Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Food Microbiology 85 (2003) 213–226
INTERNATIONAL JOURNAL OF Food Microbiology
www.elsevier.com/locate/ijfoodmicro

The survival of foodborne pathogens during domestic washing-up and subsequent transfer onto washing-up sponges, kitchen surfaces and food

Karen Mattick[a], Karen Durham[a], Gil Domingue[a], Frieda Jørgensen[a,*], Mithu Sen[b], Donald W. Schaffner[c], Tom Humphrey[d]

[a] *PHLS Food Microbiology Collaborating Laboratory, Division of Food Animal Science, School of Clinical Veterinary Science, University of Bristol, Langford House, Lower Langford, Bristol BS40 5DU, UK*
[b] *Procter & Gamble, European Technical Center, Tamselaan 100, B-1853 Strombeeek-Bever, Belgium*
[c] *Food Risk Analysis Initiative, Rutgers, The State University of New Jersey, 65 Dudley Road, New Brunswick, NJ 08901-8520, USA*
[d] *School of Clinical Veterinary Science, University of Bristol, Lower Langford, Bristol BS40 5DU, UK*

Received 9 July 2002; received in revised form 18 October 2002; accepted 28 October 2002

Abstract

In this study, the survival of *Salmonella*, *Campylobacter* and *Escherichia coli* O157: H7, when exposed to a range of constant temperatures (47–60 °C), in hard or soft water, in the presence/absence of detergent (0–0.3%) and organic matter, and during drying, was investigated. Further experiments used a washing-up process simulation, where soiled dishes contaminated with bacteria were washed in a bowl of warm water containing detergent. In addition, this study considered the risk of bacterial transfer onto (1) sterile dishes and sponges via contaminated water, (2) kitchen surfaces wiped with a contaminated sponge, (3) items placed in direct contact with a contaminated kitchen surface, (4) food placed on a contaminated dish or (5) dishes from contaminated food. A proportion of dishes remained contaminated with all pathogen types after a typical washing-up. Water hardness did not appear to affect survival. *E. coli*, and to a lesser extent *Salmonella*, survived towel- or air-drying on dishes and after towel-drying the cloth became contaminated on every occasion, regardless of the test organism. A proportion of sterile dishes washed after contaminated dishes became contaminated with pathogens but transfer from dishes onto food was rare. Washing-up sponges frequently became contaminated with pathogens. The results of this study highlight the potential for survival and cross contamination of food borne pathogens in the kitchen environment.

Keywords: Foodborne pathogens; Washing-up; *E. coli*

1. Introduction

Previous estimates suggest that 16% of food poisoning outbreaks in England and Wales may be associated with meals prepared in private houses (Cowden et al., 1995). A large proportion of fresh, raw retail chickens in England are contaminated with *Campylobacter* or

* Corresponding author. Tel.: +44-117-9289233; fax: +44-117-9289324.
E-mail address: frieda.jorgensen@bristol.ac.uk (G. Domingue).

0168-1605/02/$ - see front matter © 2002 Elsevier B.V. All rights reserved.
doi:10.1016/S0168-1605(02)00510-X

Salmonella (Jørgensen et al., 2002; Kramer et al., 2000; FSA website (www.food.gov.uk)) and, accordingly, the commonest vehicle implicated in private house outbreaks was poultry (44/101 outbreaks, Ryan et al., 1996). Cross contamination was described as a contributory factor in 28/101 outbreaks (Ryan et al., 1996) and other publications confirm that food preparation can lead to the cross contamination of other sites in the kitchen (de Boer and Hahné, 1990).

Clearly, raw ingredients that are contaminated with foodborne pathogens are handled frequently in the domestic kitchen and the process of 'washing-up' is a control point for preventing cross contamination in this environment. Washing items that have been soiled during the preparation or consumption of food serves to physically remove organic matter and associated microorganisms, such that they can be re-used with a minimal risk of causing cases of food poisoning.

A limited survey of consumer homes showed that, on average, UK consumers use a 0.12% detergent solution in the sink and at the end of an average washing up, the soiled washing-up water contained 0.4% w/v organic matter (Procter & Gamble, unpublished). No suds would be present on the surface of washing-up water containing $\geq 2.4\%$ (w/v) organic matter and typical detergent concentrations. It is unlikely that an informed consumer would continue to use such water and so 2.4% organic matter can be considered the maximum concentration likely to be present in washing-up water. The average washing-up water temperature in the UK is 48 °C at the start, as in most Northern European countries (Procter & Gamble, unpublished) and this will fall by a few degrees during the washing-up process.

The upper limit for growth of *Salmonella*, *Escherichia coli* and *Campylobacter* is reported to be approximately 46–47 °C (Anonymous, 1996, 2002; Hazeleger et al., 1998; Solomon and Hoover, 1999), so growth could not occur at the average European washing-up water temperature. The rate of death in broth or buffer over this temperature range can be predicted from the existing published literature and is likely to be relatively slow, for example the $D_{50\ ^\circ C}$ for *E. coli* O157:H7 is approximately 20 min (Stringer et al., 2000). Therefore, we hypothesise that *Salmonella*, *E. coli* O157 and *Campylobacter* may be able to survive a typical washing-up process and persist subsequently on dish surfaces or washing-up sponges. During washing-up, however, the combination of high temperature with high concentrations of detergent, organic matter and/or fat make it difficult to predict the inactivation kinetics of pathogens and experimental data are required.

If *Salmonella*, *E. coli* O157 and *Campylobacter* can survive washing-up, it will be important to determine whether they can survive subsequent stresses that might be encountered, for example desiccation associated with towel- or air-drying. Having established that cross contamination occurs frequently in the kitchen (de Boer and Hahné, 1990; Scott and Bloomfield, 1990), the potential for transfer of bacteria that have survived washing-up requires investigation. Such scenarios could include transfer from; contaminated washing-up water onto clean dishes and washing-up sponges; contaminated sponges onto kitchen surfaces during wiping; contaminated surfaces onto items placed on it; contaminated dishes to clean foods placed on it and from contaminated food to clean dishes.

In this study, the survival of *Salmonella*, *Campylobacter* and *E. coli* O157: H7 in food debris on soiled dishes, during the washing-up process and subsequent drying, and the potential for transfer of surviving pathogens was assessed. Our results demonstrate that bacterial pathogens were not always inactivated during washing-up and *E. coli* O157 in particular could withstand subsequent drying on dishes. The risk of transfer from contaminated dishes to a sterile food was low but the contamination of towels and washing-up sponges that may be used to wipe hands and work surfaces, respectively, was of more concern. This work will help to define the risks associated with bacterial survival during washing-up.

