practice in order to control *Campylobacter* and predictive models can inform processors about food treatments to eliminate such pathogenic bacteria. However, data available in the literature rarely take account of interactions between *Campylobacter* and food and it has been shown that *Campylobacter* when attached to muscle has greater heat resistance (Blankenship and Craven 1982). For this review, only four published papers were found which examined the survival of *Campylobacter* in meat products and which took into account the fact that such bacteria will often be subjected to gradual heating during processing, rather than instantaneous exposure to heat. Whilst surface contamination of chicken is important in cross-contamination during catering, particularly when bacterial levels are high, models should also recognise the public health threat from contamination of internal tissues. It is also essential to know the extent to which *Campylobacter* may grow in naturally infected tissues (see below). As data are generated in this area, internal contamination of chicken muscle is proving to be much more common than previously thought (Humphrey 1991; Berndtson *et al.,* 1992; Scherer *et al.,* 2006; Luber and Bartlett 2007). In this environment, *Campylobacter* cells will be initially protected from heat exposure and may be able to synthesise heat-shock proteins (Parkhill *et al*., 2000), which could better protect them as temperatures approach potentially lethal levels.

In addition, of the studies which have been done in media and meat, many different strains have been used. However, studies investigating strains belonging to different genetic backgrounds and from different sources are few in number. There is a real need to recognise the population biology of *Campylobacter* in such studies to provide a more thorough assessment of the range of behaviours of both *C. jejuni* and *C. coli* when subjected to challenges relating to heat, pH or other stresses. Furthermore, many of the techniques used to recover *Campylobacter* in such studies are not sufficiently sensitive to recover sublethally damaged cells and therefore may underestimate the survival of cells, which may still be a public health threat (Mihaljevic *et al.,* 2007).

The large variation in experimental design of published challenge studies, including those used to detect and enumerate *Campylobacter* and which may impact on our ability of accurately predict the response of *Campylobacter* to changes in its physical and biochemical environments mean that It is currently not possible to generate such predictive models.

We propose a guide to best practice based on research undertaken by T.J.H.

1. That the viability of test strains pre-challenge is maintained and that storing these in aliquots at -80^{o}C will help ensure their stability. Furthermore strains grown for challenge studies should be minimally passaged following resuscitation. It is important to ensure that strains do not become lab-adapted or are affected by storage at 4^{o}C prior to an experiment, unless this is part of the experiment.
2. No recovery method is too sensitive to ensure that any sub-lethally injured cells can be recovered and survival not underestimated. Therefore, all media should be subject to rigorous quality control and stored in the dark to prevent the build up of oxidative products and contain quenching agents to counteract such products. Studies have shown that such methods have much greater recovery than media without such products or where selective media has been used (Humphrey, 1986).

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Appendix 1

Relevant papers- culture broths/heat treatment (may include other stresses):

1. **Birk T, Ingmer H, et al.** Chicken juice, a food-based model system suitable to study survival of *Campylobacter jejuni*. *Letters in Applied Microbiology* 2004; **38**(1): 66-71.

   AIMS: The purpose of this study was to develop a food-based model system that resembles the environment that Campylobacter jejuni experiences on raw poultry products and use this model system to investigate growth and survival of the bacterium. METHODS AND RESULTS: Chicken juice was collected from frozen chickens and subsequently cleared by centrifugation and subjected to sterile filtration. At low temperatures (5 and 10 degrees C) C. jejuni NCTC11168 remained viable in chicken juice for a remarkably longer period of time than in the reference medium BHI. When exposed to heat stress (48 degrees C) C. jejuni NCTC11168 also showed increased viability in chicken juice compared with the reference medium. Furthermore, agar plates made with chicken juice supported growth of four clinical isolates of C. jejuni and a C. jejuni strain obtained from chicken at both 37 and 42 degrees C. CONCLUSIONS: Our work shows that minimal processed and sterilized chicken juice is an ideal environment for survival of C. jejuni and that it is useful as a food-based model system. SIGNIFICANCE AND IMPACT OF THE STUDY: The developed model system may contribute to the understanding of C. jejuni viability on poultry products and can be instrumental in the development of alternative preservation strategies.

2. **Habib I, Uyttendaele M, et al.** Survival of poultry-derived *Campylobacter jejuni* of multilocus sequence type clonal complexes 21 and 45 under freeze, chill, oxidative, acid and heat stresses. *Food Microbiology* 2010; **27**(6): 829-834.

   The application of multilocus sequence typing (MLST) for studying Campylobacter jejuni diversity reveals that MLST clonal complex (CC) 21 and CC-45 occupies significant proportion in the diverse population of C jejuni. These two complexes are ecologically abundant and represent an interesting subpopulation for studying C jejuni survival under different stress conditions. In the present study we characterize and compare 19 C. jejuni strains assigned to CC-21 and CC-45, isolated from chicken meat, based on laboratory stress models maintained in Muller-Hinton broth. Model conditions were mimicking freeze, chill, oxidative, acid and heat stresses. Results show that survival patterns varied between the strains. C. jejuni strains of CC-21 survived significantly better than C. jejuni strains of CC-45

under heat (P value = 0.022) and chill (P value = 0.001) stress models. On the other hand, C. jejuni strains of CC-45 showed significantly better survival compared to C. jejuni strains of CC-21 in response to oxidative (P value = 0.003) and freeze (P value = 0.021) stress models. C. jejuni strains assigned to the founder ST-45 showed significantly better survival (P value = 0.017) under heat stress model compared to their ancestral sequence types. However, an association between survival fitness and the diversification of a clonal group cannot be demonstrated directly from the obtained results. In conclusion, findings of the present study show that genotypic variations of C. jejuni might play a role in enabling certain lineages to be selected when encountering adverse and stressful environments. In future stress response studies, it is recommended to consider the effect of genotypic diversity among C jejuni strains as that might bias the experimental findings. (C) 2010 Elsevier Ltd. All rights reserved.

3. **Hughes RA, Cogan T, et al.** Exposure of *Campylobacter jejuni* to 6 degrees C: Effects on Heat Resistance and Electron Transport Activity. *Journal of Food Protection* 2010; **73**(4): 729-733.

   Human infection with Campylobacter jejuni is frequently associated with the consumption of foods, especially chicken meat, which have been exposed to a range of temperatures during processing, storage, and cooking. Despite the public health importance of C. jejuni. little is known about the effects of cold exposure (refrigeration) on the subsequent ability of this pathogen to survive heat challenge. This work examined the effect of rapid exposure to 6 degrees C for 24 h on the heat resistance at 52 degrees C of 19 degrees C. jejuni strains originally isolated from various sources. The resulting death curves were analyzed with the Weibull model. Unlike cold-exposed cells of Escherichia coil and Salmonella, which have been reported to show significant increased sensitivity to heat, such exposure had only a marginal effect on heat resistance of the C. jejuni strains in this study. A possible explanation for this effect is that rapid chilling renders C. jejuni cells unable to adapt to reduced temperatures in an active manner. This hypothesis is supported by the observation that exposure to 6 degrees C for 24 h resulted in a significant and marked reduction in electron transport system activity when compared with controls at 37 degrees C.

4. **Hughes RA, Hallett K, et al.** The Response of *Campylobacter jejuni* to Low Temperature Differs from that of *Escherichia coli*. *Applied Environmental Microbiology* 2009; **75**(19): 6292-6298.

Human infection with Campylobacter jejuni is often associated with the consumption of foods that have been exposed to both chilling and high temperatures. Despite the public health importance of this pathogen, little is known about the effects of cold exposure on its ability to survive a subsequent heat challenge. This work examined the effect of rapid exposure to chilling, as would occur in poultry processing, on the heat resistance at 56 C of two C. jejuni strains, 11168 and 2097e48, and of Escherichia coli K-12. Unlike E. coli K-12, whose cold-exposed cells showed increased sensitivity to 56 C, such exposure had only a marginal effect on subsequent heat resistance in C. jejuni. This may be explained by the finding that during rapid chilling, unlike E. coli cells, C. jejuni cells are unable to alter their fatty acid composition and do not adapt to cold exposure. However, their unaltered fatty acid composition is more suited to survival when cells are exposed to high temperatures. This hypothesis is supported by the fact that in C. jejuni, the ratio of unsaturated to saturated fatty acids was not significantly different after cold exposure, but it was in E. coli. The low-temperature response of C. jejuni is very different from that of other food-borne pathogens, and this may contribute to its tolerance to further heat stresses.

5. **Jasson V, Uyttendaele M, et al.** Establishment of procedures provoking sub-lethal injury of *Listeria monocytogenes*, *Campylobacter jejuni* and *Escherichia coli* O157 to serve method performance testing. *International Journal of Food Microbiology* 2007; **118**(3): 241-249.

   In this study procedures provoking sub-lethal injury for three different pathogens are described which may be used in determination of accuracy and robustness of methods, comparison studies and or validation of rapid detection methods. Three common food-borne pathogens were used, Listeria monocytogenes, Campylobacter jejuni and Escherichia coli O157. The pathogens were exposed to heat stress, cold stress, freeze stress, acid stress, oxidative stress and food stress. Sub-lethal injury was determined by plating in parallel on selective and non-selective media. The statistical significant differences in enumeration were established. The choice of stress to create sub-lethal injury to cells depended on the fact that the procedure must be easy to handle, repeatable and relevant for stress conditions in foods, but also on the micro-organism itself. Oxidative stress (1000 microM H(2)O(2)) was chosen to impose sub-lethal injury on L. monocytogenes and a specific food stress for E. coli O157. For C. jejuni a specific food stress as well as the oxidative stress (750 microM H(2)O(2)) were capable of creating a standardized procedure of provoking injury.

6. **Kelly AF, Park SF, et al.** Survival of *Campylobacter jejuni* during stationary phase: evidence for the absence of a phenotypic stationary-phase response. *Applied Environmental Microbiology* 2001; **67**(5): 2248-2254.

