Taylor & Francis
Taylor & Francis Group

Observations on *Salmonella* contamination of eggs from infected commercial laying flocks where vaccination for *Salmonella enterica* serovar Enteritidis had been used

Robert Davies* and Mark Breslin

Department of Food and Environmental Safety, Veterinary Laboratories Agency — Weybridge, New Haw, Addlestone, Surrey KT15 3NB, UK

Eggs were collected monthly from 12 cage-layer flocks on four farms where *Salmonella* Enteritidis was present in vaccinated flocks despite vaccination with an *S.* Enteritidis bacterin. Where possible, hens were also taken for culture at the end of the laying period, and faecal and environmental samples were taken from the laying houses before and after cleaning and disinfection. Twenty-four batches of six egg shells from the 13 652 tested (0.18% [0.11 to 0.26 CI^{95}] single egg equivalent) were positive for *S.* Enteritidis and 54 (0.40% [0.30 to 0.52 CI^{95}] single egg equivalent) for other serovars. Six batches of 13 640 (0.04% [0.02 to 0.10 CI^{95}] single egg equivalent) egg contents, bulked in six egg pools, contained *S.* Enteritidis and three batches contained other serovars. In addition three further batches contained *S.* Enteritidis in both contents and shells, and two other batches contained other serovars in both. The total level of contamination by *S.* Enteritidis of both contents and shells found in vaccinated flocks was therefore 33 batches/13 682 eggs(0.24% [0.17 to 0.34 CI^{95}] single egg equivalent). The total of contamination for any *Salmonella* serovar was 92 batches/13 682 eggs (0.68% [0.55 to 0.84 CI^{95}] single egg equivalent). These results contrast with the findings of testing of eggs from three unvaccinated flocks prior to this study where 21 batches of egg shells from a total of 2101 eggs (1.0% [0.63 to 1.56 CI^{95}] single egg equivalent) and six batches of contents from 2051 eggs (0.29% [0.11 to 0.64 CI^{95}] single egg equivalent) were contaminated with *S.* Enteritidis. *S.* Enteritidis was found in 67/699 (9.6%) of vaccinated spent hens and 64/562 (11.4%) of bulked fresh faecal samples taken from laying houses. Failure to adequately clean and disinfect laying houses and to control mice appeared to be a common feature on the farms.

Introduction

The global spread of *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) in chickens (Ward *et al.*, 2000; Rabsch *et al.*, 2001) has resulted in an international food poisoning pandemic (Rodrigue *et al.*, 1990; ACMSF, 2001; Almonacid *et al.*, 2002). Although statutory action has been taken at breeding flock level, contaminated eggs and egg products remain the main source of infection (Hayes *et al.*, 1999; Molbak & Neimann, 2002). The special facility of *S.* Enteritidis to cause prolonged infection of the avian reproductive tract

has been a major factor in vertical transmission of the organism from breeding flocks and internal contamination of eggs is thought to have been the major factor in its spread (Guard-Petter, 2001). *Salmonella* Enteritidis localizes in glandular parts of the reproductive tract, such as the magnum and isthmus and ovarian granulosa cells (Thiagarajan *et al.*, 1994; Keller *et al.*, 1995) in a way that other food poisoning serovars such as *Salmonella* Typhimurium do not (Baker *et al.*, 1980; Okamura *et al.*, 2001). Chickens infected at 1 day old can remain as life-long carriers, with infection increasing during

stress, especially the onset of lay (Gast & Holt, 1998; Bercheri *et al.*, 2001). In one naturally infected flock 16% of 37 birds sampled had infection in the ovaries and 27% in the oviducts (Hoop & Pospischil, 1993). Although the basis of the predilection for invasion of ovarian tissues and eggs is not fully explained, it is thought to involve factors such as binding of type 1 fimbriae to secretions of shell membrane producing glands (de Buck *et al.*, 2002), high molecular weight lipopolysaccharide and high-density growth (Petter, 1993; Parker *et al.*, 2001, 2002).