2. Materials and methods

2.1. Preparation of bacterial cells for inoculating washing-up water

The bacterial strains used in this study were *Campylobacter jejuni* strain 77 (originally isolated from poultry), *Salmonella enterica* serovar Typhimurium DT104 strain 30 (Mattick et al., 2000), *S. enterica* serovar Enteritidis PT4 strain E (Humphrey et al., 1995b) and a non-toxigenic *E. coli* 0157 NCTC12900 strain. While the present work was being carried out, other studies demonstrated that the *E. coli* O157 NCTC strain was considerably less stress-resistant than a number of other *E. coli* O157 strains (Wilde et al., 2001; Francis and O'Beirne, 2001). We therefore also carried out a number of experiments using *E. coli* O157 strain ATCC11229 (a non-pathogenic strain, Sommer et al., 2000) and compared the drying resistance of the NCTC strain to that of a verocytotoxigenic *E. coli* O157 strain E100793 isolated from beef (phage type 21, verotoxin types 1 and 2, highly stress tolerant; Wilde et al., 2001).

Bacteria were stored at -20 °C on Protect beads (Mast Diagnostics, Merseyside). To generate cultures of *E. coli* and *Salmonella*, a bead was streaked onto 5% horse blood agar (Columbia Agar Base, Oxoid, Hampshire, containing horse blood, E & O Laboratories, Bonnybridge, Scotland) and incubated at 37 °C for 24 h. Stationary phase cultures were prepared in nutrient broth (NB, Oxoid) as described previously (Mattick et al., 2000). The culture was adjusted to an optical density of 0.2 at 600 nm (equivalent to approximately 10^8 CFU ml^{-1}) and serial dilutions were prepared in maximal recovery diluent (MRD, Oxoid).

A *Campylobacter* cell suspension was prepared by streaking a bead onto blood agar, incubating in a microaerobic environment at 37 °C for 48 h, subculturing and incubating under the same conditions for a further 24 h. Growth was removed from the blood agar plate and suspended to homogeneity in MRD to an optical density of 0.1 at 600 nm (equivalent to approximately 10^8 CFU ml^{-1}). Serial dilutions were prepared in MRD, as before.

2.2. Survival of pathogens in food debris

Food residue was simulated using real grease soil (Procter & Gamble, unpublished). This was prepared by homogenising 1 tin of Tyne Brand minced beef (370 g), a whole raw egg, 101 g Smash dried potato, 36 g McDougalls sponge mix, 242 g full cream UHT milk, 14.2 g Bisto gravy granules and 105 g Mazola corn oil in a Blender (Braun). The gravy granules and corn oil were first mixed and microwaved for 1.5 min at 780 Watt. The soil was not sterilised and contaminating colonies of *Bacillus* spp. were sometimes observed but negative controls for the test pathogens ensured that those recovered were the same ones that were inoculated.

To investigate the survival of bacteria in food debris on dishes prior to washing-up, cultures or cell suspensions of *Salmonella*, *E. coli* O157 or *Campylobacter* were inoculated into soil and mixed to give an initial cell count of approximately 1×10^6 CFU ml^{-1}. Aliquots (20 μl) of inoculated soil were then placed onto small squares of kitchen-grade Formica and left exposed to the air for up to 72 h at 21 °C. Bacterial numbers were determined over time by rehydrating the soil on a Formica square in 10 ml of MRD for 15 min, then mixing vigorously to resuspend the soil. The suspension was diluted further in MRD, plated onto blood agar using the method of Miles and Misra (1938) and incubated at 37 °C for either 24 h (*Salmonella* and *E. coli*) or 48 h (*Campylobacter*, microaerobically), prior to enumeration. A selective medium was not required due to the low level of background contamination in the soil, so blood agar was used for its ability to recover injured bacteria cells (Mattick et al., 2001a,b). Data analysis was performed in Microsoft Excel 97 using a two-sample Student's *t*-test assuming equal variance.

2.3. Inactivation of bacteria in water containing detergent and soil

Aliquots of sterile tap water (50 ml) with a water hardness of 26 ppm were added to sterile honey jars. Non-antibacterial concentrated supermarket detergent was added at concentrations of 0, 0.1 or 0.3% v/v, chosen as a range of concentrations around the UK average (0.12%). Soil was added to the water samples to a final concentration of either 0 or 0.4%, the latter being the average concentration of organic matter in washing-up water (Procter & Gamble, unpublished).

The solution was mixed and the honey jars were placed into a pre-heated stirrer water bath at temperatures between 47 and 60 °C and left for 30 min to equilibrate. The water temperature was maintained at the stated temperature throughout the experiment. *Salmonella*, *E. coli* O157 or *Campylobacter* cells were inoculated to an initial bacterial cell concentration of approximately 10^6 CFU ml^{-1}. At pre-determined time intervals, aliquots of the washing-up water were removed and immediately diluted in MRD to reduce the temperature and thus prevent further bacterial death. Surviving *Campylobacter*, *Salmonella* and *E. coli* cells were enumerated as before. Regression analysis for the effect of water temperature on time to achieve a 3 $\log_{10}$ reduction was performed using Sigmaplot 2001 (SPSS, Richmond, CA, USA).

Pre-chilled cells were investigated, since meal preparation often involves handling refrigerated food ingredients, for example meat or poultry, that may be contaminated with foodborne pathogens and pre-chilling may affect pathogen survival during washing-up. Fresh cultures (*Salmonella* and *E. coli* O157) or fresh cell suspensions (*Campylobacter*) were standardised as before and refrigerated at 6 °C for 15 h, prior to inactivation in simulated washing-up water. Control cultures were not refrigerated.

Cultures of *S.* Typhimurium DT104 strain 30 were inoculated into soft (26 ppm) or hard water (230 ppm) containing 0%, 0.1% or 0.3% detergent, pre-heated to 48 °C. The local water company kindly provided the samples of soft and hard water. Subsequent work used soft water, since this was more readily available. The distribution of $\log_{10}$ reductions in *S.* Typhimurium DT104 was determined using Bestfit 4.0.2 (Palisade, Newfield, NY, USA).

2.4. Washing-up process simulation

The laboratory simulation of the washing-up process mimicked that of a typical consumer in the United Kingdom. Aliquots of sterile tap water (5 l) were used, with a water hardness of 26 ppm. Non-antibacterial concentrated supermarket detergent was added to give a concentration of 0.12% v/v, the average used in the UK. The water was pre-heated to 48 °C at the start of washing-up, and then allowed to decrease towards ambient during the process, as would happen naturally.

Aliquots (6 g) of soil were inoculated with a predetermined number of bacterial cells, mixed and spread evenly over the surface of each of 20 dishes (26 cm in diameter). Therefore, when 1000 cells were mixed with soil and spread onto 20 dishes, there was 120 g soil and 2×10^4 bacterial cells on the 20 dishes, giving a predicted maximum concentration of 2.4% w/v soil and 4 CFU ml^{-1} bacteria in the 5 l of washing-up water. After contamination, the dishes were stacked with the top dish covered and used within 1 h.

The soiled dishes were washed individually by hand (wearing rubber gloves) in the simulated washing-up water, using a washing-up brush to remove soil (eight clockwise and eight anti-clockwise strokes on the upper surface; four clockwise and four anti-clockwise strokes on lower surface). The time taken to wash 20 dishes and the final temperature were recorded, for each washing-up simulation.