When Campylobacter jejuni NCTC 11351 was grown microaerobically in rich medium at 39 degrees C, entry into stationary phase was followed by a rapid decline in viable numbers to leave a residual population of 1% of the maximum number or less. Loss of viability was preceded by sublethal injury, which was seen as a loss of the ability to grow on media containing 0.1% sodium deoxycholate or 1% sodium chloride. Resistance of cells to mild heat stress (50 degrees C) or aeration was greatest in exponential phase and declined during early stationary phase. These results show that C. jejuni does not mount the normal phenotypic stationary-phase response which results in enhanced stress resistance. This conclusion is consistent with the absence of rpoS homologues in the recently reported genome sequence of this species and their probable absence from strain NCTC 11351. During prolonged incubation of C. jejuni NCTC 11351 in stationary phase, an unusual pattern of decreasing and increasing heat resistance was observed that coincided with fluctuations in the viable count. During stationary phase of Campylobacter coli UA585, nonmotile variants and those with impaired ability to form coccoid cells were isolated at high frequency. Taken together, these observations suggest that stationary-phase cultures of campylobacters are dynamic populations and that this may be a strategy to promote survival in at least some strains. Investigation of two spontaneously arising variants (NM3 and SC4) of C. coli UA585 showed that a reduced ability to form coccoid cells did not affect survival under non-growth conditions.

7. **Klancnik A, Botteldoorn N, et al.** Survival and stress induced expression of groEL and rpoD of *Campylobacter jejuni* from different growth phases. *International Journal of Food Microbiology* 2006; **112**(3): 200-207.

Although Campylobacter jejuni is the leading cause of bacterial diarrhoeal disease in humans worldwide, its potential to adapt to the stressful conditions and survive in extra-intestinal environment is still poorly understood. We tested the effect of heat shock (55 degrees C, 3 min) and oxidative stress (3 mM H2O2 for 10 min or prolonged incubation at atmosphere oxygen concentration) on non-starved and starved cells of Campylobacter jejuni from different growth phases. Viability as assessed with the Bacterial Viability Kit LIVE/DEAD BacLighttrade mark dying before fluorescent microscopy and culturability of the cells (CFU ml(-1)) from both growth phases showed that starvation increased heat but not oxidative

resistance. High temperature and oxidative stress invoked quick transformation from culturable spiral shaped to nonculturable spiral and coccoid cells. Despite physiological changes of the cells we were not able to document clear differences in the expression of heat shock and starvation genes (dnaK, htpG, groEL), oxidative (ahpC, sodB), virulence (flaA) and housekeeping genes (16S rRNA, rpoD) after heat treatment (55 degrees C, 3 min) or oxidative stresses applied. When starving, no induction of expression of any of these genes was noticed, chloramphenicol had no influence on their gene expression. Quantitative real-time PCR analyses showed that at least 10-20 min of heat shock was necessary to evidently increase the amount of groEL and rpoD transcripts.

8. **Martinez-Rodriguez A, Kelly AF, et al.** Emergence of variants with altered survival properties in stationary phase cultures of *Campylobacter jejuni*. *International Journal of Food Microbiology* 2004; **90**(3): 321-329.

During the stationary phase of Campylobacter jejuni NCTC 11351 viable numbers fluctuate in a characteristic fashion. After reaching the maximum cell count (ca. 2 x 10(9) CFU/ml) in early stationary phase (denoted phase 1), viable numbers subsequently decrease to about 10(6) CFU/ml after 48 h and then increase again to about 10(8) CFU/ml (denoted phase 2) before decreasing once more to a value intermediate between the previous maximum and minimum values. To investigate whether the increase in viable numbers following the initial decline was due to the emergence of a new strain with a growth advantage in stationary phase analogous to the 'GASP' phenotype described in Escherichia coli [Science 259 (1993) 1757], we conducted mixed culture experiments with cells from the original culture and antibiotic-resistant marked organisms isolated from the re-growth phase. In many experiments of this type, strains isolated from phase 2 failed to out-compete the original strain and we have thus been unable to demonstrate a convincing GASP phenotype. However, strains isolated from phase 2 showed a much lower rate of viability loss in early stationary phase and a small increase in resistance to aeration, peroxide challenge and heat, indicating that the emergent strain was different from the parent. These results support the view that dynamic population changes occur during the stationary phase of C. jejuni that may play a role in the survival of this organism.

9. **Mihaljevic RR, Sikic M, et al.** Environmental stress factors affecting survival and virulence of *Campylobacter jejuni*. *Microbial Pathogens* 2007; **43**(2-3): 120-125. Enteric pathogens are constantly exposed to stressful conditions in their natural habitat in the host and even more in the extra-host environment, including food processing. The aim of this study was to evaluate the effect of selected environmental stress factors: temperature shift, starvation and atmospheric oxygen concentration on culturability/viability of two Campylobacter jejuni isolates. Additionally, after stress exposure, in an in vitro cell culture model using Caco-2 cells, the adhesion, invasion and intracellular survival of C. jejuni were studied. Nutrient insufficiency was the most powerful stress factor which significantly affected C. jejuni culturability and viability, as well as, adhesion and invasion properties. Temperature elevation induced a transient growth arrest, and temporary loss of pathogenic potential as indicated by impaired adhesion and invasion efficiency of C. jejuni. However, bacteria recovered within 24-48h inside the Caco-2 cells. Oxidative stress neither affected C. jejuni growth nor reduced the binding and invasion into Caco-2 cells. Only 5h oxygen exposure increased the invasion capability and intraepithelial survival of the clinical isolate. Modulation of C. jejuni virulence in response to environmental stress factors may have further implications in the pathogenesis of campylobacteriosis.

10. **Moore JE and Madden RH.** The effect of thermal stress on *Campylobacter coli*. *Journal of Applied Microbiology* 2000; **89**(5): 892-899.

AIM: Enteropathogenic Campylobacter jejuni, Camp. coli and Camp. lari are currently the most common cause of acute infectious diarrhoeal illness in the UK. Many domestic animals, including pigs, act as natural reservoirs for these organisms and infection may occur through the ingestion of contaminated foodstuffs. The safety of locally produced porcine liver was assessed in relation to the heat susceptibility of Campylobacter spp. present in eviscerated product. METHODS AND RESULTS: Heat susceptibility (D10) studies were performed on a wild-type strain of Camp. coli [NI39] isolated from porcine liver under standardized conditions. In addition, the effect of culture age and heating menstruum was determined. Thermal stress studies in phosphate-buffered saline showed Camp. coli NI39 to be heat sensitive (D10 = 8.-0, 30.8, 15.6, 10.3 s at 55.4, 57.4, 59.7, 61.2 degrees C, respectively; z = 6.10 degrees C). However, non-logarithmic biphasic survivor curves were observed at higher temperatures (> 56 degrees C), indicating the presence of a heat-resistant subpopulation (10(4)-10(5) cfu) which was not demonstrated when examining

either Salmonella Typhimurium or Listeria monocytogenes. CONCLUSIONS: The use of D10 values may be limited. Therefore, porcine liver, under processing, must be treated as a potential source of Campylobacter spp., and clearly defined F-values should be quantified through the use of empirically 'spiked' samples to ensure the eradication of campylobacters from the product, for each individual process being evaluated. SIGNIFICANCE AND IMPACT OF THE STUDY: It is important to define safe processing parameters in the manufacture of products which receive mild thermal processes in order to eliminate the risk of disease to man.

11. **Murphy C, Carroll C, et al.** The effect of different media on the survival and induction of stress responses by *Campylobacter jejuni*. *Journal of Microbial Methods* 2005; **62**(2): 161-166.

    The survival kinetics of Campylobacter jejuni strain CI 120 to a challenge of pH 4.5 was studied in seven different media. A medium effect was observed, showing up to a 5-log difference in stress resistance of cells. Strain variation in survival of C. jejuni was observed in Brucella broth (BBL). The ability of C. jejuni CI 120 to respond to a stress after growth in seven different media was also examined. An Adaptive Tolerance Response (ATR) was induced in only three of the seven media tested. The degree of resistance induced by the ATR varied between the different media. The production, during growth, of an extracellular component that confers stress resistance against subsequent acid challenge was observed in only four of seven media tested. Due to the direct effect of medium on stress/survival of C. jejuni, the results suggest that studies using different media may not be comparable.

12. **Nguyen H, Corry JE, et al.** Heat resistance and mechanism of heat inactivation in thermophilic campylobacters. *Applied Environmental Microbiology* 2006; **72**(1): 908-913.

    The heat resistance of Campylobacter jejuni strains AR6 and L51 and the heat resistance of Campylobacter coli strains DR4 and L6 were measured over the temperature range from 50 to 60 degrees C by two methods. Isothermal measurements yielded D55 values in the range from 4.6 to 6.6 min and z values in the range from 5.5 to 6.3 degrees C. Dynamic measurements using differential scanning calorimetry (DSC) during heating at a rate of 10 degrees C/min yielded D55 values of 2.5 min and 3.4 min and z values of 6.3 degrees C and 6.5 degrees C for AR6 and DR4, respectively. Both dynamic and isothermal methods yielded mean D55 values that were substantially greater than those reported previously (0.75 to 0.95 min). DSC analysis of each strain during heating at a rate of 10

degrees C/min yielded a complex series of overlapping endothermic peaks, which were assigned to cell wall lipids, ribosomes, and DNA. Measurement of the decline in the numbers of CFU in calorimetric samples as they were heated showed that the maximum rate of cell death occurred at 56 to 57 degrees C, which is close to the value predicted mathematically from the isothermal measurements of D and z (61 degrees C). Both estimates were very close to the peak m1 values, 60 to 62 degrees C, which were tentatively identified with unfolding of the 30S ribosome subunit, showing that cell death in C. jejuni and C. coli coincided with unfolding of the most thermally labile regions of the ribosome. Other measurements indicated that several essential proteins, including the alpha and beta subunits of RNA polymerase, might also unfold at the same time and contribute to cell death.

13. **Sagarzazu N, Cebrian G, et al.** High hydrostatic pressure resistance of *Campylobacter jejuni* after different sublethal stresses. *Journal of Applied Microbiology* 2010; **109**(1): 146-155.