Recognizing the potential for ongoing contamination of laying farms and the success achieved by the broiler industry in controlling *S*. Enteritidis (Anonymous, 2001), the British Egg Industry Council introduced a requirement for vaccination of member flocks in 1997 (ACMSF, 2001; British Egg Industry Council, 2002). The commercial killed vaccine is based on a virulent strain of *S*. Enteritidis PT4 that showed good results in laboratory and early field trials (Timms *et al.*, 1990, 1994) but given under commercial iron limitation conditions and adjuvanted with alhydrogel. This paper describes the results of testing eggs, spent hens and poultry house faecal and environmental samples from vaccinated but infected flocks for *Salmonella* in an attempt to estimate the possible risk from such flocks under field conditions.

Materials and methods

Sample collection

Laying farms where *S*. Enteritidis was present were identified from notifications to the Zoonoses Order Database. A commercial killed *S*. Enteritidis vaccine ('Salenvac'; Intervet) had been used on all the flocks, administered by intramuscular injection given as two separate 0.5 ml doses at approximately 4 and 18 weeks of age during the rearing period. When voluntary agreement for intensive sampling had been obtained, the premises were visited and between 200 and 400 samples were taken at each farm to represent the separate flocks on the site. The samples were taken directly into 225 ml buffered peptone water (BPW) (1.07228; Merck) using gauze surgical swabs (Robinson Healthcare) and consisted of approximately 25 g faecal material or 10 to 15 g dust or other dry environmental samples or surface swabs. These were returned to the laboratory under ambient conditions and culture begun immediately.

Eggs were collected from each house into new boxes and returned to the laboratory under ambient conditions and stored overnight at room temperature. Chickens at the end of their productive life ('Spent Hens') were humanely killed, returned to the laboratory and stored at 4°C for up to 16 h before aseptic postmortem and culture of separate 25 g pools of the caeca and liver/spleen/ovary/oviduct. Tissue samples were thinly sliced with sterile scissors before culture.

Salmonella culture method

Eggs were cultured in batches of six. Eggs were carefully cracked and contents released into 500 ml BPW supplemented with 7 g/l beef heart infusion and this was incubated for 48 h at 37°C. Shells were placed in 225 ml BPW. After incubation of all samples 0.2 ml broth was inoculated into the substance of 20 ml DIASSALM medium (1.09803; Merck) in a petri dish and incubated at 41.5°C for 24 h; 1 μl inoculum from the edge of the opaque growth zone was then inoculated on to Rambach agar (1.07500; Merck). The DIASSALM plates and Rambach plates were then incubated for a further 24 h. The plates were examined and any DIASSALM plates on which the growth had spread widely, but which were negative for *Salmonella* on the Rambach plates, were replated. Suspect *Salmonella* colonies were confirmed by complete serotyping at the *Salmonella* reference laboratory at VLA — Weybridge according to the Kaufmann-White Scheme (Popoff, 2001).

Results

Table 1 presents a breakdown of *Salmonella* contamination of eggs and other samples from one individual farm, coded SUT. In flock A, no *Salmonella* was found in 570 eggs although *S*. Enteritidis was found in bulked faeces and environmental samples from the house. In house B no *Salmonella* was found in the contents of 726 eggs but one batch of six shells (equivalent to a minimum contamination rate of 0.14%) contained *S*. Enteritidis and two six-shell batches contained *Salmonella* Infantis or *Salmonella* Livingstone. In house 9 no *Salmonella* was found in the contents of 930 eggs but *S*. Infantis or *S*. Livingstone were found on the shells of 1.2% of batches. In house 10, *S*. Infantis was found in the contents of two (0.2%) six-egg batches totalling 912 eggs. *S*. Enteritidis was found in two batches of shells and *S*. Infantis on 39 (equivalent to a minimum contamination rate of 4.3% of individual shells). Contamination of eggs occurred sporadically throughout the year. Samples taken from the house after cleaning and disinfection (C&D) of house B showed an increase in the prevalence of *S*. Enteritidis, and an increase in other serovars on surfaces, compared with pre-C&D sampling. In houses 9 and 10 the level of *Salmonella* was high both before and after cleaning and disinfection. Both *S*. Infantis or *S*. Livingstone were found in fresh mouse faeces collected from disinfected houses.

