ELSEVIER

Journal of Cleaner Production 9 (2001) 69–73

Journal of Cleaner Production

www.cleanerproduction.net

Notes from the field

Preliminary attempts towards production of table eggs free from *Salmonella enteritidis*

Elie K. Barbour [a,*], Liana El Jurdi [a], Carine Issa [b], Raja Tannous [b]

[a] *Department of Animal Science, Faculty of Agricultural and Food Sciences, American University of Beirut, P.O. Box 11-0236, Beirut, Lebanon*
[b] *Food Technology and Nutrition Department, Faculty of Agricultural and Food Sciences, American University of Beirut, P.O. Box 11-0236, Beirut, Lebanon*

Received 17 October 1999; accepted 30 April 2000

Abstract

The aim of this preliminary work is to reach to a cleaner production of intact shell-table eggs free from *Salmonella enteritidis* (SE) contamination, by application of pasteurization and dry heat. Three highly virulent strains of SE, previously reported to the World Animal Health Organization, were used in this study. The three strains acquired plasmid profiles similar to SE isolates reported in severe food illness of humans. The plasmid profiles of the three SE strains were: strain 1 (14.1 kb), strain 2 (14.1 and ≈50.0 kb), and strain 3 (1.8, 14.1, and ≈50.0 kb).

Each of the three SE strains grown up to 10 h (stationary phase) and adjusted to the same light transmission of 12% at a wavelength of 540 nm, was homogenized with yolk of eggs to form three individual inocula. Strain 1 inoculum to eggs resulted in initial SE count/ml of egg content equivalent to 1.80×10^{19} colony forming unit (cfu); however, strain 2 inoculum resulted in 2.3×10^{19} cfu, and strain 3 inoculum resulted in 2.20×10^{19} cfu ($P>0.05$). Treatment of inoculated intact shell eggs in a hot water bath at 57°C for 25 min, followed by application of hot air at 55°C for another 57 min resulted in the highest reduction in SE count/ml of the egg content for strain 1 (average 33.33 cfu), followed by strain 2 (average of 0.73×10^{4} cfu), and strain 3 (average of 1.60×10^{4} cfu). All counts after treatment were significantly less than the initial count before treatment ($P<0.05$).

Decreasing the density of the SE in the three inocula by about 13 logs to be 1.80×10^{6} cfu/ml of egg content for strain 1, 2.34×10^{6} for strain 2, and 2.20×10^{6} for strain 3 ($P>0.05$) and applying the same pasteurization and dry heat treatment on inoculated eggs resulted in complete absence of viability in the three strains, averaging zero cfu/ml of egg content.

The pasteurization and dry heat treatment of intact shell-table eggs results in significant reduction of the high initial count of SE strains, regardless of their plasmid profile or density of contamination. The production of table eggs completely clean of SE could be obtained by the same treatment when the initial contamination is low (around 10^{6} cfu/ml of egg content).

Keywords: *Salmonella enteritidis*; Eggs; Pasteurization; Dry heat

1. Introduction

Salmonella enteritidis (SE) infection increased in many parts of the world in the late 1970s, emerging as a major source of salmonellosis in Europe, North America, and South America by the mid-1980s [1]. The SE pandemic continued through the late 1980s, and its incidence in humans increased in two-thirds of the 35 countries reporting to the World Health Organization [2]. This higher incidence was mostly related to chicken eggs contaminated with SE, thus exacting a considerable toll on eggs, as public confidence in the safety of eggs began to decline [2]. Evidently some strains of SE had become more invasive in chickens reaching the ovary, oviducts, and eggs [3].

The estimated number of human SE cases in the USA alone is 200,000 to 1 million annually, leading to economic losses due to SE foodborne illness ranging from $200 million to $1 billion annually [4]. The economic implications of SE on human cases with foodborne illness is associated with an economic impact on egg producers. A significant example would be the food scare

* Corresponding author. Tel.: +961-3-346-194; fax: +961-1-744460.
E-mail address: eb01@aub.edu.lb (E.K. Barbour).

