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Kinetics of growth and inactivation of *Salmonella enterica* serotype Typhimurium DT104 in pasteurised liquid egg products

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ABSTRACT

The potential impact of post-pasteurisation contamination of liquid egg products with the multi-antibiotic resistant pathogen *Salmonella enterica* serotype Typhimurium definitive type 104 (DT104) was assessed by determining the viability of this bacterium in whole egg, albumen and 10% w/w sugared and salted yolk incubated at 4–42 °C. Results indicated that populations of *S.* Typhimurium DT104 were slowly inactivated in all four products when stored at 4 °C. However, based on the typical shelf-lives of cold-stored liquid egg, less than 0.6 log-kill would be achieved in those products prior to their use. Incubation at temperatures pertaining to abuse situations (10, 15, 20 and 25 °C) revealed an increasing potential for growth of *S.* Typhimurium DT104 in whole egg, albumen and sugared yolk, as indicated by trends in growth rate, lag duration and maximum population density. At even higher temperatures (30, 37 and 42 °C), growth rates of *S.* Typhimurium DT104 in whole egg and sugared yolk continued to increase. The same was true for *S.* Typhimurium DT104 in albumen except that growth was not observed at 42 °C and instead populations were inactivated within 30 h. At no temperature tested was *S.* Typhimurium DT104 able to grow in salted yolk. The influence of these growth and inactivation patterns on the risk of salmonellosis in relation to product type and storage temperature is discussed.

1. Introduction

The widespread use of antibiotics in agriculture and human medicine has raised concern about the emergence of antibiotic resistant bacteria and their impact on public health. One study conducted in the US indicated that the prevalence of strains of *Salmonella enterica* serotype Typhimurium resistant to five antibiotics increased from 0.6% in 1979/1980–34% in 1996 (Glynn et al., 1998). The growing incidence of multi-antibiotic resistances in *S.* Typhimurium appears to be a worldwide phenomenon (Evans and Davies, 1996; Hollingsworth and Kaplan, 1997; Kam, 1996; Mackie et al., 1996; Martel et al., 1995; Ramos et al., 1996). Of particular concern is a multi-antibiotic resistant strain of *S.* Typhimurium known as definitive phage type 104 (DT104) that is an increasing cause of salmonellosis infection worldwide and is associated with a greater risk of hospitalisation, invasive illness and death (Helms et al., 2005; Varma et al., 2005).

S. Typhimurium DT104 is typically resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline. Molecular studies have indicated that the genes encoding those resistances are contained on the bacterial chromosome (Ridley and Threlfall, 1998; Threlfall et al., 1994) and, therefore, it is unlikely that removal of selective pressure by antibiotics will rid this bacterium of its resistant properties compared to plasmid carriage. The identification of *S.* Typhimurium DT104 isolates that are also resistant to trimethoprim and ciprofloxacin (Threlfall et al., 1996) further limits the means by which these infections can be treated.

Humans acquire *S.* Typhimurium DT104 infection primarily through consumption of animal food products. Outbreaks have been linked to poultry, meat and meat products and unpasteurised dairy products (Cody et al., 1999; Dechet et al., 2006; Ethelberg et al., 2007; Grein et al., 1999; Villar et al., 1999). Contamination of these foods is typically *via* direct contact with faecal matter of infected animals, for example during hide removal and gut evisceration following slaughter. Currently, the control of foodborne *S.* Typhimurium DT104 infection at food processing and preparation levels relies upon well-established strategies that are typical of those for other salmonellae (Hogue et al., 1997), including adherence to good manufacturing practices, heat-treating foods during pasteurisation or cooking, avoiding cross-contamination of other foods and storage at cool temperatures. However, because infectious agents may be intentionally or unintentionally added to foods

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post-production, it is also useful to identify the potential for pathogens to survive in foods after processing, based on the inherent food environment (e.g. the presence of antimicrobial molecules or competitive microflora) and extrinsic factors (e.g. storage time and temperature, and in-house cooking).

The current project was conducted to assess the ability of *S.* Typhimurium DT104 to survive and grow in four liquid egg products including whole egg, albumen (egg white) and 10% sugared and salted yolk at a range of temperatures relevant to the manufacture and storage of these products. Parameters of viability curves were determined and can be incorporated into modelling programs that, in conjunction with risk assessment, may be used to determine the likelihood of associated foodborne disease outbreaks occurring.