Table 2 presents the results of sampling flocks in four houses on farm CK. Preliminary sampling in 2001 identified *S*. Enteritidis in contents and shells from two of the four infected flocks, and *Salmonella* Newport was found in and on eggs from a another flock. A gap in sampling occurred during the remainder of 2001 and regular sampling was resumed in February 2002, by which time all the original flocks had been replaced. Only occasional isolates of *Salmonella* were found on shells during this period. *S*. Enteritidis was only found in spent hens from house 4 and *S*. Newport was found in hens from house 2 and house 4. *S*. Enteritidis was found in bulked faeces and environmental samples from all the houses before C&D, and in both the two houses sampled after C&D the organism remained at a relatively high frequency, including in samples of mouse and rat faeces and flies.

Table 3 presents the results of testing eggs and other samples from a three-house site where both *S*. Enteritidis and *S*. Typhimurium were present. No *Salmonella* was found in 1110 eggs from house A even though *S*. Enteritidis was present in seven

Table 1. *Prevalence of* Salmonella *in eggs and other samples from vaccinated cage laying flocks: farm code SUT*

	Flock A		Flock B		Flock 9		Flock 10	
	Shells	Contents	Shells	Contents	Shells	Contents	Shells	Contents
January 2002	ND	ND	1/246 (0.4 to 2.4)	0/246	ND	ND	ND	ND
February	0/90	0/90	N/A	N/A	0/90	0/90	2/90 (2.2 to 13.3)	0[1]/90 (0)[1.1 to 6.7][a]
March	0/48	0/48	0/48#	0/48#	0[1]/90 (0)[1.1 to 6.7][a]	0/90	0[12]/90 (0)[20 to 80][a]	0/90
April	0/48	0/48	0[1]/48 (0)[2.2 to 12.5][a]	0/48	0/90	0/90	0[9]/72 (0)[12.5 to 75.0][a]	0/72
May	ND	ND	ND	ND	ND	ND	ND	ND
June	0/48	0/48	0/48	0/48	0/90	0/90	0[3]/60 (0)[5 to 30][a]	0/60
July	0/48	0/48	0/48	0/48	0[3]90 (0)[3.3 to20.0][b]	0/90	0[1]60 (0)[1.7 to 10][a]	0/60
August	0/48	0/48	0/48	0/48	0[2]/90 (0)[2.2 to 13.3][b]	0/90	0[2]/90 (0)[2.2 to 13.3][a]#	0/90#
September	0/48	0/48	0/48	0/48	0[1]/60 (0)[1.7 to 10.0][b]	0/60	0[10]/90 (0)[11.1 to 66.7][a]	0/90
October	0/48	0/48	0[1]/48 (0)[2.2 to 12.5][b]	0/48	0[4]/60 (0)[6.7 to 40][b]	0/60	0[2]90 (0)[2.2 to 13.3][a]	0/90
November	0/48	0/48	0/48	0/48	0/90	0/90	0/90	0/90
December	0/48	0/48	0/48	0/48	0/90	0/90	0/90	0/90
January	0/48	0/48	0/48	0/48	0/90	0/90	0[1]/90 (0)[1.1 to 6.7] [a]	0[1]/90 (0)1.1 to 6.7] [a]
Total	0/570	0/570	1[2]/726 (0.14 to 0.8)[0.3 to 1.7][ab]	0/726	0[11]/930 (0)[1.2 to 7.1][ab]	0/930	2[39]/912 (0.2 to 1.3)[4.3 to 25.7][a]	0[2]/912 (0)[0.2 to 1.3][a]
Spent hens	ND		1/75(1.3)[e]		ND		ND	
Bulked faeces	1[1]/20(5.0)[5.0][b]		1[3]/28(3.6) [10.7][ac]		0[15]/36 (0)[41.7][b]		0[33]/39 (0)[84.6][a]	
Environmental samples	1/20(5)		0[7]/52(0)[13.5][ab]		1[74]/84 (1.2) [88.1][b73,a]		0[55]/59 (0)[93.2][a]	
Post C&D	N/A		18[14]/79 (22.8)[17.7][a13,d]		0[41]/46 (0)[89.1][b]		0[176]/198 (0)[88.9][a]	
Wildlife (mouse droppings)	N/A		0/1		1/1[100][b]		1/1[100][a]	