Four dishes (numbers 4, 9, 14 and 19) were examined for the presence of pathogens immediately after washing-up by pouring an indicator agar over the surface, allowing it to solidify for 1 h at 21 °C and then incubating at 37 °C for 48 h. For *Salmonella*, the agar used was plate count agar (MC463, Oxoid) containing 2.5 g l^{-1} sodium thiosulphate and 1 g l^{-1} ferric ammonium citrate, with incubation for 48 h at 37 °C. For *E. coli* O157, the agar used was CLED medium (CM301) containing Andrade indicator (Oxoid CM423), with incubation for 24 h at 37 °C. For *Campylobacter*, the agar overlay method could not be used, due to the requirement for blood or charcoal in the agar, making it difficult to visualise colonies. Therefore, dishes were placed in a sterile bag and mixed with 250 ml modified Exeter broth for 1 min, avoiding unnecessary aeration. The latest modification of Exeter broth (using Bolton broth as the base) was prepared by adding 1 vial of *Campylobacter* growth supplement (SR084E, Oxoid; sodium pyruvate, sodium metabisulphite and ferrous sulphate, all at 0.125g per vial), 5 ml lysed defibrinated horse blood, 10 mg l^{-1} trimethoprim, 2 mg l^{-1} amphotericin and 15 mg l^{-1} cefoperazone to 500 ml Bolton broth (CM983, Oxoid). After rinsing the dish, the broth was decanted into a sterile container, such that little headspace remained, and placed at 37 °C for 6 h, after which 5 mg l^{-1} rifampicin and 2500 IU l^{-1} polymyxin were added. Delayed addition of rifampicin and polymixin to the Exeter broth has previously been shown to improve recovery of injured *Campylobacter* cells (Humphrey, 2002). The broths were then further incubated for 42 h at 37 °C. Aliquots of broths (10 μl) were sub-cultured onto Charcoal Cefoperazone Desoxycholate Agar (mCCDA, CM739, SR155, Oxoid)) plates and incubated at 37 °C for 48 h under microaerobic conditions. Presumptive colonies were confirmed as *Campylobacter* by sub-culturing onto blood agar and nutrient agar for purity and performing a Gram stain and an oxidase test.

After washing dish numbers 5, 10, 15 and 20, a sterile dish was dipped into the water for 5 s and the presence of pathogens was assessed as before, to assess transfer of bacteria from the water to dishes.

2.5. Effect of dish drying method on pathogen survival after washing-up

After washing dishes that had been inoculated with bacterial cells, dish numbers 5, 10, 15 and 20 were either air-dried on a washing-up rack h at 21 °C for 24 h (and 72 h in the case of *E. coli* O157). This procedure was then repeated but this time dish numbers 5, 10, 15 and 20 were dried manually with a tea-towel. After drying, dishes were assessed for the presence or absence of bacteria, by the methods described previously.

After towel-drying, the cloth was cultured for the presence or absence of the pathogens. For *Campylobacter*, the method was the same as that used for detecting this organism on the dishes. For *Salmonella*, the towel was placed into 250 ml buffered peptone water (BPW) and incubated for 24 h at 37 °C. An aliquot (0.1 ml) from each broth was sub-cultured into 10 ml Rappaport Vassiliadis enrichment broth (RV, Oxoid code CM669) and incubated at 41.5 °C for 24 h. Ten-microlitre aliquots of the selective broths were sub-cultured onto xylose lysine deoxycholate (XLD, Oxoid) and incubated at 37 °C for 24 h. Presumptive *Salmonella* isolates were streaked onto blood agar and incubated at 37 °C for 24 h to obtain pure cultures. Pure isolates were confirmed as *Salmonella* using serology (O and H antigen).

For *E. coli*, the towel was placed into 250 ml TSB and incubated at 37 °C for 24 h. Aliquots (10 μl) of the enrichment broth were then sub-cultured onto cefixime tellurite sorbitol MacConkey agar (CT-SMAC) and agar plates were incubated at 37 °C for 24 h and examined for typical colonies.

2.6. Transfer of pathogens from washing-up sponge to surfaces

Prior to use, the sponge was washed to remove all trace of preservatives and other chemicals that may have an antibacterial effect. The sponge was placed into a sterile bag with 100 ml sterile cold tap water and 2 ml washing-up liquid, and the bag contents were mixed for 60 s. The sponge was removed from the bag, rinsed thoroughly with cold water and squeezed to remove excess liquid. The sponge was then boiled in sterile distilled water for 10 min, excess water was removed and the sponge was dried overnight in an oven. The weight of the sponge was recorded.

The sponge was used to wash 20 dishes that had been evenly spread with 6 g soil per dish containing between 10^3 and 10^6 CFU dish^{-1} *S.* Typhimurium DT104 strain 30, *E. coli* NCTC12900 or *E. coli* ATCC11229. After washing-up, excess water was removed from the sponge by wringing and the sponge was weighed. The wet sponge was wiped over a Formica surface, weighed and left to dry overnight on a Petri dish at 21 °C. Three areas of the wiped surface (10×10 cm square = 100 cm^2) were swabbed immediately first using a swab pre-moistened in BPW (for *Salmonella*) or TSB (for *E. coli*) and then using a dry swab. Three further areas were left to dry overnight at 21 °C, prior to swabbing. After overnight drying, the sponge was weighed again, used to wipe three further areas and these areas were swabbed immediately.

To detect *Salmonella*, the swabs were placed into 18 ml buffered peptone water (BPW), incubated, subjected to selective enrichment and plated onto selective agar as above. To detect *E. coli*, swabs were placed into 18 ml TSB, incubated, subjected to selective enrichment and plated onto selective agar, as before. For *E. coli* (non-O157) the selective agar used was MacConkey agar (MAC).

2.7. Enumeration of pathogens in sponge rinse

After washing-up, the number of bacteria contaminating the sponge was determined. The sponge to be tested was placed into a stomacher bag containing 100 ml BPW (for *Salmonella*) or TSB (for *E. coli*). The sponge and broth were mixed for 90 s to mechanically remove pathogens. The broth was then squeezed from the sponge and decanted into a sterile container. A second rinse was produced in the same way.

A five-tube most probable number (MPN) test was performed on the rinse broth to enumerate *Salmonella*, *E. coli* or *E. coli* O157. Five aliquots of 10 ml rinse broth were added to 10 ml aliquots of double strength BPW (for *Salmonella*) or TSB (for *E. coli* or *E. coli* O157). In addition, five aliquots of 1 ml and five of 0.1 ml rinse broth were added to 10 ml aliquots of single strength BPW or TSB. All broths were incubated for 24 h. For *E. coli* O157 and *E. coli*, respectively, aliquots (10 μl) of TSB were sub-cultured onto SMAC and MAC, respectively, and incubated for 24 h at 37 °C. For *Salmonella*, an aliquot (100 μl) of BPW was sub-cultured into 10 ml RV and incubated for 24 h at 41.5

Table 1
Survival of foodborne pathogens in soil at 21 °C for 24 h

	Log_{10} reduction in cell number
S. Typhimurium DT104 strain 30	− 0.41 (± 0.05)
E. coli O157 strain E100793 (toxigenic)	− 0.53 (± 0.03)
E. coli O157 NCTC12900 (non-toxigenic)	− 1.00 (± 0.07)
C. jejuni strain 77	> − 5.00 (± 0.00)

°C. A further aliquot (10 μl) was sub-cultured to XLD and incubated for 24 h at 37 °C. The proportion of tubes yielding *Salmonella* or *E. coli* O157 for each dilution was used to calculate a CFU ml^{-1} using the five-tube MPN chart (Anonymous, 1995).