2. Materials and methods

2.1. Strains used and preparation of inocula

S. Typhimurium DT104 strains #10 TX and #7470C-1, isolated from poultry and swine, respectively, were used in this study. The strains were maintained in nutrient broth (CM0001, Oxoid, Adelaide, Australia) supplemented with 30% glycerol (Sigma, Melbourne, Australia) at −80 °C. Experimental inocula were cultured from frozen stocks and grown on brilliant green agar (CM0263, Oxoid) supplemented with 0.8 g/l sulfadiazine ("BGS agar"; Sigma) and the antibiotics ampicillin, chloramphenicol, streptomycin and tetracycline (Sigma) at 25 μg/ml each with incubation at 37 °C for 18 (±2) h. Cells were inoculated into peptone buffered saline (PBS; BR0014, Oxoid) and absorbance was determined to estimate population density. A two-strain cocktail was prepared with equal amounts of each strain in PBS to achieve a concentration of approximately 2.5×10^6 CFU/ml.

2.2. Liquid egg products used and assessment of background microflora

Four frozen and pasteurised, liquid egg products including whole egg, albumen, 10% w/w sugar yolk and 10% w/w salted yolk were obtained from an Australian egg supplier, transported under cold-storage and stored at −20 °C until use. Aliquots were thawed by incubation at 4 °C (for yolk samples) and 10 °C (for whole egg and albumen samples) for 16 (±2) h prior to use. Product specifications from the supplier indicated low numbers of aerobes (1×10^4 CFU/g), coliforms (<10 CFU/g), yeasts and moulds (<20 CFU/g) and *Salmonella* (not detected in 1 g) in the liquid egg products and this was corroborated in the current study prior to inoculation with *S.* Typhimurium DT104. Specifically, undiluted amounts (4×250 μl) of each liquid egg product were plated to plate count agar (CM0463, Oxoid; for an aerobic plate count), violet red bile glucose agar (CM0485, Oxoid; to detect Enterobacteriaceae), dichloran rose-bengal chloramphenicol agar (CM0727 and SR0078, Oxoid; for the enumeration of yeasts and moulds) and BGS agar (for the enumeration of *Salmonella* spp.). Plates were incubated at 37 °C for 24 h, except for dichloran rose-bengal chloramphenicol agar, which were incubated at 25 °C for 5 days, and CFU were enumerated.

2.3. Preparation of egg samples and inoculation with *S.* Typhimurium *DT104*

Experimental samples were prepared by weighing 50 (±0.5) g of each liquid egg product to a sterile 50 ml centrifuge tube. The tubes were incubated at the appropriate temperature (4, 10, 15, 20, 25, 30, 37 or 42 °C) for 1 h to equilibrate to temperature. In triplicate, 100 μl of the two-strain cocktail containing *S.* Typhimurium DT104 #10 TX and #7470C-1 was added to 50 g volumes of each liquid egg product, thus providing an initial cell density of approximately 5×10^3 CFU/g. The inoculated egg samples were mixed thoroughly by manual inversion and incubation on a roller deck (Luckham, Burgess Hill, UK) for 10 min. Inoculated egg samples were incubated at the appropriate temperature, which was monitored at 10 s intervals using a temperature data logger (TinyTag Ultra 2, Hastings Data Loggers, Port Macquarie, Australia). Temperatures were within ±0.7 °C of the required temperature for the duration of each trial.

2.4. Enumeration of survivors and determination of growth or inactivation rates

At appropriate intervals aliquots were withdrawn and, where necessary, serially diluted in PBS. Diluted samples were surface-plated using a spiral plater (Autoplate 4000, Spiral Biotech, Bethesda, USA) onto BGS agar with antibiotics. When cell viability was expected to be low, undiluted aliquots (0.5–0.15 g) were manually plated. Plates were incubated at 37 °C for 24 (±1) h and typical red-coloured colonies were counted. Viability was determined over a period that ranged from 24 h, when liquid eggs were incubated at 42 °C, to 3076 h (i.e. 128 d), when stored at 4 °C.