Data presented as number of six-egg batches positive for *S.* Enteritidis PT4[other serotypes]/number of batches tested (possible range percentage positive for *S.* Enteritidis)[percentage positive range for other serovars]. ND, not done; N/A, not applicable; #, new flock.

[a] *S.* Infantis, [b] *S.* Livingstone, [c] *S.* Anatum, [d] *S.* Tennessee, [e] caeca and pooled tissues (liver, spleen, ovary and oviduct).

Table 2.   *Prevalence of* Salmonella *in eggs and other samples from vaccinated cage laying flocks: farm code CK*

	Flock 2		Flock 3		Flock 4		Flock 5	
	Shells	Contents	Shells	Contents	Shells	Contents	Shells	Contents
2001	3/498 (0.6 to 3.6)[a]	8/498 (1.6 to 9.6)[a]	4/498 (0.8 to 4.8)[a,b]	1/498 (0.2 to 1.2)[a]	0/500	0/500	0[1]/498 (0)[0.2 to 1.2][e]	0[1]/498 (0)[0.2 to 1.2][e]
February, all new flocks#	2/60 (3.3 to 20.0)[d]	0/60	1/60 (1.7 to 10.0)[c]	0/60	0/60	0/60	0/60	0/60
March	0/60	0/60	1/60 (1.7 to 10.0)[a]	0/60	N/A	N/A	0/60	0/60
April	2/60 (3.3 to 20)[2]	0/60	1/60 (1.7 to 10)[d]	0/60	N/A	N/A	0/60	0/60
May	0/60	0/60	N/A	N/A	1/60# (1.7 to 10.0)[1]	0/60#	0/60	0/60
June	0/60	0/60	ND	ND	0/60	0/60	0/60	0/60
July	0/60	0/60	0/60#	0/60#	0/60	0/60	0/60	0/60
August	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60
September	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60
October	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60
November	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60
December	N/A	N/A	0/60	0/60	0/60	0/60	N/A	N/A
January	0/150#	0/150#	0/150	0/150	N/A	N/A	N/A	N/A
Total	7/1248 (0.6 to 3.4)[a,b,d]	6/1248 (0.5 to 2.9)[a]	7/1188 (0.6 to 3.5)[a,b,c]	0/1188	1/1040 (0.1 to 0.6)[a]	0/1040	0[1]/1098 (0)[0.1 to 0.5][e]	0[1]/1098 (0)[0.1 to 0.5][e]
Spent hens (pooled tissues [P] and caeca [C])	0[1]/134 (0)[0.7][e], P & C		ND		31/164(18.9)[d], P & C, 27; C only, 4		1/75(1.3)[f], C only	
Bulked faeces	6/48(12.5)[a]		1/30(3.3)[a]		3/39(7.7)[a,d]		1/21(4.8)[a]	
Environmental samples	35/154(22.7)[a]		7/40(17.5)[a]		81/200(40.5)[a,b,d]		7/81(8.6)[a]	
Post C&D	27/196(13.8)[a]		ND		53/232(22.8)[a,b]		ND	
Wildlife								
Mouse[g]	1/2[a]		ND		8/9[a]		0/1	
Rat[g]	1/1[a]		ND		ND		ND	
Flies	1/2[a]		ND		ND		ND	