0959-6526/01/$ - see front matter
PII: S0959-6526(00)00033-0

initiated by a senior U.K. health official's comment linking SE-outbreaks in humans with eggs; this declaration immediately resulted in a 60% decline in table-egg consumption in the UK [5].

The surge of SE cases and outbreaks in humans resulted in implementation of an emergency test and slaughter program in Great Britain in 1988, with compensation to the owners of SE-positive layer flocks [3]. This program was discontinued a few years later, after millions of pounds had been spent and hundreds of thousands of birds destroyed. A less drastic program was initiated in the USA in 1990 to trace back from human SE outbreaks where eggs were implicated, to the flocks of origin, to test them for SE, and to divert the eggs from positive flocks to plants specializing in pasteurization of liquid-egg contents [3]. Evaluation of both programs showed that SE was still widely distributed in egg-layer flocks, that the rates had not decreased, and that SE was even more prevalent in table eggs than before [3].

This failure of implemented programs by the UK and the USA demands focus on treatment of eggs to kill the invasive SE organisms in their contents, to reach for a cleaner production. Surveys by the US Department of Agriculture demonstrated recovery of SE from liquid-egg samples before pasteurization [6]. The proportion of liquid-egg samples positive for SE was 23%, detected in 630 randomly tested samples in egg-breaking plants. Unfortunately, phage type 4 of SE was detected in 21 (12%) of the liquid-egg samples collected throughout the USA in 1995. This phage type 4-SE variant had not been found previously in poultry in the USA, but was the type mainly responsible for the rapid spread and increase in European countries.

Actually, phage type 4 is the invasive type of SE carrying plasmid of ≈50 kb [7]. It is worth mentioning that the possession of a plasmid by SE organisms of about 50 kb (38 MDa) is essential for its high virulence and invasiveness, helping the infection to reach the ovary and oviducts and consequently to the egg content and hatched offspring [7].

The objective of this preliminary work is to study the impact of pasteurization and dry heat treatment on production of intact shell-table eggs free from *Salmonella enteritidis*.

2. Materials and methods

2.1. SE strains

Three SE strains were used in this study. The three strains were previously reported to the World Animal Health Organization as highly invasive and virulent to chickens with the capability of transmission to the internal contents of the egg through infected oviducts of chickens [8]. The three strains were having plasmid profiles similar to SE isolates reported in fooborne human SE infections [9,10]. The plasmid profiles of the three SE strains were: strain 1 (14.1 kb), strain 2 (14.1 and ≈50.0 kb), and strain 3 (1.8, 14.1 and ≈50.0 kb). The three strains were stored individually on triple sugar iron (TSI) agar slants, at −20°C.

2.2. SE growth to stationary phase

In order to determine the time needed for each of the three strains to reach to stationary phase, counts of the colony forming units per milliliter of a broth culture were determined at different times. More specifically, the frozen TSI agar cultures of the three strains were left at room temperature for an hour, and each culture was subcultured individually into a separate 100 ml of tryptose phosphate broth (TPB) medium, using a sterile loop. The TPB cultures were left at 37°C. SE counts were done at 4, 6, 8, 10, 12, 14, and 16 h of incubation. Bacterial count was done using a microtiter plate-serial dilution, with a dilution factor of 1:10. The maximum dilution was 1 in 10^{20}. The diluent used in each well of the microtiter plate was 90 μl of sterile saline. An amount of 20 μl of each dilution was plated on brilliant green agar plate and incubated at 37°C for 48 h. Only plated dilutions showing 30 to 300 colonies were considered for count. Calculation of counts of each of the three SE strains was done according to the following formulae:

$$\textit{Count}(\textit{colony forming units per milliliter}) = \textit{colony count of plated dilution} \times \textit{dilution factors} \times 50$$

The multiplication by 50 is done to change from count per 20 μl to count per 1000 μl, or one milliliter.