AIMS: To study the development of resistance responses in Campylobacter jejuni to high hydrostatic pressure (HHP) treatments after the exposure to different stressful conditions that may be encountered in food-processing environments, such as acid pH, elevated temperatures and cold storage. METHODS AND RESULTS: Campylobacter jejuni cells in exponential and stationary growth phase were exposed to different sublethal stresses (acid, heat and cold shocks) prior to evaluate the development of resistance responses to HHP. For exponential-phase cells, neither of the conditions tested increased nor decreased HHP resistance of C. jejuni. For stationary-phase cells, acid and heat adaptation-sensitized C. jejuni cells to the subsequent pressure treatment. On the contrary, cold-adapted stationary-phase cells developed resistance to HHP. CONCLUSIONS: Whereas C. jejuni can be classified as a stress sensitive micro-organism, our findings have demonstrated that it can develop resistance responses under different stressing conditions. The resistance of stationary phase C. jejuni to HHP was increased after cells were exposed to cold temperatures. Significance AND IMPACT OF THE STUDY: The results of this study contribute to a better knowledge of the physiology of C. jejuni and its survival to food preservation agents. Results here presented may help in the design of combined processes for food preservation based on HHP technology.

14. **Sorqvist S.** Heat resistance of *Campylobacter* and *Yersinia* strains by three methods. *Journal of Applied Bacteriology* 1989; **67**(5): 543-549.

    Two methods of determining the heat resistance of bacteria, a glass cup and a test tube method, were compared with a method using capillary tubes. Three strains of Yersinia enterocolitica, one of Campylobacter jejuni and two of C. coli were tested in physiological saline. The differences between the results obtained by the glass cup method and the reference method were not statistically significant for five strains and were small also for the other, a Yersinia strain. D values obtained by the glass cup method at 58, 60 and 62 degrees C were 1.4-1.8, 0.40-0.51 and 0.15-0.19 min (zeta values 4.00-4.52 degrees C) for the Yersinia strains, and 0.42, 0.13 and 0.07 min (zeta value 5.07 degrees C) for one C. coli strain. For the other Campylobacter strains, D values of 0.71-0.78, 0.24-0.28 and 0.12-0.14 min (zeta values 4.94 and 5.60 degrees C) were recorded at 56, 58 and 60 degrees C. D values obtained at 60 degrees C by the test tube method were 2.7-5.0 min and were considered to be unrealistic.

Relevant papers- culture broths/other stresses (cold/freeze, pH, etc):

15. **Birk T, Rosenquist H, et al.** A comparative study of two food model systems to test the survival of *Campylobacter jejuni* at -18 degrees C. *Journal of Food Protection* 2006; **69**(11): 2635-2639.

    The survival of Campylobacter jejuni NCTC 11168 was tested at freezing conditions (-18 degrees C) over a period of 32 days in two food models that simulated either (i) the chicken skin surface (skin model) or (ii) the chicken juice in and around a broiler carcass (liquid model). In the skin model, cells were suspended in chicken juice or brain heart infusion broth (BHIB) and added to 4-cm2 skin pieces, which were subsequently stored at -18 degrees C. In the liquid model, cells were suspended in chicken juice or BHIB and stored at -18 degrees C. The decrease in the number of viable C. jejuni NCTC 11168 cells was slower when suspended in chicken juice than in BHIB. After freezing for 32 days, the reductions in the cell counts were 1.5 log CFU/ml in chicken juice and 3.5 log CFU/ml in BHIB. After the same time of freezing but when inoculated onto chicken skin, C. jejuni NCTC 11168 was reduced by 2.2 log units when inoculated in chicken juice and 3.2 log units when inoculated into BHIB. For both models, the major decrease occurred within the first 24 h of freezing. The results obtained in the liquid model with chicken juice were comparable to the reductions of Campylobacter observed for commercially processed chickens. The survival at

-18 degrees C in the liquid model was also tested for three poultry isolates and three human clinical isolates of the serotypes 1.44, 2, and 4 complex. As observed for C. jejuni NCTC 11168, all the strains survived significantly better in chicken juice than in BHIB and were not notably influenced by serotype or origin. The findings indicate that the composition of the medium around the bacteria, rather than the chicken skin surface, is the major determining factor for the survival of C. jejuni at freezing conditions. The liquid model with chicken juice was therefore the best model system to study the freezing tolerance in Campylobacter strains.

16. **Chan KF, Le Tran H, et al.** Survival of clinical and poultry-derived isolates of *Campylobacter jejuni* at a low temperature (4 degrees C). *Applied Environmental Microbiology* 2001; **67**(9): 4186-4191.

    Campylobacter jejuni is a leading cause of bacterial gastroenteritis in humans, and contamination of poultry has been implicated in illness. The bacteria are fastidious in terms of their temperature requirements, being unable to grow below ca. 31 degrees C, but have been found to be physiologically active at lower temperatures and to tolerate exposure to low temperatures in a strain-dependent manner. In this study, 19 field isolates of C. jejuni (10 of clinical and 9 of poultry origin) were studied for their ability to tolerate prolonged exposure to low temperature (4 degrees C). Although substantial variability was found among different strains, clinical isolates tended to be significantly more likely to remain viable following cold exposure than poultry-derived strains. In contrast, the relative degree of tolerance of the bacteria to freezing at -20 degrees C and freeze-thawing was strain specific but independent of strain source (poultry versus clinical) and degree of cold (4 degrees C) tolerance.

17. **Chaveerach P, tur Huurne AA, et al.** Survival and resuscitation of *Campylobacter jejuni* and *Campylobacter coli* under acid conditions. *Applied Environmental Microbiology* 2003; **69**(1): 711-714.

    The culturability of 10 strains of Campylobacter jejuni and Campylobacter coli was studied after the bacteria were exposed to acid conditions for various periods of time. Campylobacter cells could not survive 2 h under acid conditions (formic acid at pH 4). The 10 Campylobacter strains could not be recovered, even when enrichment media were used. Viable cells, however, could be detected by a double-staining (5-cyano-2,3-ditolyl tetrazolium chloride [CTC]-4',6'-diamidino-2-phenylindole [DAPI]) technique, demonstrating

that the treated bacteria changed into a viable but nonculturable (VBNC) form; the number of VBNC forms decreased over time. Moreover, some VBNC forms of Campylobacter could be successfully resuscitated in specific-free-pathogen fertilized eggs via two routes, amniotic and yolk sac injecting.

18. **Garenaux A, Jugiau F, et al.** Survival of *Campylobacter jejuni* strains from different origins under oxidative stress conditions: effect of temperature. *Current Microbiology* 2008; **56**(4): 293-297.

    Campylobacter jejuni is a microaerophilic pathogen but is able to survive oxidative stress conditions during its transmission to the human host. Strains of different origins (reference, poultry, or human clinical) were tested for survival under oxidative stress conditions. C. jejuni strains were grown in Mueller Hinton broth to obtain late exponential-phase cultures. Then they were exposed to 2 different stresses: (1) cultures were either plated on Columbia agar plates and exposed to atmospheric oxygen or (2) paraquat (a chemical oxidizing agent) was added to liquid cultures to reach a 500-microM concentration. Both of these experimental conditions were realized at 3 different temperatures: 4 degrees C, 25 degrees C, and 42 degrees C. Results obtained with paraquat and atmospheric oxygen were similar. Surprisingly, C. jejuni was found to be very sensitive to oxidative stress at 42 degrees C, which is its optimal growth temperature, whereas it was more resistant at 4 degrees C. A strain effect was observed, but no relationship was found between the origin of the strains and level of resistance. High temperature (42 degrees C) combined with oxidative stress allowed a rapid decrease in the C. jejuni population, whereas low temperature considerably decreased the effect of oxidative stress.

19. **Klancnik A, Guzej B, et al.** Stress response and pathogenic potential of *Campylobacter jejuni* cells exposed to starvation. *Research in Microbiology* 2009; **160**(5): 345-352.

    Campylobacter jejuni is a Gram-negative, fragile, spiral bacterium, known worldwide to be a major cause of acute human enteritis. Like many other food-borne bacteria, campylobacters must be able to survive under diverse conditions both inside the host and in the environment. Understanding stress response mechanisms provides information necessary for improving food processing and strategies that enhance food safety as well as clarifying the pathogenesis of campylobacteriosis. We investigated the relation between stress response to starvation and pathogenic potential in C. jejuni. Starvation changed the morphology and physiology of C. jejuni cells. However, the lower metabolic activity of 5-h-

starved culture was not a dormant state, but probably a viable but non-culturable (VBNC) form of the cells, since starved C. jejuni induced heat stress resistance. The health hazard potential of starved cells is still unclear. We showed that, in spite of starvation, C. jejuni survived in vitro within Caco-2 enterocites up to 4 days and caused systemic campylobacteriosis in vivo in a mouse model. However, bacterial numbers in investigated organs were significantly lower and the infection was resolved sooner. Our results show that nutrient insufficiency is responsible for C. jejuni transformation, influencing but not abolishing its survival and virulence properties while in the VBNC state.

20. **Rajkovic A, Smigic N, et al.** Resistance of *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Campylobacter jejuni* after exposure to repetitive cycles of mild bactericidal treatments. *Food Microbiology* 2009; **26**(8): 889-895.

    While maintaining nutritional and sensorial attributes of fresh foods mild processing technologies generally deliver microbiologically perishable food products. Currently little information exists on possible increase in the resistance of pathogens after repetitive exposure to mild (sub-lethal) treatments. Multiple strain-cocktails of Listeria monocytogenes, Escherichia coli O157:H7 and Campylobacter jejuni were exposed to 20 consecutive cycles of sub-lethal inactivation by three different techniques. Used techniques comprised inactivation with lactic acid (LA), chlorine dioxide (ClO(2)) and intense light pulses (ILP). Results showed that the selection of resistant cells was both species and technique dependent. While repetitive cycles of ClO(2) treatment did not result in increased resistance, repetitive inactivation with LA yielded L. monocytogenes culture of higher resistance in comparison to the parental culture. The increased resistance, expressed as decreased level of reduction in bacterial counts in subsequent inactivation cycles, was also observed with ILP for both L. monocytogenes and E. coli O157:H7 strains. Visual trend observations were confirmed through statistical linear regression analysis. No such effects were noted for C. jejuni which became undetectable after first 2-5 cycles. Current findings indicate the ability of foodborne pathogens to adapt to mild bactericidal treatments creating new challenges in risk assessment and more specifically in hazard analysis.