Data presented as number of six-egg batches positive for *S*. Enteritidis[other serotypes]/number of batches tested (possible range percentage positive for *S*. Enteritidis)[percentage positive range for other serovars]. ND, not done; #, new flock.
S. Enteritidis Phagetypes: [a] PT21B, [b] PT35, [c] PT4, [d] PT6.
Other serotypes: [e] *S*. Newport, [f] *S*. Agona.
[g] Faeces.

Table 3. *Prevalence of* Salmonella *in eggs and other samples from vaccinated cage laying flocks: farm code SGS*

	Flock A		Flock B		Flock C	
	Shells	Contents	Shells	Contents	Shells	Contents
2001	0/240	0/240	0/240	0/240	3/240 (1.25 to 7.5)	0/240
January 2002	0/90#	0/90#	0[1]/90 (0)#[1.1 to 6.7][a]	0/90#	N/A	N/A
February	0/90	0/90	0[1]/90 (0)[1.1 to 6.7][b]	0/90	N/A	N/A
March	0/90	0/90	0/90	0/90	0/90#	0/90#
April	0/90	0/90	0/90	0/90	0/90	0/90
May	0/90	0/90	0/90	0/90	0/90	0/90
June	0/90	0/90	ND	ND	0/90	0/90
July	0/60	0/60	ND	ND	0/60	0/60
August	0/30	0/30	0/30#	0/30#	0/30	0/30
September	0/60	0/60	0/60	0/60	0/60	0/60
October	0/90	0/90	0/90	0/90	0/90	0/90
November	N/A	N/A	0/30	0/30	0/30	0/30
December	ND	ND	ND	ND	ND	ND
January	0/90	0/90	0/90	0/78	0/90	0/90
Total	0/1110	0/1110	0[1]/990 (0)[0.1 to 0.6][ab]	0/978	3/960 (0.3 to 1.9)	0/960
Spent hens (pooled tissues [P] and caeca [C])	7/100(7.0), P = 6, C = 2, P+C = 1		19[2]/76 (25.0)[2.6][a], P = 6, C = 9, P+C = 6		8/75(10.7), C = 5, P = 5, C+P = 2	
Bulked faeces	9[5]/90 (10.0)[5.6][a]		16[18]/80 (20.0)[22.5][a]		19[6]/69 (27.5)[8.7][ac]	
Environmental samples	21[1]/45 (46.7)[2.2][a]		30[43]/85 (35.3)[50.6][a]		37[3]/44 (84.1)[6.8][a]	
Post C&D	14[1]/196 (7.1)[0.5][a]		61[76]/280 (21.8)[27.1][a]		58[3]/138 (42.0)[2.2][a]	
Wildlife						
Mouse faeces	0/2		1[5]/6(16.7)[83.3][a]		3/3(100.0)	
Flies	0/3		3[1]/5(60.0)[20.0][a]		N/A	
Litter beetles	0/2		N/A		N/A	

Data presented as number of six-egg batches positive for *S.* Enteritidis PT4[other serotypes]/number of batches tested (possible range percentage positive for *S.* Enteritidis)[percentage positive range for other serovars]. #, new flock; ND, not done; N/A, not applicable.
Other serotypes: [a] *S.* Typhimurium DT104, [b] *S.* Typhimurium DT204B, [c] *S.* Yoruba.

of 100 spent hens and in bulked faeces and environmental samples. In house B no *S*. Enteritidis was found in 990 shells and 978 contents but it was present in 19/76 (25%) of spent hens. *S*. Typhimurium was found in two batches (0.2%) of shells and in 2.6% of 76 spent hens. In house C, again no *Salmonella* was found in egg contents but *S*. Enteritidis was found in three batches (0.3% single egg equivalent) of 960 eggs and in eight batches (10.7%) of spent hens. Both *S*. Enteritidis and *S*. Typhimurium were found in bulked faeces in all the houses and in environmental samples both before and after C&D.