In addition, the bacterial density in the TPB culture creating turbidity was assessed at different incubation times by measuring the percent light transmission in the three individual SE cultures at a wave length of 540 nm, using a spectrophotometer (Spectronic 20, Bausch and Lomb, Rochester, New York, USA).

2.3. SE-egg inoculation in high count

The three individual inocula of the three SE strains were prepared in their determined stationary phase, reached after 10 h of TPB culture—incubation at 37°C. More specifically, the frozen TSI cultures of the three individual SE strains were kept at room temperature for an hour, and each strain was subcultured by a sterile loop into individual 100 ml of TPB. The TPB cultures of the three SE strains were left at 37°C for 10 h. Each of the three SE cultures was adjusted to a similar light transmission of 12% at a wavelength of 540 nm, using sterile saline diluent. Yolks of three table eggs free of *Salmonella* were pooled in a sterile beaker and mixed together

with a sterile magnetic stirrer. An amount of 1 ml of each 10 h-TPB SE culture was added over 9 ml of the pooled yolk (dilution of 1 in 10), thus finalizing the preparation of the SE inocula. Each of three eggs, from a layer flock free of *Salmonella* infection, was injected through the shell and inside the yolk with a 50 μl of each inoculum, using tuberculin syringe with 20-gauge needle. The number of three eggs per treatment used in this study is universally approved in food microbiology evaluation research [11]. House cement was used to seal the hole in the egg shell after delivering the inoculum. The initial counts before treatment (cfu/ml of egg content) were determined based on the count in the prepared inoculum and the average volume of contents of four eggs, equivalent to 56.75 ml.

2.4. SE-egg inoculation in low count

The same procedure described before was used in preparation of the three inocula of the three SE strains, using 10 h incubation of TPB cultures at 37°C to reach to the stationary phase. The light transmission was adjusted to 12% for all isolates; however, a further dilution of 1 in 10^{13} was used for each isolate after light transmission was adjusted, using sterile saline. A volume of 1 ml of the 1 in 10^{13} dilution of SE culture was added over 9 ml of pooled egg yolk.

The rest of the procedure for egg inoculation, using the same number of eggs per treatment, is as mentioned previously. The initial counts before treatment in the inoculated eggs were determined based on the count of the prepared inoculum and the average volume of contents of four eggs, equivalent to 56.75 ml.

2.5. Egg treatment and SE recovery

Eggs injected by high or low counts of the three SE strains were treated in a hot water bath set at 57°C for 25 min, followed by transfer of the eggs to a hot oven set at 55°C for another 57 min. Three control eggs were left uninjected at room temperature for a period of 82 min.

SE recovery (count) from internal content of each egg was attempted by mixing the content of the egg for 1 min in a separate sterile beaker, using a sterile magnetic stirrer. Serial dilution of the egg content was done on a microtiter plate using a dilution factor of 1 in 10. The maximum dilution used was 1 in 10^{10}. The diluent used in each well was 180 μl of sterile saline. An amount of 25 μl of each dilution was plated on Brilliant Green agar plate and incubated at 37°C for 48 h. Plated dilutions showing 30–300 colonies were considered for count. Calculation of SE count was done according to the formulae:

Count(*colony forming units per milliliter of egg content*)

$$= \textit{colony count of plated dilution} \times \textit{dilution factor} \times 40$$

The multiplication by 40 is to change from count per 25 μl to count per 1000 μl, or one milliliter.

2.6. Statistics

The Chi square (χ^2) was used to compare between SE counts. This procedure was accomplished using a statistical computing program (M Stat Computer Program, Michigan State University, Michigan, USA).