2.8. Cross contamination from washed dishes to foods

To prepare contaminated, simulated washing-up water, a culture of *E. coli* O157 NCTC12900 was pre-chilled at 6 °C for 15 h, diluted in MRD and inoculated into simulated washing-up water containing 2.4% soil and 0.12% detergent to achieve the initial cell concentrations of 100, 500, 1000 and 5000 CFU $dish^{-1}$.

Twenty dishes were surface sterilised using alcohol wipes and dipped individually into the water for 5 s per dish. After this, dishes were placed on a draining rack to air dry. Ten dishes were air dried for 15 h and 10 for 72 h. After air-drying, small Petri dishes completely filled with solidified CLED agar containing Andrade indicator (Oxoid CM423) were used to assess transfer from dish to food. The water activity of the agar was measured. The agar was placed onto each dish and used to investigate four different contact times (1, 5, 10 and 20 min). The small Petri dishes were then removed from the dish and incubated agar side up in a larger Petri dish with a lid at 37 °C for 24 h. Two dishes from each set of 10 were also covered with CLED agar containing Andrade medium in order to observe whether *E. coli* cells were present on the dish but had failed to transfer to the small Petri dishes containing agar. Contact with pressure was examined by placing an evenly spread weight (100 g) on top of the agar block during contact. Transfer to MacConkey agar (Oxoid) was also examined, to compare agars with different selectivity and as an indication of the level of injury of the transferred cells.

In addition, a comparison of towel drying and air-drying for 15 h was made. Four small Petri dishes containing CLED with Andrade indicator were inverted statically on the surface of dishes that had either been towel-dried (60 s contact time) or air-dried (20 min).

2.9. Transfer from food to dishes

Five hundred grams of minced beef (20% fat) was contaminated with pre-chilled *E. coli* 0157 NCTC12900 or *E. coli* ATCC11229 at three levels (10^3, 10^4 and 10^5 cells). The contaminated beef was then used to prepare meat patties on a dish. The patties were then removed and CLED agar containing Andrade indicator was poured over the dish. The agar was allowed to solidify, the dishes were incubated at 37 °C for 24 h and typical colonies of *E. coli* 0157 were recorded photographically.

3. Results

3.1. Survival of pathogens in food debris

Cell numbers of *E. coli* 0157 and *S.* Typhimurium DT104 decreased by only approximately 0.5 log_{10}

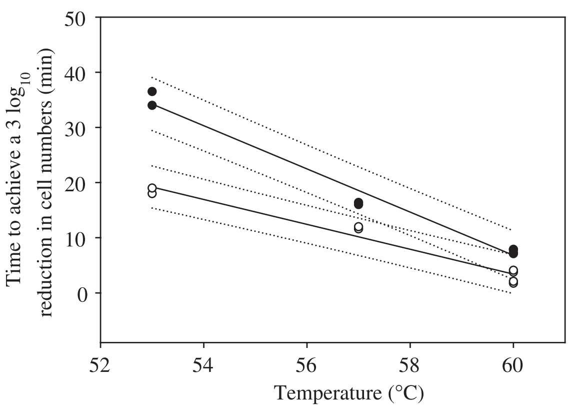

Fig. 1. Time to achieve a 3 log_{10} reduction on *Salmonella* Typhimurium DT104 in washing-up water from 53 to 60 °C. Closed circles represent plain water; open circles represent water containing 0.4% soil and 0.1% detergent. Regression lines are solid, and 95% prediction interval lines are dotted.

Table 2
Survival of *S.* Typhimurium DT104 strain 30 in water or simulated washing-up water at constant elevated temperature

Temperature (°C)	Time to achieve a 3 $\log_{10}$ reduction in cell numbers (min)	
	Water	Water plus 0.4% soil and 0.1% detergent
47	>60 (± 0.00)	>60 (± 0.00)
50	>60 (± 0.00)	>60 (± 0.00)
53	35.3 (± 0.72)	18.5 (± 0.29)
57	16.2 (± 0.12)	11.8 (± 0.12)
60	7.5 (± 0.17)	2.9 (± 0.48)

CFU ml^{-1} during 24 h of air-drying in soil at 21 °C, whereas *Campylobacter* decreased by >5.0 $\log_{10}$ CFU ml^{-1} (Table 1). Cell numbers of toxigenic *E. coli* O157 strain E100793 decreased less during air-drying in soil on a surface for 24 h at 21 °C than the non-toxigenic *E. coli* 0157 strain NCTC12900 ($P = 0.006$, Table 1).

3.2. Effect of detergent and soil concentration, pre-chilling and water hardness on inactivation of Salmonella during washing-up

Preliminary experiments using *Salmonella* showed < 3 $\log_{10}$ reduction in *S.* Typhimurium cell numbers when suspended in washing-up water at 47 or 50 °C for 60 min, even in the presence of 0.4% soil and 0.1% detergent (data not shown). At the higher temperatures tested (53–60 °C), however, cell death in water was much faster and the addition of soil and detergent resulted in an increased cell death (Fig. 1). As temperature increased, the difference due to added soil and detergent decreased, for example, at 60 °C the time to achieve a 3 $\log_{10}$ reduction in cell numbers was 7.5 min for water alone or 2.9 min for water containing 0.4% soil and 0.1% detergent (Table 2). Regression analysis of the effect of temperature on time to 3 $\log_{10}$ reduction show good correlations for *Salmonella* survival in water alone ($r^2 = 0.9817$) and in water with soil and detergent ($r^2 = 0.9659$). Care should be taken in extrapolating inactivation rates to lower temperatures, because predicted inactivation times at 47 or 50 °C were shorter than those observed.

Pre-chilled cells usually died more rapidly than fresh ones and this appeared to be particularly evident in water containing detergent (Table 3). The detrimental effect of detergent was confirmed, with a typically greater $\log_{10}$ reduction in cell numbers when detergent was present, regardless of whether the cells were fresh or pre-chilled. A detergent concentration of 0.3% v/v did not appear to be significantly more detrimental to cell viability than 0.1%. The two *Salmonella* strains showed similar levels of inactivation, giving confidence that the *Salmonella* data in this study may be representative for the genus. There was no clear difference in survival of *S.* Typhimurium DT104 strain 30 when soft (26 ppm) or hard (230 ppm) water was used for washing-up, regardless of detergent concentration (Table 4).