Viability curves were constructed by plotting viable numbers ($\log_{10}$ CFU/g) against time. For each product type and temperature combination, the viability of *S.* Typhimurium DT104 was determined in triplicate, providing 96 growth or, in some instances, inactivation curves. When viable cells could not be detected in a 0.5 g amount of an undiluted sample (i.e. < 1.30 $\log_{10}$ CFU/g) the data were not used to estimate the inactivation rate. When appropriate, the inactivation rate was determined by linear regression fitted to the data using Microsoft® Excel. Where growth occurred, the viability data were fitted using the Baranyi D model (Baranyi and Roberts, 1994) in DMFit Version 2.1 software kindly provided by the Institute of Food Research, Norwich, UK. This primary model was used to obtain estimates of growth rate (maximum potential rate), lag period and maximum population density (MPD). The effect of temperature on those parameters was tested using various regression models starting with a separate slopes model for each liquid egg product using SAS.

3. Results

The viability of *S.* Typhimurium DT104 in commercially-sourced whole egg, albumen, sugared yolk and salted yolk stored at specific temperatures ranging from 4 to 42 °C was determined (see Fig. 1 for examples of the typical growth or inactivation curves obtained). The ability of *S.* Typhimurium DT104 to survive and grow in liquid egg, as indicated by inactivation or growth rate, lag period and MPD, is shown in Table 1. At 15–37 °C, the growth of *S.* Typhimurium DT104 in albumen occurred at two distinct rates. That is, a faster rate of growth preceded a slower rate that commenced when the cell density was between 10^6 and 10^7 $\log_{10}$ CFU/g. When this occurred, the viability curve was fitted with two log-linear models, providing a growth rate for the first and second phases of growth.

Results indicated that there was no growth of *S.* Typhimurium DT104 in any of the liquid egg products stored at 4 °C. Instead, viability decreased by 2.25 $\log_{10}$ CFU/g to below detectable limits (i.e. 1.30 $\log_{10}$ CFU/g) in all three replicates within approximately 2400 h (i.e. 100 d) in albumen and salted yolk products and within 3000 h (i.e. 125 d) in whole egg and sugared yolk (data not shown).

As shown in Table 1, when eggs were incubated at 10–37 °C, growth was observed in whole egg, sugared yolk and albumen, although a relatively slower rate of growth was observed in the albumen at each temperature. In salted yolk incubated at 10–37 °C, the *S.* Typhimurium DT104 population was inactivated and the

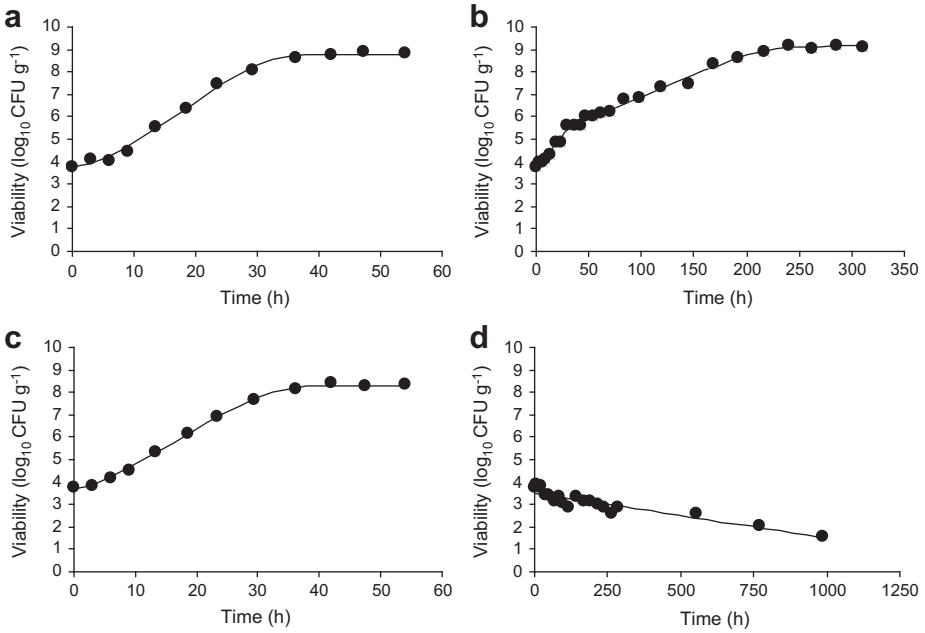

Fig. 1. Representative viability curves, with fitted models, for *S.* Typhimurium DT104 in liquid (a) whole egg, (b) albumen, (c) 10% sugared yolk and (d) 10% salted yolk incubated at 20 °C. Data pertaining to a single replicate, typical of triplicate samples, are shown for each product type.

mean rate of inactivation increased with temperature. At 42 °C, growth of *S.* Typhimurium DT104 was observed only in whole egg and sugared yolk, whereas inactivation occurred in the other two product types. In all instances where growth of *S.* Typhimurium DT104 occurred, the mean MPD achieved was between 7.95 and 9.23 $\log_{10}$ CFU/g.