3. Results and discussion

The growth of the three SE strains in tryptose phosphate broth between 4–16 h of incubation at 37°C is shown in Table 1. The stationary phase of the three strains followed the log phase at 10–12 h of incubation. Strains acquiring three plasmids of 1.8, 14.1, and ≈50 kb was able to reach to a higher count at its stationary phase (3.95×10^{22} cfu/ml) than that obtained by strain 1 acquiring one plasmid of 14.1 kb (2.20×10^{20} cfu/ml) and by strain 2 acquiring two plasmids of 14.1 and ≈50 kb (2.35×10^{20} cfu/ml). This is in agreement with previous reports suggesting that different SE strains may express different growth potentials [12–14].

The recovery of SE from internal contents of eggs after injection by a similar ($P>0.05$) high initial count of each of the three strains (around 10^{19} cfu/ml of egg content) followed by pasteurization and dry heat treatment, is shown in Table 2. As the number of acquired plasmid(s) by SE increased the resistance to pasteurization and dry heat treatment increased accordingly. The average counts of SE (cfu/ml of egg content) following treatment of eggs for the three strains was: strain 3 (1.60×10^4) > strain 2 (7.3×10^3) > strain 1 (3.3×10). It is worth mentioning that the number of plasmids in strains 1, 2, and 3 was 1, 2, and 3, respectively. The ≈50 kb plasmid was present in strains 2 and 3 only, associated with a certain degree of resistance to this treatment of eggs. More elaborate investigation is needed to study the role of the presence of ≈50 kb plasmid in SE strains to resistance to pasteurization and dry heat treatment. The control-non injected eggs were shown to be free of *Salmonella* organisms.

The recovery of SE from internal contents of eggs after injection by a similar low initial count ($P>0.05$) of each of the three strains (around 10^6 cfu/ml of egg content) followed by pasteurization and dry heat treatment, is shown in Table 3. Regardless of the number of plasmid(s) acquired by SE strains, the pasteurization and dry heat treatment was able to completely clean the eggs from low numbers of contaminating SE organisms. This is a promising result to the egg industry, especially that natural infection levels of SE-positive eggs is reported

Table 1
Growth of the three SE strains differing in their plasmid profiles and identification of approximate time needed to reach the stationary phase[a]

Incubation time (h)	SE strains and their plasmid profiles Strain 1 (14.1 kb) Av.* count	%light trans.†	Strain 2 (14.1 and ≈50 kb) Av. Count	%light trans.	Strain 3 (1.8, 14.1 and ≈50 kb) Av. Count	% light trans.
4	1.65×10^{12}	88	2.25×10^{12}	91	1.40×10^{12}	81
6	1.75×10^{16}	21	1.90×10^{16}	23	1.65×10^{16}	18
8	1.80×10^{18}	17	2.00×10^{18}	18	1.85×10^{18}	17
10	1.80×10^{20}	16	2.35×10^{20}	18	2.20×10^{20}	17
12	2.20×10^{20}	16	2.65×10^{18}	17	3.95×10^{22}	16
14	2.10×10^{16}	16	2.30×10^{16}	17	2.55×10^{17}	16
16	1.55×10^{12}	16	1.75×10^{12}	17	1.60×10^{12}	16

[a] *Average of two plating for each dilution.†Light transmission at a wave length of 540 nm.

Table 2
Recovery (count) of SE from internal contents of eggs after injection of a high initial count of each of the three SE strains followed by pasteurization and dry heat treatment*[a]

SE strain	Plasmid profile (kb)	Egg	Recovery (count) of SE (cfu/ml of egg content) Initial count	Count following treatment
1	14.1	1	1.80×10^{19}	0.0
		2	1.80×10^{19}	1.0×10^{2}
		3	1.80×10^{19}	0.0
		Av.	$(1.80\times10^{19})^{\dagger}$	$(3.3\times10)^{\ddagger}$
2	14.1 and ≈50.0	1	2.34×10^{19}	6.8×10^{3}
		2	2.34×10^{19}	5.6×10^{3}
		3	2.34×10^{19}	9.6×10^{3}
		Av.	$(2.34\times10^{19})^{\dagger}$	$(7.3\times10^{3})^{\ddagger}$
3	1.8, 14.1 and ≈50.0	1	2.20×10^{19}	1.60×10^{4}
		2	2.20×10^{19}	1.56×10^{4}
		3	2.20×10^{19}	1.64×10^{4}
		Av.	$(2.20\times10^{19})^{\dagger}$	$(1.60\times10^{4})^{\ddagger}$
	Control§	1	0.0	0.0
		2	0.0	0.0
		3	0.0	0.0
		Av.	0.0†	0.0†