Data from 35 individual $\log_{10}$ reduction observations and the exponential distribution that describes them are shown in Fig. 2. Seventeen of the 35 observations showed essentially no reduction in *S.* Typhimurium DT104 after 15 min at 48 °C. Progressively fewer samples showed a greater reduction, with one sample showing about a 0.5 $\log_{10}$ decrease. The distribution of differences in $\log_{10}$ reductions was

Table 3
$\log_{10}$ reduction in cell number during heat challenge at 48 °C for 10 min

Detergent concentration (% v/v)	$\log_{10}$ reduction in cell numbers							
	E. coli O157 NCTC 12900		*C. jejuni* strain 77		*S.* Typhimurium DT104 strain 30		*S.* Enteritidis PT4 strain E	
	Fresh	Pre-chilled	Fresh	Pre-chilled	Fresh	Pre-chilled	Fresh	Pre-chilled
0	− 0.05 (± 0.03)	− 0.81 (± 0.19)	− 0.43 (± 0.01)	− 0.40 (± 0.00)	0.00	− 0.23	− 0.01	− 0.12
0.1	− 0.23 (± 0.03)	− 1.21 (± 0.11)	> − 4.30 (± 0.00)	> − 4.30 (± 0.00)	− 0.03 (± 0.01)	− 1.83 (± 0.06)	− 0.21 (± 0.01)	− 2.03 (± 0.05)
0.3	− 0.09 (± 0.02)	− 1.29 (± 0.01)	> − 4.30 (± 0.00)	> − 4.30 (± 0.00)	− 0.01 (± 0.00)	− 1.87 (± 0.12)	− 0.20 (± 0.01)	− 2.00 (± 0.04)

Initial level 10^6 CFU ml^{-1}.

Table 4
Survival of *S.* Typhimurium DT104 strain 30 after 15 min at 48 °C with different detergent concentrations in soft and hard water

Detergent concentration (% v/v)	Log_{10} reduction in cell numbers	
	Soft (26 ppm)	Hard (230 ppm)
0	− 0.11 (± 0.05)	− 0.07 (± 0.03)
0.1	− 0.08 (± 0.03)	− 0.19 (± 0.07)
0.3	− 0.23 (± 0.09)	− 0.10 (± 0.04)

described by an exponential distribution with a mean (β) of 0.15250. The goodness of fit for this distribution, by root-mean squared error, was 0.0129. This particular example demonstrates the descriptive power available when larger datasets are used. Since 35 observations were available, the distribution of cell reductions could be calculated with a high degree of precision. The creation of distributions like that shown in Fig. 2 is very useful later in the development of quantitative risk assessments (Montville et al., 2002).

3.3. Washing-up simulation

During washing-up of dishes that had been inoculated with organic matter and bacterial pathogens, the temperature of the washing-up water decreased with time and the concentration of organic matter and

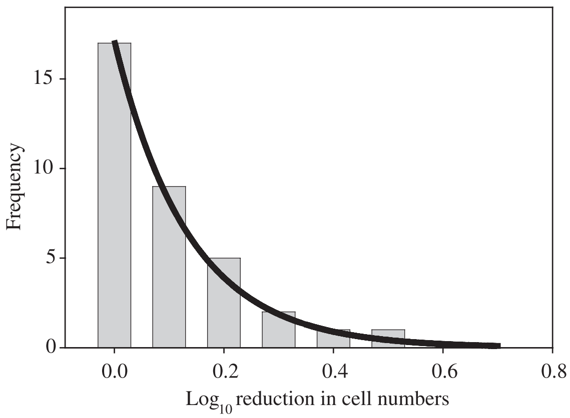

Fig. 2. Frequency distribution of *Salmonella* Typhimumrium DT104 log_{10} reductions after 15 min in 48 °C water. Bars represent 35 individual observations and solid line represents an exponential distribution with a mean (β) of 0.1525.

pathogens in the water increased. The washing-up process took 12 min on average and the water temperature was observed to drop approximately 10 °C (from 48 to 38 °C).

With bacteria inoculated at a concentration of 10^3 CFU $dish^{-1}$, a proportion of the dishes were still contaminated with *C. jejuni*, *E. coli* O157 or *S.* Typhimurium DT104 after washing (Table 5, Fig. 3). Therefore, these bacteria could survive the washing-up process, although *Campylobacter* appeared to survive least well and *E. coli* O157 appeared to survive best (Table 5). Not surprisingly, when dishes were inoculated with 10^6 CFU $dish^{-1}$, a higher proportion of dishes had viable *Salmonella* cells present after washing-up, compared with the situation when dishes were inoculated with 10^3 CFU $dish^{-1}$ (Table 5).

3.4. Survival of bacteria on dishes during drying

Campylobacter cells were very sensitive to towel- or air-drying and were not recovered on the dishes after drying (Table 5), whereas *E. coli* O157 survived better than the other bacteria tested and was still recovered from dishes after air-drying for 72 h (data not shown). *Salmonella* cells did not survive air-drying but a proportion of dishes remained contaminated after towel drying. When dishes were inoculated with a higher concentration of *Salmonella* cells, all dishes remained contaminated after towel drying but only half after air-drying. When dishes were dried using a towel, the cloth became contaminated on every occasion, regardless of the test organism.

Table 5
Proportion of dishes containing viable cells after washing-up and subsequent drying, and proportion of sterile dishes that became contaminated when washed subsequent to the inoculated dishes

	Initial CFU $dish^{-1}$	Wet, washed dish	Towel dried, washed dish	Air dried, washed dish	Sterile dishes contaminated during washing
C. jejuni strain 77	10^3	2/4	0/4	0/4	1/4
E. coli O157 NCTC12900	10^3	4/4	3/4	4/4	4/4
S. Typhimurium strain 30	10^3	3/4	1/4	0/4	1/4
S. Typhimurium strain 30	10^6	4/4	4/4	2/4	4/4

Fig. 3. A dish contaminated with *Salmonella* (the black areas) after washing-up (left) and the control (right). The indicator agar (plate count agar (MC463, Oxoid) containing 2.5 g l^{-1} sodium thiosulphate and 1 g l^{-1} ferric ammonium citrate) was poured over the surface of the dishes and these were then incubated at 37 °C for 48 h. The *Salmonella*-positive dish was contaminated during a simulated washing-up procedure, using dishes inoculated with *Salmonella*. The indicator agar was poured on the dish immediately after washing up.

3.5. Transfer of bacteria to sterile dishes during washing-up

A proportion of sterile dishes washed subsequent to the contaminated dishes became contaminated with *E. coli*, *Salmonella* or *Campylobacter* (Table 5). Transfer of *S.* Typhimurium DT104 strain 30 to sterile dishes occurred more frequently when there was a higher number of bacteria in the washing-up water (Table 5).

3.6. Transfer of pathogens from washing-up sponge to surfaces

Before the washing-up process, unused, dry, clean sponges weighed 5.8 g (± 0.12) on average. After washing-up and squeezing out the excess water, sponges weighed 20.1 g (± 1.9) on average, having gained approximately 16 g of potentially contaminated washing-up water. After wiping a kitchen surface (approximately 20 × 30 cm, or 600 cm^2), the sponges weighed 19.3 g (± 1.9) on average, so 0.8 g liquid had been deposited onto the 600 cm^2 surface (equivalent to 0.0013 g cm^{-2} on average). After standing at 21 °C for 15 h (overnight), the sponges weighed 7.4 g (± 0.5), indicating that on average 11.9 g moisture had been lost through evaporation.