Fig. 2 shows the relationship between temperature and rate of growth of *S.* Typhimurium DT104 in the three liquid egg products in which growth occurred. The growth rate data for *S.* Typhimurium DT104 in whole egg and sugared yolk are well described by the respective square root models (average $R^2 = 0.9737$), which indicated that growth rate significantly ($p > 0.001$) increased with temperature in the range 10–42 °C. The models fitted to the data for *S.* Typhimurium DT104 in whole egg and sugared yolk are described in Equations (1) and (2), respectively.

$$\sqrt{\text{growth rate } (\log_{10}\text{CFU/g/h})} = -0.0737 + [0.0268 \times \text{temp.}(^{\circ}\text{C})] \quad (R^2 = 0.9787) \qquad (1)$$

$$\sqrt{\text{growth rate } (\log_{10}\text{CFU/g/h})} = -0.0326 + [0.0215 \times \text{temp.}(^{\circ}\text{C})] \quad (R^2 = 0.9787) \qquad (2)$$

The slopes of the two curves are significantly different ($p > 0.001$) indicating that the effect of temperature in each product type varies. In contrast, the rate of growth of this bacterium in albumen, which included two rates at some temperatures, was not well described by linear regression fitted to the square root growth rate data ($R^2 = 0.3564$).

Overall, a large amount of variability was observed in the duration of the lag phase in growing *S.* Typhimurium DT104 populations in liquid egg products. Analysis of the effect of temperature on the lag period (Fig. 3) indicated that, for temperatures in the 10–42 °C range, a single model (Equation (3)) could be fitted to the combined data sets for whole egg and sugared yolk.

$$\sqrt{[1/\text{lag(h)}]} = 0.1021 + [0.0182 \times \text{temp.}(^{\circ}\text{C})] \quad (R^2 = 0.8056) \qquad (3)$$

Except at 10 and 15 °C, the growth of *S.* Typhimurium DT104 in albumen did not include a lag phase and, therefore, was not modelled. At those temperatures, the mean lag period for *S.* Typhimurium DT104 in albumen was 1380.10 h (i.e. 57.5 d) at 10 °C and 40.79 h at 15 °C.

The relationship between temperature and MPD reached by *S.* Typhimurium DT104 populations in liquid egg is given in Fig. 4. In the temperature range 10–42 °C, the mean MPD achieved in each product was always lowest at 10 °C. The mean MPD was greatest (9.23 $\log_{10}$ CFU/g) when inoculated into whole egg and stored at 25 °C. However, when this bacterium was in albumen or sugared yolk, the maximum mean MPD achieved occurred at 20 and 42 °C, respectively, indicating a varied relationship between temperature and MPD in each product type. The quadratic models fitted to the data for whole egg, albumen and sugared yolk are given in Equations (4)–(6), respectively.

$$\text{MPD } (\log_{10}\text{CFU/g/h}) = 6.9045 + [0.1664 \times \text{temp.}(^{\circ}\text{C})] - [0.003 \times \text{temp.}(^{\circ}\text{C})]^2 \quad (R^2 = 0.6981) \qquad (4)$$

$$\text{MPD } (\log_{10}\text{CFU/g/h}) = 6.0893 + [0.2283 \times \text{temp.}(^{\circ}\text{C})] - [0.0047 \times \text{temp.}(^{\circ}\text{C})]^2 \quad (R^2 = 0.7411) \qquad (5)$$

$$\text{MPD } (\log_{10}\text{CFU/g/h}) = 7.3285 + [0.077 \times \text{temp.}(^{\circ}\text{C})] - [0.0009 \times \text{temp.}(^{\circ}\text{C})]^2 \quad (R^2 = 0.9056) \qquad (6)$$

4. Discussion

To ensure the production of microbiologically sound foods, the processes of manufacture, distribution and storage use well-placed deleterious stresses to either inhibit or inactivate contaminating microorganisms. The potential for pathogenic microorganisms to

Table 1
Growth and inactivation characteristics of *S.* Typhimurium DT104 in liquid egg products stored at 4–42 °C.