21. **Smigic N, Rajkovic A, et al.** Survival of lactic acid and chlorine dioxide treated *Campylobacter jejuni* under suboptimal conditions of pH, temperature and modified atmosphere. *International Journal of Food Microbiology* 2010; **135**(2): 136-143.

As mild decontamination treatments are gaining more and more interest due to increased consumer demands for fresh foods, it is of great importance to establish the influence of decontamination treatments on the subsequent bacterial behaviour under suboptimal storage conditions. For this purpose Campylobacter jejuni cells treated with lactic acid (LA, 3% lactic acid, pH 4.0, 2 min) or chlorine dioxide (ClO(2), 20 ppm, 2 min) were inoculated in Bolton broth (pH 6.0) and incubated under 80% O(2)/20% N(2), 80% CO(2)/20% N(2), air or micro-aerophilic (10% CO(2)/85% N(2)/5% O(2)) atmosphere, at 4 degrees C during 7 days. Treatment with water served as a control. The most suppressive atmosphere for the survival of C. jejuni was O(2)-rich atmosphere, followed by air, micro-aerophilic and CO(2)-rich atmosphere. The survival of C. jejuni was dependent on the type of initial decontamination treatment, with water treated cells showing the greatest survival followed by LA and ClO(2) treated cells. Intracellular pH (pH(i)) of individual C. jejuni cells was determined using Fluorescence Ratio Imaging Microscopy (FRIM). At all tested conditions, different subpopulation of the cells could be distinguished based on their pH(i) values. The pH(i) response was independent on the surrounding atmosphere since similar distribution of the subpopulations was observed for all tested atmospheres. However, the pH(i) response was dependent on the initial decontamination treatment. The investigation of intracellular parameters gave an insight into pathogen behaviour under stressful conditions at intracellular level. The results obtained in this study highlighted the importance of combining decontamination technologies with subsequent preservation techniques to the control survival and growth of foodborne pathogens.

22. **Smigic N, Rajkovic A, et al.** Intracellular pH as an indicator of viability and resuscitation of *Campylobacter jejuni* after decontamination with lactic acid. *International Journal of Food Microbiology* 2009; **141**(1): 140-146.

The aim of the study was to determine intracellular pH (pH(i)) as an indicator of the physiological state of two Campylobacter jejuni strains (603 and 608) at the single cell level after bactericidal treatment with lactic acid (3% v/v lactic acid, pH 4.0, 0.85% w/v NaCl) and during recovery and survival using Fluorescence Ratio Imaging Microscopy (FRIM). After exposure to lactic acid solution a decline in pH(i) to 5.5 (FRIM detection limit) was observed in the majority of cells (75-100%) within 2 min. The enumeration data revealed that after 2 min of lactic acid exposure, approx. 90% of the initial population became unculturable. In the following 10 min of exposure, a further decrease in the cell count was observed resulting in 3.53 and 3.21 log CFU/ml reduction of culturable cells at the end of the treatment. On the

contrary, the FRIM results revealed that the subpopulations with pH(i)>5.5 increased between 2 and 12 min of exposure to lactic acid. Removing the acid stress and incubating the cells suspension under the more favourable conditions resulted in an immediate increase in cell population with pH(i)>pH(ex) for both C. jejuni strains. Further 24 h incubation at 37 degrees C resulted in increased pH(i) and colony count (recovery study). On the contrary, 24 h incubation at suboptimal temperature of 4 degrees C, showed pH(i) decrease to pH(ex)=6.0 (no pH gradient) in the whole population of C. jejuni cells. Rather than dying, cells exposed for longer time (72 and 120 h) to 4 degrees C increased the subpopulation of the cells with positive pH gradient, mostly comprised of the cells with DeltapH>0.5, indicating the ability of C. jejuni cells to regulate their metabolic activity under suboptimal conditions.

Relevant papers- inoculated meat/heat treatment (may include other stresses):

23. **Bergsma NJ, Fischer ARH, Van Asselt ED, Zwietering MH and De Jong AEI.** Consumer food preparation and its implication for survival of *Campylobacter jejuni* on chicken. *British Food Journal* 2007; **109**(7): 548-561.

    Purpose – The disease burden caused by Campylobacter jejuni may be decreased by reduced consumption of undercooked chicken meat. However, little is known about consumer preparation of poultry and the effects of commonly applied cooking times on bacterial inactivation. This study aimed to answer these questions.

    Design/methodology/approach – Surveys were mailed in The Netherlands and analysed and laboratory inactivation experiments were conducted for the most frequent preparation method.

    Findings – The surveys revealed that the predominant way of chicken meat cooking was (stir)frying fillets and that consumers were generally aware of the presence of bacteria on chicken meat. Thorough heating of meat was considered important, which was often checked by visual inspection. In the laboratory, D-values for C. jejuni were obtained at frying temperatures: D was 1.95 min for artificially contaminated whole and D 0.59 min for diced fillets, respectively under practically relevant conditions. Large variability in survival was found, however.

    Originality/value – The paper shows that by combining consumer research and food microbiology it was concluded that the actual risk of consumption of chicken breast fillets that contain surviving C. jejuni is higher than previously assumed.

24. **Blankenship LC and Craven SE.** *Campylobacter jejuni* survival in chicken meat as a function of temperature. *Applied Environmental Microbiology* 1982; **44**(1): 88-92.Recognition of Campylobacter fetus subsp. jejuni (referred to hereafter as C. jejuni) as an important human pathogen and its isolation from meat products indicate the need for knowledge of its survival characteristics in meats. Thermal death times (D-values) for a single strain and a five-strain composite were determined in 1% peptone and autoclaved ground chicken meat at temperatures ranging from 49 to 57 degrees C. Survival was determined for these strains in chicken meat at 4, 23, 37, and 43 degrees C. Survival was also determined on raw chicken drumsticks stored at 4 degrees C in either an ambient or a CO2 atmosphere. D-values were greater in chicken meat than in peptone in all cases. D-values in peptone for strain H-840 at 49, 51, 53, 55, and 57 degrees C were 15.2, 4.90, 1.71, 0,64, and 0.25 min, respectively. The corresponding D-values in ground chicken meat were 20.5, 8.77, 4.85, 2.12, and 0.79 min, respectively. Similar results were obtained with a composite of five strains. When sterile ground chicken meat was inoculated with approximately 10(6) to 10(7) C. jejuni cells per g and stored at 37 degrees C in an ambient atmosphere, a 1-to 2-log count increase occurred during the first 4 days, followed by a gradual decline of about 1 log during the remainder of the 17-day storage period. No growth was observed among similarly inoculated samples that were stored at 4, 23, and 43 degrees C but counts declined by about 1 to 2 logs at 4 degrees C (17 day), by 2.5 to 5 logs at 23 degrees C (17 days), and to undetectable levels at 43 degrees C (between 10 and 16 days). Survival on raw chicken drumsticks stored at 4 degrees C in CO2 and in an ambient atmosphere declined by about 1.5 and 2.0 logs, respectively, during 21 days of storage. The effect of temperature on the survival of C. jejuni in chicken meat was similar to that reported in other natural and laboratory milieus. Ordinary cooking procedures that destroy salmonellae would be expected to destroy C. jejuni.

25. **Gill CO and Harris LM.** Survival and growth of *Campylobacter fetus* subsp. *jejuni* on meat and in cooked foods. *Applied Environmental Microbiology* 1982; **44**(2): 259-263.

Twelve strains of Campylobacter fetus subsp. jejuni isolated from humans and animals grew at temperatures ranging from 34 to 45 degrees C and pH minima between 5.7 and 5.9. Only one strain grew at pH 5.8 with lactic acid present at a concentration similar to that in meat. All strains had decimal reduction times of less than 1 min at 60 degrees C. Further examination of a typical strain showed that it grew at 37 degrees C on high-pH meat but not at 37 degrees C on normal-pH meat. Bacterial numbers on both high (6.4)-pH and

normal (5.8)-pH inoculated meat declined at a similar rate when the meat was stored at 25 degrees C. At -1 degree C, the rate of die-off was somewhat slower on normal-pH meat but was very much slower on high-pH meat. The initial fall in bacterial numbers that occurred when meat was frozen was also greater for normal-pH meat than for high-pH meat. The organism exhibited a long lag phase (1 to 2 days) when grown in cooked-meat medium at 37 degrees C and died in meat pies stored at 37 or 43 degrees C. Evaluation of the risk of Campylobacter contamination of red-meat carcasses to human health must take into account the limited potential of the organism to grow or even survive on fresh meats and in warm prepared foods.

26. **Sampers I, Habib I, et al.** Survival of *Campylobacter* spp. in poultry meat preparations subjected to freezing, refrigeration, minor salt concentration, and heat treatment. *International Journal of Food Microbiology* 2010; **137**(2-3): 147-153.