When washing-up water was inoculated at an initial cell concentration of 10^3 CFU *Salmonella* $dish^{-1}$ and the washing-up sponge was used to wipe a kitchen surface, no *Salmonella* cells were recovered from the surface, even when it was wiped immediately after washing-up and swabs were taken straight away (Table 6). At the same initial cell concentration, some *E. coli* cells were detected by surface swabbing but strain ATCC11229 was recov-

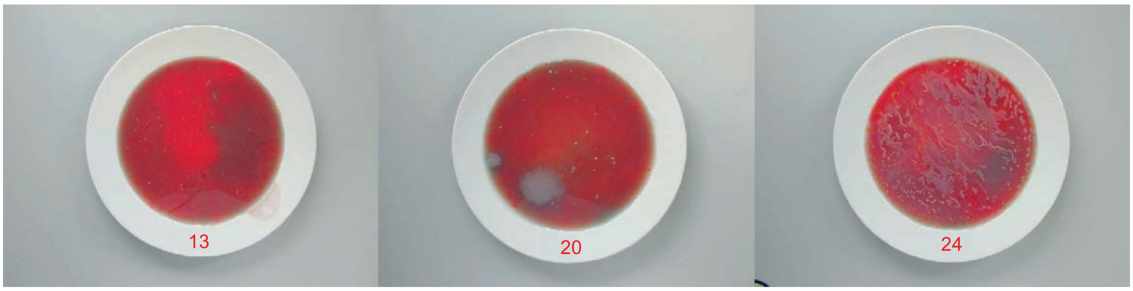

Fig. 4. *E. coli* ATCC 11229 on dishes contaminated during the preparation of meat patties, spiked with 10^3 (left), 10^4 (middle) or 10^5 (right) *E. coli* cells. Indicator agar (CLED medium (CM301) containing Andrade indicator (Oxoid CM423)) was poured over the surface of the dishes after the contamination and the dishes were then incubated for 24 h at 37 °C.

Table 6
Proportion of positive surface swabs taken after wiping surface with a washing-up sponge

Bacteria	Strain	Initial cell concentration (CFU dish^{-1})	Timepoint at which sponge used to wipe surface (h)	Timepoint at which surface swab taken (h)	Proportion of swabs positive
S. Typhimurium	DT104 strain 30	10^3	0	0	0/9
			0	24	0/9
			24	0	0/9
		10^4	0	0	3/3
			0	24	0/3
			24	0	0/3
		10^5	0	0	3/3
			0	24	1/3
			24	0	0/3
E. coli	NCTC12900	10^3	0	0	0/12
			0	24	1/12
			24	0	1/12
	ATCC11229	10^3	0	0	5/6
			0	24	4/6
			24	0	3/6
	ATCC11229	10^6	0	0	6/6
			0	24	3/6
			24	0	3/6

ered from surfaces much more frequently than strain NCTC12900 (Table 6). When higher initial levels of *Salmonella* or *E. coli* ATCC11229 were used to contaminate the organic matter spread onto the dishes, the surfaces were more frequently contaminated with bacteria (Table 6).

When the sponge was rinsed twice in broth, *Salmonella* and *E. coli* inoculated at a level of 10^3 CFU sponge^{-1} were recovered from the liquid on every occasion but the second rinse always had a lower level of contamination than the first (Table 7). When *E. coli* ATCC11229 was inoculated at 10^6 CFU sponge^{-1} the concentration of bacteria in the first rinse was the same as when inoculated at the lower level but the concentration in the second rinse appeared slightly higher (Table 7).

Table 7
Number of viable bacteria in sponge rinses after washing-up

	Strain	Initial cell concentration (CFU dish^{-1})	Concentration bacteria (CFU ml^{-1}) Rinse 1	Rinse 2
S. Typhimurium	DT104 strain 30	10^3	0.2	0.1
E. coli	NCTC12900	10^3	0.1	< 0.02[a]
	ATCC11229	10^3	8.2	4.6
	ATCC11229	10^6	8.2	8.0

[a] Positive by enrichment.

3.7. Transfer of *E. coli* O157 from washed dishes to simulated foods

Food was simulated by using a block of CLED agar containing Andrade indicator agar. The water activity of this agar was 0.99. The frequency of transfer of *E. coli* 0157 NCTC12900 from the surface of a washed, air-dried dish to a simulated food was assessed and found to be low (Table 8). In addition, when the simulated food was replaced by selective agar, no cells were recovered, indicating that the cells undergoing transfer were injured (data not shown). Control washed, dried dishes were overlaid with indicator agar and indicated that viable *E. coli* O157 cells were present on the dishes but failed to transfer to the food. The application of pressure (designed to simulate the weight of food) sometimes appeared to increase the chance of transfer but the relationship was not clear (Table 8).

A comparison of the transfer frequency of bacteria from towel- or air-dried washed dishes onto food

Table 8
The influence of contact time, application of weight and bacterial concentration on the proportion of successful transfers of *E. coli* O157 NCTC12900 from contaminated dishes to a surrogate food

Initial CFU dish^{-1}	Weight	Contact time (min)			
		5	20	60	900
100	–	2/4	2/4	0/4	2/4
	+	0/4	0/4	0/4	0/4
500	–	0/4	0/4	0/4	0/4
	+	2/4	2/4	2/4	0/4
1000	–	0/4	0/4	0/4	0/4
	+	0/4	0/4	0/4	0/4
5000	–	0/4	0/4	0/4	0/4
	+	0/4	2/4	2/4	1/4

indicated that transfers occurred more frequently from towel-dried dishes to food than from air-dried dishes. With *E. coli* ATCC11229 and *E. coli* 0157 NCTC12900, respectively, 1/20 and 2/20 transfers occurred following towel drying, whereas 0/20 and 0/20 transfers occurred following air-drying.

3.8. Transfer from food to dishes

The transfer of bacteria from minced beef (inoculated with different levels of *E. coli* O157) to sterile dishes was investigated. Visual inspection of the dishes overlaid with agar demonstrated that increasing the level of *E. coli* strains NCTC12900 or ATCC11229 present in the minced beef gave rise to an increase in the contamination level on the dish (Fig. 4).

4. Discussion

This study investigated factors affecting the survival of *Campylobacter*, *Salmonella* and *E. coli* O157 during a typical washing-up process and the subsequent potential for transfer of bacteria to sites in the kitchen. The water temperature, detergent concentration and organic matter concentration was designed to simulate the range used by a typical UK consumer, identified during preliminary studies.

Air-drying in organic matter, as would occur if dirty dishes were left for some time prior to washing-up, showed that *Campylobacter* survived very poorly in comparison with *Salmonella* and *E. coli*. This confirms previous reports that *Campylobacter* is very sensitive to desiccation (Humphrey et al., 1995a), whereas *Salmonella* can survive air-drying for at least 24 h (Humphrey et al., 1995b; Bradford et al., 1997) and *E. coli* may survive even longer (Bale et al., 1993).