Product	Temp. (°C)	Rate (log_{10} CFU/g/h)	Lag time (h)	MPD (log_{10} CFU/g)
Whole egg	4	−0.00053 (±0.00015)	–	–
	10	0.028 (±0.002)	5.84 (±7.40)	8.07 (±0.04)
	15	0.103 (±0.005)	15.23 (±4.28)	9.18 (±0.10)
	20	0.182 (±0.012)	3.66 (±1.39)	8.80 (±0.02)
	25	0.459 (±0.004)	4.21 (±0.26)	9.23 (±0.10)
	30	0.574 (±0.025)	2.39 (±0.38)	9.14 (±0.10)
	37	0.860 (±0.020)	2.91 (±0.24)	9.04 (±0.04)
	42	0.993 (±0.033)	1.46 (±0.18)	8.65 (±0.31)
Albumen	4	−0.00067 (±0.00003)	–	–
	10	0.011 (±0.005)	1380.10 (±690.20)	7.98 (±0.24)
	15	0.073 (±0.006)[a] 0.009 (±0.001)[b]	40.79 (±1.26)	8.13 (±0.16)
	20	0.061 (±0.008)[a] 0.021 (±0.001)[b]	0	9.13 (±0.11)
	25	0.098 (±0.005)[a] 0.040 (±0.021)[b]	0	8.75 (±0.24)
	30	0.107 (±0.014)[a] 0.041 (±0.011)[b]	0	8.57 (±0.08)
	37	0.091 (±0.017)[a] 0.055 (±0.019)[b]	0	8.08 (±0.21)
	42	−0.02191 (±0.02863)	–	–
10% sugared yolk	4	−0.00102 (±0.00055)	–	–
	10	0.028 (±0.001)	28.08 (±7.92)	7.95 (±0.47)
	15	0.067 (±0.003)	10.36 (±3.60)	8.46 (±0.03)
	20	0.158 (±0.001)	3.04 (±0.11)	8.34 (±0.04)
	25	0.333 (±0.012)	3.50 (±0.10)	8.69 (±0.03)
	30	0.427 (±0.011)	1.78 (±0.41)	8.92 (±0.04)
	37	0.490 (±0.012)	1.25 (±0.24)	8.85 (±0.07)
	42	0.759 (±0.035)	1.27 (±0.23)	8.99 (±0.03)
10% salted yolk	4	−0.00069 (±0.00006)	–	–
	10	−0.00095 (±0.00012)	–	–
	15	−0.00100 (±0.00019)	–	–
	20	−0.00231 (±0.00072)	–	–
	25	−0.00254 (±0.00018)	–	–
	30	−0.00487 (±0.00185)	–	–
	37	−0.00505 (±0.00055)	–	–
	42	−0.23910 (±0.01718)	–	–

MPD, Maximum population density.
Each value is the mean (±standard deviation) of triplicate product samples.
– Not applicable because growth did not occur.
[a] Rate for first phase of growth, where multiple phases occurred.
[b] Rate for second phase of growth.

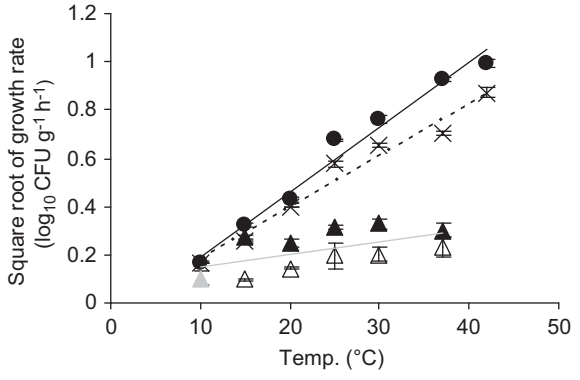

Fig. 2. Square root model showing the effect of temperature (10–42 °C) on the mean growth rate of *S.* Typhimurium DT104 in liquid whole egg (●), albumen (▲ for first phase of growth; △ for second phase; and ▲ when only one phase was observed) and 10% sugared yolk (×). Bars indicate standard deviation ($n = 3$). The regression equations fitted to the data for liquid whole egg (black line), albumen (combined data; grey line) and 10% sugared yolk (dashed line) are $y = 0.0268x - 0.0737$ ($R^2 = 0.9787$), $y = 0.0054x + 0.0914$ ($R^2 = 0.3564$) and $y = 0.0215x - 0.0326$ ($R^2 = 0.9687$), respectively.