    The survival of Campylobacter spp. under defined conditions of freezing (-22 degrees C) was studied in naturally contaminated chicken skin and minced chicken meat. A decline of approximately one log(10) cfu/g was observed after 1 day of freezing. No further significant reduction was achieved by prolonged storage in the freezer, although a tendency for further gradual reduction of the numbers of Campylobacter spp. present was noted. Campylobacter spp. could still be detected qualitatively (per 0.1g) after 84 days. In a second part of this study, the survival of Campylobacter spp. in a typical minced meat preparation (minced meat supplemented with 1.5% salt (NaCl)) stored at refrigeration (4 degrees C) or frozen (-22 degrees C) was studied. No significant reduction of the pathogen was observed if the minced chicken meat was kept at 4 degrees C for 14 days, opposite to approximately one log(10) cfu/g reduction after 1 day when the minced meat preparation was stored in the freezer (-22 degrees C) for 14 days. The latter reduction is imputed to the effect of freezing as mentioned above and not due to the supplementation of NaCl to minced meat or the combination of NaCl and freezing, because similar reductions of Campylobacter spp. were noticed when minced meat (without addition of NaCl) was frozen. Finally, in a third part of the study, the survival of Campylobacter spp. subjected to a heat treatment, conform to consumer-based pan-frying, in inoculated (4.5+/-0.2 cfu/g) as well as naturally contaminated chicken burgers (2.1+/-0.1 cfu/g) was studied. The Campylobacter spp. numbers declined after 2 min (internal temperature reached circa 38 degrees C), where after 4 min (internal temperature reached circa 57.5 degrees C) they dropped below detectable levels (<10 cfu/g).

Relevant papers- inoculated meat/other stresses (cold/freeze, pH, etc):

27. **Bhaduri S and B Cottrell.** Survival of cold-stressed Campylobacter jejuni on ground chicken and chicken skin during frozen storage. *Applied Environmental Microbiology* 2004; **70**(12): 7103-7109.

    Campylobacter jejuni is prevalent in poultry, but the effect of combined refrigerated and frozen storage on its survival, conditions relevant to poultry processing and storage, has not been evaluated. Therefore, the effects of refrigeration at 4 degrees C, freezing at -20 degrees C, and a combination of refrigeration and freezing on the survival of C. jejuni in ground chicken and on chicken skin were examined. Samples were enumerated using tryptic soy agar containing sheep's blood and modified cefoperazone charcoal deoxycholate agar. Refrigerated storage alone for 3 to 7 days produced a reduction in cell counts of 0.34 to 0.81 log10 CFU/g in ground chicken and a reduction in cell counts of 0.31 to 0.63 log10 CFU/g on chicken skin. Declines were comparable for each sample type using either plating medium. Frozen storage, alone and with prerefrigeration, produced a reduction in cell counts of 0.56 to 1.57 log10 CFU/g in ground chicken and a reduction in cell counts of 1.38 to 3.39 log10 CFU/g on chicken skin over a 2-week period. The recovery of C. jejuni following freezing was similar on both plating media. The survival following frozen storage was greater in ground chicken than on chicken skin with or without prerefrigeration. Cell counts after freezing were lower on chicken skin samples that had been prerefrigerated for 7 days than in those that had been prerefrigerated for 0, 1, or 3 days. This was not observed for ground chicken samples, possibly due to their composition. C. jejuni survived storage at 4 and -20 degrees C with either sample type. This study indicates that, individually or in combination, refrigeration and freezing are not a substitute for safe handling and proper cooking of poultry.

28. **Curtis LM, Patrick M, et al.** Survival of Campylobacter jejuni in foods and comparison with a predictive model. *Letters in Applied Microbiology* 1995; **21**(3): 194-197. Campylobacter jejuni was inoculated into a range of raw and cooked foods and survival determined during storage at 2 degrees, 10 degrees and 20 degrees C for up to 56 d. To facilitate easy enumeration, two antibiotic-resistant strains of Camp. jejuni, which had similar survival characteristics to the parent strain, were used. Campylobacter jejuni survived for longer at lower temperatures in all foods and inactivation was most rapid in pate. There was generally good agreement between the survival data and predictions from a Camp. jejuni survival model (Food MicroModel).

29. **Davis MA and Conner DE.** Survival of Campylobacter jejuni on poultry skin and meat at varying temperatures. *Poultry Science* 2007; **86**(4): 765-767.

Researchers have recently found a much higher prevalence of Campylobacter on skin-on poultry products vs. skinless products. These data suggest that contamination is associated primarily with poultry skin, and Campylobacter may not survive on skinless poultry meat. Therefore, the objective of this study was to quantify the survival of Campylobacter poultry skin vs. meat under differing storage conditions. Skin and meat were irradiated to eliminate native microflora and inoculated with Campylobacter jejuni (~5.0 x 10(5) cfu/mL). Meat and skin samples were packaged in polystyrene trays, covered with Cryovac film, and then subjected to 1 of the following storage conditions: 1) 4 degrees C for 11 d; 2) 4 degrees C for 1 d, then -3 degrees C for 10 d; 3) 4 degrees C for 1 d, -3 degrees C for 1 d, then 4 degrees C for 9 d; or 4) 4 degrees C for 1 d, -3 degrees C for 1 d, 20 degrees C for 1 h on d2, then 4 degrees for 9 d. On d 0, 2, 3, 5, 7, 9, and 11, populations of Campylobacter were determined. The experiment was replicated 3 times. In each experiment, populations of surviving Campylobacter were not affected by storage conditions (P > or = 0.05), and there was no interaction between temperature treatments and sample type. Surviving Campylobacter populations were affected (P < or = 0.05) by sample type (skin vs. meat). Campylobacter, in the absence of competing microflora, survived well on poultry skin and meat at the temperatures tested. In all experiments, higher populations were established on the inoculated skin vs. inoculated meat. These populations remained consistently 0.4 to 0.9 log(10) cfu/g higher on skin vs. meat. Poultry skin topography may account, in part, for these higher populations on skin.

30. **Duffy L and Dykes GA.** Growth temperature of four *Campylobacter jejuni* strains influences their subsequent survival in food and water. *Letters in Applied Microbiology* 2006; **43**(6): 596-601.

AIM: To determine if Campylobacter jejuni grown at 37 and 42 degrees C have different abilities to survive on beef and chicken, and in water. METHODS AND RESULTS: Beef, chicken and water were separately inoculated with four Camp. jejuni (two poultry and two beef) strains grown at 37 or 42 degrees C. The matrices were stored at approximately 4 degrees C and Camp. jejuni numbers were monitored over time by plate counts. On beef there was a greater decrease in number for two strains ($P < 0.05$; approximately 0.7 and 1.3 log CFU cm(-2)) grown at 37 degrees C as compared with 42 degrees C. By contrast on chicken there was a decrease in numbers for two strains ($P < 0.05$; approximately 1.3 and 1 log CFU g(-1)) grown at 42 degrees C as compared with 37 degrees C. In water there was a greater decrease in numbers for all strains ($P < 0.05$; approximately 3-5.3 log CFU ml(-1)) grown at 42 degrees C as compared with 37 degrees C. CONCLUSIONS: Growth temperature influences the survival of Camp. jejuni on food and in water. SIGNIFICANCE AND IMPACT OF THE STUDY: Campylobacter jejuni survival studies need to consider growth temperature to avoid erroneous results. Campylobacter jejuni grown at 37 degrees C, the body temperature of humans and cattle, may represent a greater public health risk in water than those grown at 42 degrees C, the body temperature of poultry.

31. **Eideh AM and Al-Qadiri HM.** Effect of refrigerated and frozen storage on the survival of *Campylobacter jejuni* in cooked chicken meat breast. *Journal of Food Science* 2011; **76**(1): 17-21.

This experimental work aimed to examine the survivability of Campylobacter jejuni in cooked chicken breast under several conditions: storage for 1, 3, and 7 d at refrigerated temperatures (4 degrees C) and for 20 d at frozen temperatures (-18 degrees C). In addition, storage at ambient temperature (26 to 28 degrees C) was involved. Chicken samples were inoculated with a mixed culture of C. jejuni strains (ATCC: 29428 and 33219) of known concentrations (50 and 500 CFU/g). Bacterial cells were recovered and enumerated using standard procedure (Preston method). Bacteria were not detected in the majority of samples stored at ambient temperature. Refrigeration reduced survivals in 95, 90, and 77.5% for samples inoculated with 500 CFU/g and kept for 1, 3, and 7 d, respectively. The maximum reduction reached 1 log(10) cycle for all refrigeration durations. It was observed that bacteria died in 17.5% of samples kept for 7 d at 4 degrees C. However, survivors in

samples inoculated with 50 CFU/g were not detected in 50, 65, and 55% of samples kept for 1, 3, and 7 d, respectively. Freezing rendered survivors not detectable in 70% of samples inoculated with 50 CFU/g, while survived viable counts were reduced in 92.5% of samples inoculated with 500 CFU/g. These findings suggested that C. jejuni could be killed or just sublethally injured with or without reduction in viable counts under the investigated storage temperatures, which may indicate the ability of this bacterium to survive in chicken meat stored under refrigerated and frozen conditions.

32. **El-Shibiny A, Connerton P, et al.** Survival at refrigeration and freezing temperatures of *Campylobacter coli* and *Campylobacter jejuni* on chicken skin applied as axenic and mixed inoculums. *International Journal of Food Microbiology* 2009; **131**(2-3): 197-202.

    Campylobacter is considered to be the most common cause of bacterial diarrhoeal illness in the developed world. Many cases are thought to be acquired from consumption of undercooked poultry. The aim of this study was to compare the effect of the rate of cooling on the survival, at 4 degrees C and -20 degrees C, of Campylobacter coli and Campylobacter jejuni strains, inoculated on chicken skin from axenic culture or as mixed inoculums. Strains chilled in a domestic refrigerator varied in their tolerance to storage at 4 degrees C. Statistically significant differences between strains applied as axenic or mixed inoculums were observed for specific strain combinations using two-way ANOVA, including the enhanced survival of antibiotic resistant C. coli 99/367 at 4 degrees C. The use of rapid cooling (at -20 degrees C/min) enhanced the survival of all the Campylobacter strains chilled to 4 degrees C compared to standard refrigeration. Freezing to -20 degrees C reduced viable counts by 2.2-2.6 log10 CFU/cm(2) in 24 h. Rapid cooling to -20 degrees C (at -30 degrees C/min) enhanced the survival of C. coli 99/367 compared to freezing in a domestic freezer. Statistically significant interaction terms between specific strains were observed in mixed inoculums chilled to -20 degrees C by freezing in a domestic freezer and by rapid chilling to -20 degrees C. Rapid chilling of poultry, particularly for 4 degrees C storage may enhance survival of Campylobacter and although this is an issue that affects meat quality, it should be considered by poultry processors.