The survival of these bacteria during a simulated washing-up was then investigated. The bacterial concentrations used are probably higher than many real situations, for example very low numbers of *Salmonella* are present on raw, retail poultry (Jørgensen et al., 2002), but the high levels enabled an accurate description of the bacterial inactivation during washing-up to be made. Using this model, we found that a small elevation in washing-up water temperature could bring about a large increase in bacterial death rate and it is unlikely that any of the pathogens tested would survive a standard cycle in a dish-washing machine, which usually involves temperatures of approximately 60 °C. The addition of detergent (0.1%) ensured a more rapid decline in bacterial numbers than washing in the absence of detergent even in the presence of organic matter. Detergent has been shown to promote cell lysis of cell membranes, for example in molecular biology applications (Popowska et al., 1999); however, increasing the amount of detergent further (from 0.1% to 0.3%) was unlikely to significantly affect bacterial survival. Organic matter may have no effect on *Salmonella* heat tolerance, for example in chicken scald-tanks (Humphrey, 1981) but the ‘real grease’ soil had a high fat content which has been reported to protect microorganisms against high temperature (Senhaji, 1977; Line et al., 1991; Juneja and Eblen, 2000), although other reports are less conclusive (Kadan et al., 1963; Kotrola and Connor, 1997). Clearly, if soil did have a protective effect, then this was masked by the detrimental effect of the detergent.

Bacteria contaminating perishable food products are likely to have been exposed to chill temperatures in the refrigerator prior to food preparation and the washing-up process. In this study, pre-chilled cells of *Salmonella* and *E. coli* O157 survived exposure to the high temperature conditions associated with washing-up less well than control cells, as reported previously in other substrates (Humphrey, 1990; Semanchek and Golden, 1998). There was no clear effect of pre-chilling with *Campylobacter* cells in this study and this may be due to its atypical response to growth arrest and stressful conditions (Kelly et al., 2001).

Water hardness varies by region in the UK and hard water has been reported to detrimentally affect detergent performance, with effectively less detergent present (Shere, 1948; Asbury, 1983). In this study, however, there was no clear effect of water hardness on microbial survival during washing-up.

A laboratory simulation of the washing-up process was subsequently developed, using a washing-up bowl, implements and a draining rack. A proportion of dishes that had been inoculated with *E. coli*, *Salmonella* or *Campylobacter* remained contaminated after a typical washing-up process, so not all bacteria were physically removed or inactivated. The transfer of pathogens onto dishes and sponges from contaminated water and then onto kitchen surfaces wiped with a contaminated sponge, onto items placed on contaminated surfaces, from contaminated dishes to a sterile food placed on it or from contaminated food placed on a sterile dish was subsequently investigated. *E. coli* O157, *Salmonella* and *Campylobacter* were detected on a proportion of sterile dishes washed subsequent to contaminated dishes, indicating that transfer of bacteria via contaminated washing-up water occurred. The potential risk associated with the survival of *Campylobacter* during washing-up and cross contamination in the kitchen environment was considered to be small due to its sensitivity to detergent and desiccation, thus subsequent studies focussed on *Salmonella* and *E. coli*.

When an initial cell concentration of 10^3 CFU $dish^{-1}$ was used to contaminate the organic matter smeared onto dishes, *E. coli* transferred onto washing-up sponges and onto surfaces wiped with a contaminated sponge but *Salmonella* was not recovered from surfaces. Fresh sponges were used for these experiments, so the possible build-up of pathogens on washing-up cloths that may occur in real kitchens was not considered, for example previous studies have reported a high prevalence and number of potential pathogens in dishcloths (Tebbutt, 1984, 1986; Beumer et al., 1996). With higher initial levels of bacteria, both *E. coli* and *Salmonella* were recovered from kitchen surfaces and a higher proportion of swabs was contaminated. The two *E. coli* strains investigated (NCTC12900 and ATCC11229) were recovered from surfaces with differing frequencies, despite surviving similarly in washing-up water at 48 °C and during air-drying for 72 h at 20 °C. It appears that *E. coli* 0157 NCTC12900 may attach to the sponge more firmly and therefore transfer less frequently than *E. coli* ATCC11229, highlighting the importance of strain choice when using one isolate to represent a larger group of bacteria. Bacteria transferred from dishes onto food more frequently when the dish had been towel-dried rather than air-dried but the overall frequency of transfer was low. Moisture was lost during overnight storage of a wet sponge at 21 °C, probably exposing remaining bacterial contaminants to increased desiccation stress. This could explain the decrease in the proportion of contaminated surface swabs when the stored sponge was used to wipe the surface.

From this study, we recommend that washing-up water is used at the maximum possible temperature (using gloves to achieve this) and, if possible, a dish-washing machine is used that can reach much higher temperatures. There is a relatively small risk of viable bacteria that have survived washing-up and drying on dish surfaces being able to contaminate food placed onto the dish but the contamination of tea towels and washing-up sponges has implications for domestic hygiene. Frequent replacement or decontamination of tea towels and washing-up sponges is recommended.

Acknowledgements

We would like to thank Procter & Gamble for funding this work.

References

Anonymous, 1995. Enumeration of micro-organisms. In: Roberts, D., Hooper, W., Greenwood, M. (Eds.), Practical Food Microbiology, 2nd ed. The Public Health Laboratory Service, London, UK, pp. 102–107.

Anonymous, 1996. Intestinally pathogenic *Escherichia coli*. In: Roberts, T.A., Baird-Parker, A.C., Tompkin, R.B. (Eds.), Micro-organisms in Foods—Microbiological Specifications of Food Pathogens, 1st ed. Blackie Academic & Professional, London, UK, pp. 126–140.

Anonymous, 2002. Factors affecting growth and survival of *Salmonella*. In: Bell, C., Kyriakides, A. (Eds.), *Salmonella*—A Practical Approach to the Organism and its Control in Foods, 1st ed. Blackwell, London, UK, pp. 84–98.

Asbury, E.D., 1983. Methods for testing sanitizers and bacteriostatic substances. In: Block, S.S. (Ed.), Disinfection, Sterilization and Preservation, 3rd ed. Lea & Febiger, Philadelphia, pp. 964–980.

Bale, M.J., Bennett, P.M., Beringer, J.E., Hinton, M., 1993. The survival of bacteria exposed to desiccation on surfaces associated with farm buildings. Journal of Applied Bacteriology 75, 519–528.

Beumer, R.R., te Giffel, M.C., Spoorenberg, E., Rombouts, F.M., 1996. *Listeria* species in domestic environments. Epidemiology and Infection 117, 437–442.

Bradford, M.A., Humphrey, T.J., Lappin-Scott, H.M., 1997. The cross contamination and survival of *Salmonella enteritidis* PT4 on sterile and non-sterile foodstuffs. Letters in Applied Microbiology 24, 261–264.

Cowden, J.M., Wall, P.G., Adak, G., Evans, H., Le Baigue, S., Ross, D., 1995. Outbreaks of foodborne infectious intestinal disease in England and Wales: 1992 and 1993. Communicable Disease Report Review 5, R109–R117.

de Boer, E., Hahné, M., 1990. Cross-contamination with *Campylobacter jejuni* and *Salmonella* spp. from raw chicken products during food preparation. Journal of Food Protection 53, 1067–1068.