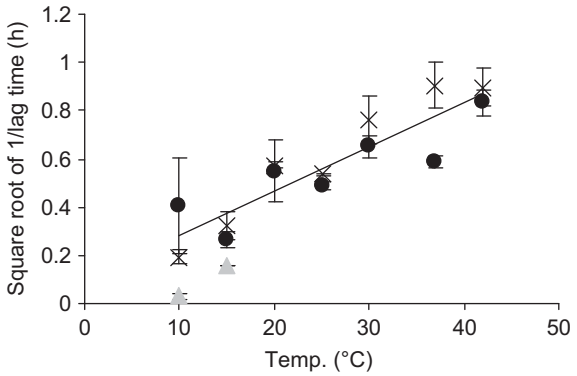

Fig. 3. Square root model showing the effect of temperature (10 – 42 °C) on the mean lag period of *S.* Typhimurium DT104 in liquid whole egg (●), albumen (▲) and 10% sugared yolk (×). Bars indicate standard deviation ($n = 3$). The regression equation fitted to the combined data for liquid whole egg and 10% sugared yolk (black line) is $y = 0.0182x + 0.1021$ ($R^2 = 0.8056$).

be introduced to food sources after some, or all, of the inimical treatments are applied is a major concern in food security. In order to focus efforts to protect food supplies from contamination, information pertaining to the ability of a pathogen to survive and multiply in foods is required. In this study, four commonly used liquid egg products were assessed for their potential to support the growth and survival of the multi-antibiotic resistant *S.* Typhimurium DT104 when stored at temperatures relevant to commercial egg processing or potential temperature abuse.

When liquid egg products were stored at 4 °C, as advised by the product manufacturer, the viability of *S.* Typhimurium DT104 gradually decreased. However, the rates of inactivation observed were low. The manufacturer's guidelines for the use of the frozen liquid egg products used in this study recommend that whole egg, albumen and 10% sugared yolk be used within 72 h of thaw and liquid 10% salted yolk within five weeks. From the current trial, the maximum amount of kill of *S.* Typhimurium DT104 that is likely to be achieved in those times is 0.04–0.07 log_{10} CFU/g in the unsalted products and 0.58 log_{10} CFU/g in salted yolk. A higher level of inactivation might be achieved in unfrozen, liquid egg products due to increased duration of storage at 0–4 °C added by the distribution stage. Despite some level of inactivation of *S.* Typhimurium DT104 occurring in liquid egg products at 4 °C, there is potential for this

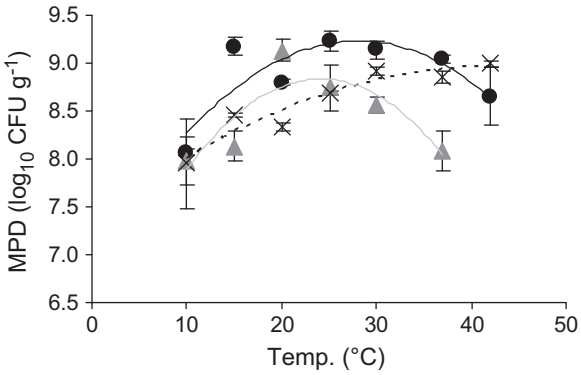

Fig. 4. The effect of temperature (10–42 °C) on the mean MPD of *S.* Typhimurium DT104 in liquid whole egg (●), albumen (▲) and 10% sugared yolk (×). Bars indicate standard deviation ($n = 3$). The regression equations fitted to the data for liquid whole egg (black line), albumen (grey line) and 10% sugared yolk (dashed line) are $y = -0.003x^2 + 0.1664x + 6.9045$ ($R^2 = 0.6981$), $y = -0.0047x^2 + 0.2283x + 6.0893$ ($R^2 = 0.7411$) and $y = -0.0009x^2 + 0.077x + 7.3285$ ($R^2 = 0.9056$), respectively.

bacterium to survive until time of use, depending on the initial level and timing of contamination.