33. **Gonzalez M, Skandamis PN, et al.** A modified Weibull model for describing the survival of *Campylobacter jejuni* in minced chicken meat. *International Journal of Food Microbiology* 2009; **136**(1): 52-58.

Campylobacter is one of the leading causes of foodborne bacterial enteritis. Since chicken meat may be an important source of C. jejuni, the aims of this study were (i) to evaluate the survival/inactivation of C. jejuni strain 49/7R and its antimicrobial resistant variants (49/7RAT and 49/7RATCIP32) in minced chicken meat during extended storage at temperatures ranging from -20 degrees C to 25 degrees C and (ii) to test the suitability of the Weibull model for predicting the inactivation of C. jejuni in minced chicken meat in a wide range of temperatures. Minced chicken meat samples were inoculated with C. jejuni and log CFU/g were counted after different storage times at -20 degrees C, -5 degrees C, 4 degrees C, 15 degrees C or 25 degrees C. The log-linear and the Weibull models were used to fit a total of 15 inactivation curves. The mean value of R(2)(adjusted) for the correlation between the surviving bacterial cells observed and predicted by the Weibull model ranged from 0.986 to 0.994, and from 0.895 to 0.925 for the log-linear model, indicating closer agreement between the data and the Weibull model than for the log-linear one. From the Weibull model, p and delta parameters were described in a secondary model as a function of temperature using third-order polynomial fitting curves. Information from the secondary model served to predict survival curves for C. jejuni in minced chicken meat for an independent set of storage temperatures. Additionally, since delta parameter of the Weibull model is related to the D concept it served to determine the time (days) needed for the 1-log reduction of CFU/g; within the above mentioned temperature range. The results revealed that antimicrobial resistant variants survived longer than did the parent strains at all temperatures studied, indicated by the 1-log reduction time estimates.

34. **Hanel CM and Atanassova V.** Impact of different storage factors on the survivability of *Campylobacter jejuni* in 34 turkey meat. *FEMS Immunology and Medical Microbiology* 2007; **49**(1): 146-148.

Campylobacter jejuni is often prevalent in turkey and poultry, but the effects of storage temperatures and storage periods and the interruption of the cooling chain on its survival have not been evaluated so far. In this study, 700 samples of turkey meat were artificially contaminated by inoculating their surface with 10(3) CFU of C. jejuni per sample, wrapped in airtight cellophane bags, and stored under different chilling and freezing conditions for various storage periods; this was followed by analysis of the cultures. Subsequent to incubation at 25 degrees C for 48 h, C. jejuni was reisolated in only 7% of the samples. When the samples were stored under refrigerator conditions at 4 degrees C, the organism was reisolated in 42% of the samples after 1 week, and in 28% of the samples after

2 weeks. The recovery rates in the samples that had been stored frozen at -20 degrees C without interruption of the cooling chain were 68% after 2 weeks and 24% after 4 weeks. Different storage conditions were simulated in order to examine the impact of an interruption of the cooling chain on the survival of Campylobacter.

35. **Lee A, Smith SC, et al.** Survival and growth of *Campylobacter jejuni* after artificial inoculation onto chicken skin as a function of temperature and packaging conditions. *Journal of Food Protection* 1998; **61**(12): 1609-1614.

    Campylobacter jejuni is one of the major causes of food poisoning in humans. C. jejuni is also widespread in food animals, and meat and meat products derived from food animals are the most common vector of bacterial transmission to humans. To determine the role of packing and storage conditions on the replication of C. jejuni on chicken, the virulent strain C. jejuni 81116 was artificially inoculated onto chicken skin pieces (1 cm2) and stored at different temperatures and under various packaging conditions. C. jejuni 81116 remained viable at -20 and -70 degrees C and was able to replicate at 4 degrees C and at ambient room temperature. C. jejuni 81116 was also inoculated onto chicken skin and subjected to repeated freeze thawing and the viability of the inoculum was quantified. C. jejuni 81116 could withstand repeated freeze thawing similar to that which may occur in the domestic home. Under all freezing conditions, C. jejuni 81116 retained a high level of viability and quickly replicated to levels which exceeded Australian food authorities' permitted bacteria level on raw food products after the sample was thawed.

36. **Oyarzabal OA, Oscar TP, et al.** Survival of *Campylobacter jejuni* and *Campylobacter coli* on retail broiler meat stored at -20, 4, or 12 degrees C and development of Weibull models for survival. *Journal of Food* Protection 2010; **73**(8): 1438-1446.

    Survival of Campylobacter jejuni and Campylobacter coli isolated from broiler meat was investigated and modeled on retail breast meat. Meat portions were inoculated with C. jejuni or C. coli at 6.4 to 6.8 log CFU/g followed by storage at -20 degrees C for 84 days or at 4 or 12 degrees C for 14 days. Kinetic data within a species and temperature were fitted to the Weibull model. When >or=70% of the residuals were in an acceptable prediction zone from -1 (fail-safe) to 0.5 (fail-dangerous) log units, the model was considered to have

acceptable performance. Survival of Campylobacter was highest at 4 degrees C, lowest at 12 degrees C, and intermediate at -20 degrees C. Survival of C. jejuni and C. coli was similar at -20 degrees C but was lower (P<0.05) for C. jejuni than for C. coli at 4 and 12 degrees C. The Weibull model provided acceptable predictions for four of six sets of dependent data with unacceptable performance for survival of C. jejuni at -20 and 12 degrees C. A difference in survival was observed between the two strains of C. jejuni tested. Comparison of Weibull model predictions with data for C. jejuni archived in ComBase revealed mostly unacceptable performance, indicating that C. jejuni and C. coli survival on raw broiler breast meat differs from published results for other strains and growth media. Variation in Campylobacter survival among replicate storage trials was high, indicating that performance of the models can be improved by collection of additional data to better define the survival response during storage at temperatures from -20 to 12 degrees C.

37. **Rajkovic A, Tomic N, et al.** Survival of *Campylobacter jejuni* on raw chicken legs packed in high-oxygen or high-carbon dioxide atmosphere after the decontamination with lactic acid/sodium lactate buffer. *International Journal of Food Microbiology* 2010; **140**(2-3): 201-206.

Quantitative risk assessment studies performed elsewhere showed the importance of reducing counts of Campylobacter jejuni on chicken carcasses for decrease of incidence of human campylobacteriosis. The current study indicated that 1.8 log CFU/g reduction of inoculated C. jejuni (6 log CFU/g) can be achieved by decontamination with lactic acid buffered with sodium lacatate (LA/NaLA, 10% w/v, pH 3.0). Subsequent packaging under modified atmosphere of 80% O(2)/20%N(2) resulted in additional reduction of approximately 1.2 log CFU/g. These results were confirmed in naturally contaminated samples (2-3 log CFU/g) resulting in immediate reduction of present C. jejuni under the limit of enumeration (1 log CFU/g). However, enrichment showed presence of C. jejuni in 10g of sample. Under 80% O(2) LA/NaLA treated C. jejuni remained detectable per 10g until day 7, after which no positive samples were found until the end of the two-weeks storage. Under 80% CO(2) LA/NaLA treated C. jejuni remained fluctuating at 10 CFU/g until the end of two-weeks storage. Control cells were reduced by approx. 1.5 log CFU/g during storage under 80% O(2)/20% N(2), whereas no reduction was observed under 80% CO(2)/20% N(2). The present study showed the potential of buffered lactic acid and high-O(2) MAP to reduce C. jejuni both on inoculated and naturally contaminated samples. The immediate effect of decontamination was further extended by additive, not synergistic, effect of 80% O(2),

suggesting the practical value of the tested concept in combating C. jejuni on chicken carcasses.

38. **Ritz M, Nauta MJ, et al.** Modelling of *Campylobacter* survival in frozen chicken meat. *Journal of Applied Microbiology* 2007; **103**(3): 594-600.

AIMS: To model the survival kinetics of Campylobacter jejuni on frozen chicken meat. METHODS AND RESULTS: Three different types of chicken meat surface (skin, skinned muscle and cut muscle) were inoculated with stationary phase cells of C. jejuni (8 log(10) CFU cm(-2)) and frozen for 5 weeks at -20 degrees C. Bacterial numbers were determined weekly using two different methods of enumeration to quantify uninjured and injured cells. Analysis of variance of the results showed that the type of chicken surface and the method used to enumerate surviving cells were the most significant sources of variations in the numbers recovered ($P < 0.0001$), much more than the freezing time. To identify an appropriate model for the description of effects of freezing on survival over time, several models were fitted to the count data. Decay was found to be nonlinear. In general, survival was least on skin, better on skinned muscle and best on cut muscle. After 2 weeks, additional inactivation by freezing appeared to be negligible. CONCLUSION: Because of the variability of survival it was not possible to fit and select a general model useful for all the different surfaces types. SIGNIFICANCE AND IMPACT OF THE STUDY: The injured state of the cells leads to variability and the underestimation of bacterial survival. This is an essential factor for the assessment of Campylobacter-associated risk.

39. **Solow BT, Cloak OM, et al.** Effect of temperature on viability of *Campylobacter jejuni* and *Campylobacter coli* on raw chicken or pork skin. *Journal of Food Protection* 2003; **66**(11): 2023-2031.