Francis, G.A., O'Beirne, D., 2001. Effects of vegetable type, package atmosphere and storage temperature on growth and survival of *Escherichia coli* O157:H7 and *Listeria monocytogenes*. Journal of Industrial Microbiology and Biotechnology 27, 111–116.

Hazeleger, W.C., Wouters, J.A., Rombouts, F.M., Abee, T., 1998. Physiological activity of *C. jejuni* far below the minimal growth temperature. Applied and Environmental Microbiology 64, 3917–3922.

Humphrey, T.J., 1981. The effects of pH and levels of organic matter on the death rates of *Salmonella* in chicken scald-tank water. Journal of Applied Bacteriology 51, 27–39.

Humphrey, T.J., 1990. Heat resistance in *Salmonella enteritidis* phage type 4: the influence of storage temperatures before heating. Journal of Applied Bacteriology 69, 493–497.

Humphrey, T.J., 2002. *Campylobacter* spp.: not quite the tender flowers we thought they were? Microbiology Today 29, 7–8.

Humphrey, T.J., Mason, M., Martin, K., 1995a. The isolation of *Campylobacter jejuni* from contaminated surfaces and its survival in diluents. International Journal of Food Microbiology 26, 295–303.

Humphrey, T.J., Slater, E., McAlpine, K., Rowbury, R.J., Gilbert, R.J., 1995b. *Salmonella enteritidis* phage type 4 isolates more tolerant of heat, acid or hydrogen peroxide also survive longer on surfaces. Applied and Environmental Microbiology 61, 3161–3164.

Jørgensen, F., Bailey, R., Williams, S., Henderson, P., Wareing, D.R.A., Bolton, F.J., Frost, J.A., Ward, L., Humphrey, T.J., 2002. Prevalence and numbers of *Salmonella* and *Campylobacter* spp. on raw, whole chickens in relation to sampling methods. International Journal of Food Microbiology 76, 151–164.

Juneja, V.K., Eblen, B.S., 2000. Heat inactivation of *Salmonella typhimurium* DT104 in beef as affected by fat content. Letters in Applied Microbiology 30, 461–467.

Kadan, R.S., Marten, W.H., Micklesen, R., 1963. Effects of ingredients used in condensed and frozen dairy products on thermal resistance of potentially pathogenic staphylococci. Applied Microbiology 11, 45–49.

Kelly, A.F., Park, A.F., Bovill, R., Mackey, B.M., 2001. Survival of *Campylobacter jejuni* during stationary phase: evidence for the absence of a phenotypic stationary-phase response. Applied and Environmental Microbiology 67, 2248–2254.

Kotrola, J.S., Connor, D.E., 1997. Heat inactivation of *Escherichia coli* O157:H7 in turkey meat as affected by sodium chloride, sodium lactate, polyphosphate and fat content. Journal of Food Protection 60, 898–902.

Kramer, J.M., Frost, J.A., Bolton, F.J., Wareing, D.R.A., 2000. *Campylobacter* contamination of raw meats and poultry at retail sale: identification of multiple types and comparison with isolates from human infection. Journal of Food Protection 63, 1654–1659.

Line, J.E., Fain, A.R., Moran, A.B., Martin, L.M., Lechowich, R.V., Carosella, J.M., Brown, W.L., 1991. Lethality of heat to *Escherichia coli* O157:H7: *D*-value and *Z*-value determinations in ground beef. Journal of Food Protection 54, 762–766.

Mattick, K.L., Jørgensen, F., Legan, J.D., Cole, M.B., Porter, J., Lappin-Scott, H.M., Humphrey, T.J., 2000. Survival and filamentation of *Salmonella enterica* serovar Enteritidis PT4 and *Salmonella enterica* serovar Typhimurium DT104 at low water activity. Applied and Environmental Microbiology 66, 1274–1279.

Mattick, K.L., Jørgensen, F., Legan, J.D., Lappin-Scott, H.M., Humphrey, T.J., 2001a. Habituation of *Salmonella* spp. at reduced water activity and its effect on heat tolerance. Applied and Environmental Microbiology 66, 4921–4925.

Mattick, K.L., Jørgensen, F., Legan, J.D., Lappin-Scott, H.M., Humphrey, T.J., 2001b. Improving recovery of *Salmonella enterica* serovar Typhimurium DT104 cells injured by heating at different water activity values. Journal of Food Protection 64, 1472–1476.

Miles, A.A., Misra, S.S., 1938. The estimation of bactericidal power of blood. Journal of Hygiene 38, 732–749.

Montville, R., Chen, Y.H., Schaffner, D.W., 2002. Risk assessment of hand washing efficacy using literature and experimental data. International Journal of Food Microbiology 73, 305–313.

Popowska, M., Kloszewska, M., Gorecka, S., Markiewicz, Z., 1999. Autolysis of *Listeria monocytogenes*. Acta Microbiologica 48, 141–152.

Ryan, M.J., Wall, P.G., Gilbert, R.J., Griffen, M., Rowe, B., 1996. Risk factors for outbreaks of infectious intestinal disease linked to domestic catering. Communicable Disease Report Review 6, R179–R183.

Scott, E., Bloomfield, S.F., 1990. The survival and transfer of microbial contamination via cloths, hands and utensils. Journal of Applied Bacteriology 68, 271–278.

Semanchek, J.J., Golden, D.A., 1998. Influence of growth temperature on inactivation and injury of *Escherichia coli* O157:H7 by heat, acid and freezing. Journal of Food Protection 61, 395–401.

Senhaji, A.F., 1977. The protective effect of fat on the heat resistance of bacteria. Journal of Food Technology 12, 203–216.

Shere, L., 1948. Some comparisons of the disinfectant properties of hypochlorites and quaternary ammonium compounds. Milk Plant Monthly 37, 66–69.

Solomon, E.B., Hoover, D.G., 1999. *Campylobacter jejuni*: a bacterial paradox. Journal of Food Safety 19, 121–136.

Sommer, R., Lhotsky, M., Haider, T., Cabaj, A., 2000. UV inactivation, liquid-holding recovery, and photoreactivation of *Escherichia coli* O157 and other pathogenic *Escherichia coli* strains in water. Journal of Food Protection 63, 1015–1020.

Stringer, S.C., George, S.M., Peck, M.W., 2000. Thermal inactivation of *Escherichia coli* O157:H7. Journal of Applied Microbiology Symposium Supplement 88, 79S–89S.

Tebbutt, G.M., 1984. A microbiological study of various food premises with an assessment of cleaning and disinfection practices. Journal of Hygiene (London) 93, 365–375.

Tebbutt, G.M., 1986. An evaluation of various working practices in shops selling raw and cooked meats. Journal of Hygiene (London) 97, 81–90.

Wilde, S., Jørgensen, F., Mattick, K.L., Humphrey, T.J., 2001. Development and study of tests to differentiate between tolerant and sensitive isolates of *Salmonella* and *Escherichia coli* O157. Food Standards Agency report code B01007.