The risk of liquid egg products, stored at 4 °C, acting as vehicles for salmonellosis will depend largely on the hurdles to survival that are applied following storage, such as cooking. The ability of thermal treatments to kill salmonellae cells is well established and, therefore, unless massive levels of contamination have occurred, it appears unlikely that liquid egg products contaminated with *S.* Typhimurium DT104, stored at 4 °C and adequately cooked prior to consumption, will lead to large outbreaks of illness. A greater food biosecurity risk is associated with cold-stored, liquid egg products that are consumed uncooked or 'lightly cooked', typically as ingredients in hollandaise sauce, meringue, home-made ice cream and mayonnaise, and raw cookie dough. Outbreaks of salmonellosis associated with some of those foods (CDC, 1990, 1994, 2000; Mitchell et al., 1989) is indicative of the capacity for *Salmonella* to survive in these products. Those incidences of infection, in conjunction with the current work, suggest that risk prevention strategies for salmonellae in liquid eggs should consider post-pasteurisation contamination of cold-stored liquid egg products that are not cooked.

To assess the effect of temperature abuse on the growth and survival of *S.* Typhimurium DT104 in liquid egg products, inoculated samples were stored at temperatures above that recommended for distribution and storage, including 10 and 15 °C and ambient temperatures (20 and 25 °C). Higher temperatures (30, 37 and 42 °C) were also included to allow for patterns in temperature effects on the viability of *S.* Typhimurium DT104 to be observed. It was found that the relationship between temperature and the potential for *S.* Typhimurium DT104 to grow and survive was highly dependent on the liquid egg product involved, with growth most likely to occur in whole egg and 10% sugared yolk followed by albumen. Growth was not observed at any temperature tested when *S.* Typhimurium DT104 was inoculated into 10% salted yolk.

Growth of *S.* Typhimurium DT104 was observed in whole egg and sugared yolk when stored at 10–42 °C and in albumen when stored at 10–37 °C. Growth characteristics such as growth rate, lag time and MPD were most similar between whole egg and sugared yolk and it was observed that in these egg products increasing temperature caused an increase in growth rate. *S.* Typhimurium DT104 populations increased by 5-log units within two days when stored at temperatures of 20 °C and above. Even at 15 °C, viable numbers increased by 5-log units within 72 h from thaw and, within the same time frame, increased by 2-log units when stored at 10 °C. Similar findings using other *Salmonella* serovars have indicated the potential for growth of these species in whole egg and yolk (Bradshaw et al., 1990; Clay and Board, 1991; Gast and Holt, 2000; Kim et al., 1989; Saeed and Koons, 1993) and, along with the current work, highlight the necessity to store these products at 4 °C or less to prevent the growth of pathogenic bacteria, regardless of the mode or timing of contamination.

The higher pH, iron restriction and presence of antimicrobial compounds (e.g. lysozyme) in egg white may account for the reduced rate of growth observed in growing populations of *S.* Typhimurium DT104 in liquid albumen relative to that in whole egg and sugared yolk at each temperature (Baron et al., 1997, 1999; Garibaldi, 1960; Garibaldi et al., 1969; Kang et al., 2006). While these characteristics of albumen inhibit growth of *S.* Typhimurium DT104 in the temperature range 10–37 °C, they are not actually lethal to the cell. Therefore, the viability of this bacterium could be expected to remain constant or to increase, depending on the duration of storage and presence or absence of a lag phase, following post-processing contamination when stored at temperatures of 10 °C or greater. Again, these results emphasise the importance of storing liquid egg products at 4 °C.

In this study, a single log-linear profile did not always describe the growth of *S.* Typhimurium DT104 populations in albumen because at 15, 20, 25 and 37 °C two distinct rates of growth occurred. Similar biphasic growth curves have been observed in pure cultures suspended in broth and have been attributed to the bacterial population using two carbon sources, one of which was exhausted prior to the second one being used (Monod, 1947). In the more complex environment of albumen the emergence of two exponential growth phases might also be caused by the concomitant increase in background microflora that results in direct competition for nutrients and/or produces compounds that inhibit the growth of *S.* Typhimurium DT104. Alternatively, the increasing number of *S.* Typhimurium DT104 cells might create competition for exogenously-limited compounds, such as iron, regardless of the presence of background microbiota. Identification of the trigger for the second, slower phase of growth might be useful in developing new methods to inhibit the potential growth of pathogens in liquid egg.