To determine growth and survival of Campylobacter jejuni and Campylobacter coli on chicken and pork, Campylobacter spp. (10(4) CFU/cm2) were inoculated on pieces of raw, irradiated chicken or pork skin and exposed to temperatures ranging from -20 to 42 degrees C under either microaerobic or aerobic conditions. Viable counts over 48 h declined 2 to 3 log CFU/cm2 at -20 degrees C and 1 to 2 log CFU/cm2 at 25 degrees C regardless of skin type, species of Campylobacter, or level of oxygen. At 4 degrees C, there was no significant change in the number of Campylobacter over 48 h. At both 37 and 42 degrees C, the number of viable Campylobacter increased significantly (2 to 3 log CFU/cm2, $P < 0.0001$) under microaerobic conditions but decreased 0.5 to 1.5 log CFU/cm2 in air. Preincubation of skins

for 24 h at 42 degrees C under microaerobic conditions to establish Campylobacter on the surface prior to lowering the temperature to -20, 4, or 25 degrees C and incubating in air resulted in a decline in viability for the first 4 h (0.5 to 1 log CFU/cm2). However, after this initial drop in viability, no additional effect on viability was observed compared with incubation at -20, 4, or 25 degrees C in air without microaerobic preincubation at 42 degrees C. Preincubation of inoculated skins at -20, 4, or 25 degrees C in air for 24 h followed by a shift in temperature to 42 degrees C for 4, 8, 24, or 48 h and a shift to microaerobic conditions resulted in an overall decline in viability on raw pork skin but not on raw chicken skin. In contrast, preincubation of inoculated skins at -20, 4, or 25 degrees C for 24 h in air followed by a shift in temperature to 37 degrees C and microaerobic conditions did not result in a decrease in viable counts for either chicken or pork skins. Overall, viability of C. coli and C. jejuni on chicken and pork skins was similar. Therefore, a lower incidence of Campylobacter spp. in pork than in poultry postslaughter, despite a similar prevalence in live animals, is not due to differences in viability of C. coli versus C. jejuni on raw chicken or pork skin.

40. **Yoon KS, Burnette CN, et al.** Development of predictive models for the survival of *Campylobacter jejuni* (ATCC 43051) on cooked chicken breast patties and in broth as a function of temperature. *Journal of Food Protection* 2004; **67**(1): 64-70.

The objective of this study was to model the kinetics of the survival of Campylobacter jejuni on cooked chicken breast patties and in broth as a function of temperature. Both patties and broth were inoculated with 10(6) stationary-phase cells of a single strain of C. jejuni (ATCC 43051) and incubated at constant temperatures from 4 to 30 degrees C in 2 degrees C increments under aerobic conditions. In most cases, a three-phase linear model fit the primary survival curves well (r2 = 0.97 to 0.99) at all incubation temperatures regardless of model medium, indicating the presence of a resistant subpopulation of C. jejuni that would not be eliminated without thermal processing. Secondary models predicting lag time (LT) and specific death rate (SDR) as functions of temperature were also developed. The Davey and Boltzmann models were identified as appropriate secondary models for LT and SDR, respectively, on the basis of goodness of fit (Boltzmann model, r2 = 0.96; Davey model, r2 = 0.93) and prediction bias and accuracy factor tests. The results obtained indicate that C. jejuni can survive well at both refrigeration and ambient temperatures regardless of model medium. Reduced survival of C. jejuni, characterized by shorter lag times and faster death rates, was observed both on patties and

in broth at ambient temperatures. In addition, the average maximum reduction of C. jejuni at 4 to 30 degrees C was 1.5 log units regardless of storage temperature or model medium. These findings suggest that C. jejuni found on contaminated poultry products has the potential to survive under conditions that are not permissive for growth and thus could cause foodborne illness if the poultry is not sufficiently cooked.

Associated papers-criteria not met:

41. **Alter T, Bori A, et al.** Influence of inoculation levels and processing parameters on the survival of *Campylobacter jejuni* in German style fermented turkey sausages. *Food Microbiology* 2006*;* **23**(7): 701-707.

    This study investigated the influence of inoculum levels and manufacturing methods on the survival of Campylobacter (C.) jejuni in raw fermented turkey sausages. Sausages were prepared and inoculated with C. jejuni. After inoculation, these sausages were processed and ripened for 8 days. Samples were taken throughout the ripening process. The presence of C. jejuni was established bacteriologically. Additionally, lactic acid bacteria were enumerated, pH values and water activity were measured to verify the ripening process. To detect changes in genotype and verify the identity of the recovered clones, AFLP analysis was carried out on the re-isolated strains. Whereas no C. jejuni were detectable when inoculating the sausages with the lowest inoculum (0.08-0.44 log(10) cfu/g sausage emulsion), C. jejuni were detectable for 12-24h by enrichment when inoculated with approximately 2 log(10) cfu/g. After inoculation with 4 and 6 log(10) cfu/g respectively, C. jejuni were detectable without enrichment for 12-48 h and by enrichment for 144 h at the most. The greatest decrease of the C. jejuni population occurred during the first 4 h of ripening. Only a very high inoculum level allowed the survival of the organism during a fermentation process and during ripening to pose a potential risk for consumers. Lower initial Campylobacter inoculums will be eliminated during proper ripening of the sausages, if sufficient decrease in water activity and pH-value is ensured.

42. **Barrell RA.** The survival *of Campylobacter coli/jejuni* in unpasteurised milk. *Journal of infection* 1981; **3**(4): 348-352.

The survival of seven strains of *Camylobacter coli/jejuni* (six isolated from human and one from bovine faeces) was studied in unpasteurised milk held at refrigeration temperatures and at 21C. The numbers of the majority of strains decreased in the interval between 10 and 24 hours storage; four strains were still detectable after 48 hours at refrigeration temperatures but only two strains were recoverable after 48 hours at 21C.

43. **Baylis CL, MacPhee S, et al.** Comparison of three enrichment media for the isolation of *Campylobacter* spp. from foods. *Journal of Applied Microbiology* 2000; **89**(5): 884-891.

AIM: This study compared the performance of three Campylobacter enrichment broths: Bolton broth (BB), Campylobacter Enrichment broth (CEB) and Preston broth (PB). METHODS AND RESULTS: Pure cultures of target and competitor organisms, and naturally-contaminated food samples, were used to establish the performance of these media. In pure culture the PB supported the growth of the greatest number of strains of Campylobacter spp. but failed to inhibit some competitor organisms. The CEB showed the opposite result, inhibiting all 15 competitor organisms used but failing to support the growth of five Campylobacter strains. By comparison, BB showed the best compromise between inhibition of competitors and growth of Campylobacter. CONCLUSIONS: Plates inoculated with BB and CEB food enrichments resulted in more Campylobacter growth than those inoculated with PB, which supported significantly less typical growth (P < or = 0.001). The most common competitor organism isolated from PB was Escherichia coli, and Pseudomonas spp. were frequently isolated from BB and CEB. Both BB and CEB were better than PB for the isolation of Campylobacter from naturally-contaminated foods, although BB yielded more confirmed Campylobacter growth than CEB. SIGNIFICANCE AND IMPACT OF THE STUDY: This study highlighted differences in performance of media used to isolate Campylobacter spp. from foods.

44. **Boysen L, Knochel S, et al.** Survival of *Campylobacter jejuni* in different gas mixtures. *FEMS Microbiology Letters* 2007*;* **266**(2): 152-157.

Campylobacter jejuni in fresh chilled chicken meat is known to be a major risk factor for human gastrointestinal disease. In the present study, the survival under chilled conditions of different C. jejuni strains exposed to different gas mixtures usually used for gas

packaging of food was examined. Bolton broth and fresh, skinless chicken fillets were inoculated with six and four strains, respectively, and exposed to the gas mixtures 70/30% O(2)/CO(2), 70/30% N(2)/CO(2), and 100% N(2) (the latter only investigated in broth) at refrigeration temperature (4-5 degrees C). In broth culture, the strains survived significantly longer when exposed to 100% N(2) and 70/30% N(2)/CO(2) than in the oxygen-containing gas mixture, 70/30% O(2)/CO(2) (P<0.0001). For the two anaerobic gas mixtures, the reductions only reached 0.3-0.8 log(10) CFU mL(-1) within the same period. In the presence of oxygen, the numbers of C. jejuni were reduced by a minimum of 4.6 log(10) CFU mL(-1) over 21 days. When inoculated onto chicken fillets, the C. jejuni strains also died significantly faster in the oxygen-containing gas mixture, 70/30% O(2)/CO(2) (P<0.0001), reaching reductions of 2.0-2.6 log(10) CFU g(-1) after 8 days. In the gas mixture without oxygen (70/30% N(2)/CO(2)), no reductions were observed.

45. **Cools I, Uyttendaele M, et al.** Survival of *Campylobacter jejuni* strains of different origin in drinking water. *Journal of Applied Microbiology* 2003; **94**(5): 886-892.

AIMS: The aim of the study was to measure the survival of 19 Campylobacter jejuni strains of different origins, including two reference strains, four poultry-derived isolates, nine human isolates and four water isolates, in sterilized drinking water. METHODS AND RESULTS: Pure cultures of 19 C. jejuni strains were inoculated in sterile drinking water and incubated at 4 degrees C for 64 days. Survival was determined by culturability on both selective (Karmali agar) and non-selective [Columbia blood agar (CBA)] media. Culturability was shown to be strain and origin-dependent. Campylobacter jejuni showed prolonged survival on a non-selective than on a selective medium. CONCLUSIONS: The origin of the strain is a determining factor for the survival of C. jejuni in drinking water at 4 degrees C. Poultry isolates showed a prolonged survival, which could be an indication that these strains could play an important role in the transmission of campylobacteriosis through water. In addition, culture conditions are an important factor for evaluating the survival of C. jejuni in drinking water at 4 degrees C. The non-selective agar (CBA) allowed growth of C. jejuni over a longer period of time than the selective agar (Karmali). Furthermore, an enrichment broth (Bolton) allowed the recovery of all 19 C. jejuni strains during the 64 days of incubation at 4 degrees C. SIGNIFICANCE AND IMPACT OF THE STUDY: This study highlighted differences in culturability depending on culture conditions and on strain origin.

46. **Hilbert F, Scherwitzel M, et al.** Survival of *Campylobacter jejuni* under conditions of atmospheric oxygen tension with the support of *Pseudomonas* spp. *Applied Environmental Microbiology* 2010*;* **76**(17): 5911-5917.