[a] *Treatment of SE-injected eggs was in a water bath at 57°C for 25 min followed by a transfer to a hot oven set at 55°C for another 57 min.§Control eggs were uninjected and left at room temperature for 82 min.†,‡Averages significantly different ($P<0.05$).

Table 3
Recovery (count) of SE from internal contents of eggs after injection of a low initial count of each of the three SE strains followed by pasteurization and dry heat treatment*[a]

SE strain	Plasmid profile (kb)	Egg	Recovery (count) of SE (cfu/ml of egg content) Initial count	Count following treatment
1	14.1	1	1.80×10^{6}	0.0
		2	1.80×10^{6}	0.0
		3	1.80×10^{6}	0.0
		Av.	$(1.80\times10^{6})^{\dagger}$	$(0.0)^{\ddagger}$
2	14.1 and ≈50.0	1	2.34×10^{6}	0.0
		2	2.34×10^{6}	0.0
		3	2.34×10^{6}	0.0
		Av.	$(2.34\times10^{6})^{\dagger}$	$(0.0)^{\ddagger}$
3	1.8, 14.1 and ≈50.0	1	2.20×10^{6}	0.0
		2	2.20×10^{6}	0.0
		3	2.20×10^{6}	0.0
		Av.	$(2.20\times10^{6})^{\dagger}$	$(0.0)^{\ddagger}$
	Control§	1	0.0	0.0
		2	0.0	0.0
		3	0.0	0.0
		Av.	0.0†	0.0†

[a] *Treatment of SE-injected eggs was in a water bath at 57°C for 25 min followed by a transfer to a hot oven set at 55°C for another 57 min.§Control eggs were uninjected and left at room temperature for 82 minutes.†,‡Averages significantly different ($P<0.05$).

to be less than 10 cfu/egg [15,16]. Another report showed that natural infection levels in SE-positive eggs is less than 100 cfu/100 g of yolk [17]; It was also shown that SE location in eggs from naturally infected flocks is in both albumen and yolk [15]. This justifies the approach followed in this study in which samples were homogenized from yolk and albumen content of each egg for assessing recovery of SE post treatment of intact shell-table eggs.

A previous report on the use of the same nature of treatment of water bath heating at 57°C for 25 min followed by hot air heating at 55°C for another 57 min resulted in acceptable overall functionality of pasteurized intact shell eggs, based on examination of lysozyme activity and other physical properties of eggs [11]. This is another promising evidence proving that the use of the same nature of treatment will not affect the physical properties of the intact shell eggs, rendering them acceptable by the consumer.

In conclusion, this treatment can produce intact shell-table eggs completely clean of SE, regardless of the nature of acquired plasmid(s); this result could be obtained on the condition that the initial contamination by SE is low (around 10^6 cfu/ml of egg content). This treatment will produce eggs free of *Salmonella enteritidis* and most likely other *Salmonellae*, thus preventing the occurrence

of salmonellosis outbreaks in humans during egg handling and consumption, which will help in significant reduction in *Salmonella* propagation inside the gastrointestinal tracts of infected people, and by that reduce significantly the fecal shedding of these organisms to the environment.

References

[1] Rodrigue DC, Tauxe RV, Rowe B. International increase in *Salmonella enteritidis*: a new pandemic? Epidemiol Infect 1990;105:21–7.