Similarly to incubation at 4 °C, the viability of *S.* Typhimurium DT104 in albumen was reduced when stored at chicken body temperature (42 °C). At the higher temperature, inactivation may result from the combination of heat stress with high pH, reduced iron availability and the antimicrobial activity of proteins such as ovotransferin and lysozyme (Ibrahim et al., 2000, 2001). Nevertheless, this pathogen was able to survive in albumen at 42 °C for over 30 h, which is longer than the average length of time for egg formation in the chicken oviduct (Johnson, 2000). The implications of this in regards to vertical transmission of *S.* Typhimurium DT104 is unclear since this model is based solely on albumen composition and temperature and other factors are likely to affect the viability of *Salmonella* in the chicken oviduct. Indeed, in chickens experimentally-infected with *S.* Typhimurium, Keller et al. (1997) reported a high incidence of this bacterium in forming eggs (i.e. eggs recovered from the chicken oviduct) but no viable cells were found in laid eggs.

The addition of 10% salt to egg yolk creates an additional hurdle to the growth and survival of bacteria (Leistner, 1994) and, in this study, caused populations of *S.* Typhimurium DT104 to be inactivated during storage at 4–42 °C. The rate of inactivation increased with temperature. The strong effect of non-lethal temperature on the inactivation rate of bacteria exposed to growth-preventative conditions has been described elsewhere (Ross et al., 2008). Unlike the other liquid egg products assessed during the current investigation, characteristics of 10% salted yolk completely prevent the growth of *S.* Typhimurium DT104 and, therefore, incubation of this food at temperatures above 4 °C will not have a detrimental impact on its microbiological safety. However, storage at any non-lethal temperature cannot be relied upon to eliminate *S.* Typhimurium DT104 and cooking, or some other post-pasteurisation stress, is required. With that said, without added salt, egg yolk is a suitable medium for *Salmonella* growth (Clay and Board, 1991; Humphrey, 1990) and, therefore, the addition of salt during manufacture is an important means of preventing proliferation of contaminating pathogens should temperature abuse occur during distribution and storage.

The data generated in this study were compared to 82 records similarly derived and sourced from ComBase (www.combase.cc; accessed 3rd December, 2008). Growth/no growth trends observed in the ComBase data were identical to the current investigation except when *S.* Typhimurium DT104 was in albumen at 10 °C. That discrepancy can be attributed to differences in experiment duration, since the mean lag time observed under those conditions in this study (i.e. 1380.10 h) extended beyond the time that viability was assessed in the ComBase data (i.e. 260 h). Quantitatively, there were some minor discrepancies in the mean rate, lag period and

MPD values for equivalent products and storage temperatures. The quantitative variability could not be attributed to experimental aspects, since they were consistent between studies, but may have been due to the liquid egg products being sourced from separate countries. Differences between those products would likely include background microflora, diet-driven nutrient components and pasteurisation processes that affect antimicrobial activities and other attributes. That such differences between studies did not significantly affect the viability of *S.* Typhimurium DT104 is validation of the general growth and survival trends described above. The identification of these patterns in behaviour will be useful in implementing guidelines to reduce the risk of foodborne illness from post-processing contamination.

This study has assessed the potential impact of post-processing contamination of four pasteurised, liquid egg products with the multi-antibiotic resistant *S.* Typhimurium DT104. From our results, when these products are stored at 4 °C, used within the recommended time-frame and cooked prior to consumption, there is unlikely to be a significant risk of illness in consumers. However, because liquid egg products are pasteurised during manufacture, they are often used as ingredients in foods that are not heat-treated during food preparation, representing an increased risk to consumer safety.

Further investigations highlighted the potential for growth of *S.* Typhimurium DT104 in liquid whole egg, albumen and 10% sugared yolk when proper cold-storage at 0–4 °C is not used. As reported by others (Bradshaw et al., 1990; Clay and Board, 1991; Gast and Holt, 2000; Kim et al., 1989; Saeed and Koons, 1993), under those conditions, the potential for outbreaks of infection are increased because typical heat treatments may not reduce the pathogen load to a safe level. Studies have indicated that the number of poultry isolates of *S.* Typhimurium DT104 is increasing at a greater rate than in any other reservoir (Hogue et al., 1997; Poppe et al., 2002; Threlfall, 2002) and, therefore, so too is the potential for contamination of liquid egg products at various stages during manufacture. The information obtained during the current investigation will be used in combination with modelling programs to provide a more detailed assessment of the risk of foodborne disease outbreaks associated with liquid egg products.

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