Campylobacter jejuni is a major food-borne pathogen. Despite causing enteritis in humans, it is a well-adapted intestinal microorganism in animals, hardly ever generating disease symptoms. Nevertheless, as a true microaerophilic microorganism it is still puzzling how Campylobacter cells can survive on chicken meat, the main source of human infection. In this study, we demonstrate that C. jejuni is able to withstand conditions of atmospheric oxygen tension when cocultured with Pseudomonas species, major food-spoiling bacteria that are frequently found on chicken meat in rather high numbers. Using an in vitro survival assay, interactions of 145 C. jejuni wild-type strains and field isolates from chicken meat, broiler feces, and human clinical samples with type strains and food isolates of Pseudomonas spp., Proteus mirabilis, Citrobacter freundii, Micrococcus luteus, and Enterococcus faecalis were studied. When inoculated alone or in coculture with Proteus mirabilis, Citrobacter freundii, Micrococcus luteus, or Enterococcus faecalis type strains, Campylobacter cells were able to survive ambient oxygen levels for no more than 18 h. In contrast, Campylobacter bacteria inoculated with type strains or wild-type isolates of Pseudomonas showed a prolonged aerobic survival of up to >48 h. This microbial commensalism was diverse in C. jejuni isolates from different sources; isolates from chicken meat and humans in coculture with Pseudomonas putida were able to use this survival support better than fecal isolates from broilers. Scanning electron microscopy revealed the development of fiberlike structures braiding P. putida and C. jejuni cells. Hence, it seems that microaerophilic C. jejuni is able to survive ambient atmospheric oxygen tension by metabolic commensalism with Pseudomonas spp. This bacterium-bacterium interaction might set the basis for survival of C. jejuni on chicken meat and thus be the prerequisite step in the pathway toward human infection.

47. **Holler C and Martin W.** Evaluation of the direct viable count method for temperature stressed *Campylobacter coli*. *Journal of Microbiological Methods* 1998; **33**(2): 157-162.

Human pathogens, such as campylobacters, transform into the nonculturable state more or less rapidly in the environment. The question arises, whether these cells are still viable. The direct cell count (DVC) method has been used widely for enumeration and viability testing of different bacterial species. The influence of temperature induced bacterial injury on the applicability of the method, however, has not been examined. Two different

Campylobacter coli strains were pre-incubated at low temperatures. The necessary nalidixic acid concentration was strain specific, and they became more sensitive to the inhibitory effect of the antibiotic after temperature downshift. The method was not applicable for cultures that had been pre-incubated at intermediate temperatures, although nonculturability had not yet set in at the beginning of the experiments. (C) 1998 Elsevier Science B.V.

48. **Hudson WR and Mead GC.** Factors affecting the survival of *Campylobacter jejuni* in relation to immersion scalding of poultry. *Veterinary Record* 1987; **121**(10): 225-227.

Potential measures for reducing the survival of campylobacters during commercial scalding of poultry have been evaluated in a series of laboratory trials. At 50 degrees C, the lower temperature limit of commercial scalding, raising the pH of a buffered heating medium from 6.0 to 9.0 markedly increased the heat sensitivity of Campylobacter jejuni but the effect was largely nullified in the presence of 1 per cent 'organic material' (50:50 horse blood and milk). Either in the presence or absence of organic material a more rapid rate of kill was observed at 60 degrees C and it was again enhanced by raising the pH to 9.0. Use of a mild detergent at a concentration of 1000 ppm had little effect on the survival of C jejuni at 50 degrees C, but the addition of a cationic quaternary ammonium product at 50 to 100 ppm was highly effective in enhancing the rate of kill, even in the presence of organic material. It is suggested that such products should be evaluated in commercial scalding systems as a possible means of preventing the spread of campylobacters and other organisms of significance to public health.

49. **Humphrey TJ.** Techniques for the optimum recovery of cold injured *Campylobacter jejuni* from milk or water. *Journal of Applied Bacteriology* 1986*;* **61**(2): 125-132.

When broth was inoculated with cells of Campylobacter jejuni that had been injured by chilling there was a fall in the viable population of up to 90%. It was greater at 43 degrees than 37 degrees C and in the presence of certain antibiotics and in some cases resulted in a surviving population that was below the minimum inoculum for subsequent growth. A technique of pre-enrichment in non-selective culture broth at 37 degrees C for 2 h before the addition of antibiotics and incubation at 43 degrees C was found to significantly reduce

the fall in numbers and to improve the detection of C. jejuni in samples of raw milk and water.

50. **Humphrey TJ and Cruickshank JG.** Antibiotic and deoxycholate resistance in *Campylobacter jejuni* following freezing or heating. *Journal of Applied Bacteriology* 1985*;* **59**(1): 65-71.

    The surviving populations of Campylobacter jejuni serotypes following freezing or heat were found to be more sensitive to rifampicin and sodium deoxycholate on subsequent culture. Thus while control cultures had an IC50 of greater than 20 micrograms/ml rifampicin those of injured cells were less than 5 micrograms/ml. Treatment with EDTA caused almost identical changes in resistance suggesting that the altered resistance pattern of injured cells was due to loss of the barrier properties of the bacterial outer membrane.

51. **Humphrey T, Mason M, et al.** The isolation of *Campylobacter jejuni* from contaminated surfaces and its survival in diluents. *International Journal of Food Microbiology* 1995; **26**(3): 295-303.

    The isolation rates of campylobacters from contaminated surfaces were improved if swabs were placed directly into selective media rather than being stored in diluents before culture. Storage in diluents resulted in a loss of viability and the remaining viable campylobacter cells were often sub-lethally injured which sensitised them to selective agents in culture media and reduced isolation rates. Campylobacter jejuni, suspended in small drops of blood, was capable of prolonged survival on work surfaces if the drops remained liquid but the bacterium died rapidly once drops had dried.

52. **Isohanni PM and Lyhs U.** Use of ultraviolet irradiation to reduce *Campylobacter jejuni* on broiler meat. *Poultry Science* 2009; **88**(3): 661-668.

    The effects of UV irradiation at a wavelength of 254 nm on the survival of Campylobacter jejuni on the surfaces of broiler meat, skin, and carcasses were studied. On broiler carcasses, the effects of UV were also studied in combination with activated oxygen. The surfaces were inoculated with varying counts of C. jejuni and treated with UV irradiation using doses ranging between 9.4 and 32.9 mW/s per square centimeter. The log reductions in C. jejuni counts were determined by dilution plating. The effects of both treatments on the sensory quality of broiler meat, including visual appearance, odor, and fatty acid composition, were also evaluated. On broiler meat, the maximum reduction achieved was 0.7 log and on broiler skin 0.8 log. On broiler carcasses, the maximum reduction using UV

irradiation was 0.4 log, and using UV in combination with activated oxygen 0.4 log. No significant differences were found in the sensory quality between the samples and the controls. The use of UV irradiation alone or in combination with activated oxygen cannot be recommended as a primary decontamination method for C. jejuni on broiler carcasses. The use of these methods in combination with other decontamination techniques, and processing with proper processing plant sanitation and hygiene, might be more effective in reducing the C. jejuni counts on broiler carcass surfaces than the use of these methods only.

53. **Kelly AF, Martinez-Rodriguez A, et al.** (2003) Description of a phoenix phenomenon in the growth of *Campylobacter jejuni* at temperatures close to the minimum for growth. *Applied Environment Microbiology*; **69**(8): 4975-4978.

    When Campylobacter jejuni cultures that had been grown in broth at 39 degrees C were subcultured into fresh medium at 30 degrees C, there was a transient period of growth followed by a decline in viable-cell numbers before growth resumed once more. We propose that this complex behavior is the net effect of the growth of inoculum cells followed by a loss of viability due to oxidative stress and the subsequent emergence of a spontaneously arising mutant population that takes over the culture.

54. **Li Y, Yang H, et al.** Effect of high-temperature inside-outside spray on survival of *Campylobacter jejuni* attached to prechill chicken carcasses. *Poultry Science* 2002; **81**(9): 1371-1377.

    Prechill chicken carcasses, inoculated with Campylobacter jejuni, were sprayed in an inside-outside birdwasher at 20, 55, or 60 C, with or without 50 ppm chlorine, in a poultry processing pilot plant. Carcasses were sprayed for 12 s at 80 pounds per square inch (psi). Next, carcasses were placed in a chiller filled with 50 ppm chlorinated ice water at 4 C for 50 min. Most probable numbers of C. jejuni were determined based on chicken carcass wash water before and after the spray treatment. The skin color of chicken carcasses was measured. The results of this study showed that the 55 and 60 C water spray treatments significantly reduced C. jejuni by more than 0.78 log cfu/carcass compared with the 20 C water spray treatment. However, all of the 50 ppm chlorine spray treatments at three different temperatures were not significantly different. The skin color of chicken carcasses did not change significantly after the spray treatments at temperatures less than 60 C. The chilling process with 50 ppm chlorinated ice water at 4 C further reduced more C. jejuni

(approximately 1 log cfu/carcass) among the water spray treatments but did not result in greater reduction of C. jejuni among the chlorine spray treatments.

55. **Stern NJ and Kotula AW.** Survival of *Campylobacter jejuni* inoculated into ground beef. *Applied Environment Microbiology* 1982; **44**(5): 1150-1153.

Ground beef was inoculated with mixed cultures of Campylobacter jejuni, and the samples were subjected to various cooking and cold-storage temperatures. When samples were heated in an oven at either 190 or 218 degrees C, approximately 10(7) cells of C. jejuni per g were inactivated (less than 30 cells per g) in less than 10 min after the ground beef reached an internal temperature of 70 degrees C. When the samples were held at -15 degrees C over 14 days of storage, the numbers of C. jejuni declined by 3 log10. When inoculated samples were stored with an equal amount of Cary-Blair diluent at 4 degrees C, no changes in viability were observed over 14 days of storage. Twenty-five times as much C. jejuni was recovered from inoculated ground beef when either 10% glycerol or 10% dimethyl sulfoxide was added to an equal amount of ground beef before freezing as was recovered from peptone-diluted ground beef. Twice as much inoculated C. jejuni was recovered from ground beef plus Cary-Blair diluent as was recovered from ground beef plus peptone diluent.