[2] Gomez TM, Molarjemi Y, Miyagawa S, Kaferstein FK, Stohr K. Foodborne salmonellosis. World Health Stat Q 1997;50:81–9.

[3] Mason J. Introduction: *Salmonella enterica* serovar Enteritidis current status and a recommendation for its prevention and control. In: Saeed AM, Gast RK, Potter ME, Wall PG, editors. *Salmonella enterica* serovar Enteritidis in humans and animals. Ames, IA: Iowa State University Press, 1999:267–9.

[4] Morales RA, McDowell RM. Economic consequences of *Salmonella enterica* serovar Enteritidis infection in humans and the U.S. egg industry. In: Saeed AM, Gast RK, Potter ME, Wall PG, editors. *Salmonella enterica* serovar Enteritidis in humans and animals. Ames, IA: Iowa State University Press, 1999:271–90.

[5] Kinderlerer JL. *Salmonella* in eggs. BNF Nutr Bull 1994;19:11–8.

[6] Ebel ED, Hogue AT, Schlosser WD. Prevalence of Salmonella enterica serovar Enteritidis in unpasteurized liquid eggs and aged laying hens at slaughter: implications on epidemiology and control of the disease. In: Saeed AM, Gast RK, Potter ME, Wall PG, editors. *Salmonella enterica* serovar Enteritidis in humans and animals. Ames, IA: Iowa State University Press, 1999:341–51.

[7] Chart H, Threlfall EJ, Rowe B. Virulence of *Salmonella enteritidis* phage type 4 is related to the possession of a 38 Mda plasmid. Fed Eur Microbiol Soc Microbiol Lett 1989;58:299–304.

[8] Barbour EK, Jurdi LH, Talhouk R, Qatanani M, Eid A, Sakr W, Bouljihad M, Spasojevic R. Emergence of *Salmonella enteritidis* outbreaks in broiler chickens of Lebanon: epidemiologic markers and competetive exclusion control. Sci Tech Rev J WAHO 1999;18(3):710–8.

[9] Bichler LA, Nagaraja KV, Pomeroy BS. Plasmid diversity in *Salmonella enteritidis* of animal, poultry, and human origin. J Food Protect 1994;57:4–11.

[10] Saeed AM, Nair US. Use of molecular biological markers in the epidemiological study of *Salmonella enterica* servar Enteritidis infections in humans and animals. In: Saeed AM, Gast RK, Potter ME, Wall PG, editors. *Salmonella enterica* serovar Enteritidis in humans and animals. Ames, IA: Iowa State University Press, 1999:161–70.

[11] Hou H, Singh RK, Muriana PM, Stadelman WJ. Pasteurization of intact shell eggs. Food Microbiol 1996;13:93–101.

[12] Gast RK, Beard CW. Detection and enumeration of *Salmonella enteritidis* in fresh and stored eggs laid by experimentally infected hens. J Food Protect 1992;55:152–6.

[13] Humphrey TJ, Chapman PA, Rowe B, Gilbert RJ. A comparative study of the heat resistance of salmonellas in homogenized whole eggs, egg yolk or albumen. Epidemiol Infect 1990;104:237–41.

[14] Saeed AM, Koons CW. Growth and heat resistance of *Salmonella enteritidis* in refrigerated and abused eggs. J Food Protect 1993;56:927–31.

[15] Humphrey TJ, Baskerville A, Mawer S, Rowe B, Hopper S. *Salmonella enteritidis* from the contents of intact PT4 eggs: a study involving naturally infected hens. Epidemiol Infect 1989;103:415–23.

[16] Humphrey TJ, Witehead A, Growler AHL, Henley A. Numbers of *Salmonella enteritidis* in the contents of naturally contaminated hens eggs. Epidemiol Infect 1991;106:489–96.

[17] Hopper SA, Mawer S. *Salmonella enteritidis* in a commercial layer flock. Vet Rec 1988;123:351.