The results of testing samples from a single continuously occupied cage layer house are presented in Table 4. Vaccination was introduced gradually as various tiers of cages were depopulated and re-filled. Eggs were taken from vaccinated birds only. No egg contents contained *Salmonella* out of 2880 tested and only three batches of shells (0.1% single egg equivalent) were positive. In contrast *S*. Enteritidis was found in bulked faeces and environmental samples taken during various stages of repopulation with vaccinated birds. The prevalence of *Salmonella* decreased during this period but was still found in 7/62(11.3%) bulked faeces when the whole house had been re-stocked with vaccinated birds. *S*. Enteritidis was also isolated from mouse faeces and flies.

Table 5 presents the overall total of the results of culture of samples from all the 12 houses. Twenty-four batches of six eggs shells from the 13 652 tested (0.18% [0.11 to 0.26 CI[95]] single egg equivalent) were positive for *S*. Enteritidis and 54 (0.40% [0.30 to 0.52 CI[95]] single egg equivalent) for other serovars. Six batches of 13 640 egg contents (0.04% [0.02 to 0.10 CI[95]] single egg equivalent) contained *S*. Enteritidis and three batches contained other serovars. In addition (data not shown), three further batches contained *S*. Enteritidis in both

contents and shells, and two other batches contained other serovars in both. The total level of contamination by *S*. Enteritidis of both contents and shells found in vaccinated flocks was therefore 33 batches/13 682 eggs (0.24% [0.17 to 0.34 CI[95]] single egg equivalent). The total of contamination for any *Salmonella* serovar was 92 batches/13 682 eggs (0.67% [0.55 to 0.84 CI[95]] single egg equivalent). These results contrast with the findings of testing of eggs from three unvaccinated flocks prior to this study (data not shown) where 21 batches of egg shells from a total of 2101 eggs (1.0% [0.63 to 1.56 CI[95]] single egg equivalent) and six batches of contents from 2051 eggs (0.29% [0.11 to 0.64 CI[95]] single egg equivalent) were contaminated with *S*. Enteritidis. *S*. Enteritidis was found in 67/699 (9.6%) of vaccinated spent hens and 64/562 (11.4%) of bulked fresh faecal samples taken from laying houses. Serotypes other than *S*. Enteritidis were found in 0.4% of spent hens and 14.4% of bulked faeces samples, but the presence of *Salmonella* in the flocks was most readily detected by testing environmental samples such as spillage from egg belts, beneath cage stacks and dust, of which 25.6% and 19.0% of 961 samples contained *S*. Enteritidis or other serovars, respectively.

Table 6 presents a monthly breakdown of the contamination of egg shells and contents during a 13-month period when flocks were sampled each month. No *S*. Enteritidis was found in egg contents during this time but two batches contained other serovars. The contamination rate of shells was variable, ranging from 0 to 0.45 of batches by month, with no apparent pattern.

Discussion

In most studies of egg production from chickens infected with *S*. Enteritidis, shell contamination has exceeded that of contents (Bichler *et al.*, 1996;

Table 4. *Prevalence of* Salmonella *in eggs and other samples from vaccinated cage laying flocks: farm code CAV*

| | Shells | Contents | Bulked faeces | Environmental samples | Wildlife | |
					Mouse faeces	Flies
June 2002[h]	1/360 (0.3 to 1.7)[a]	0/360	N/A	N/A	N/A	N/A
July[f]	0/360	0/360	46/97 (47.0)[a]	46/98 (46.9)[c]	1/3[d]	1/3[a]
August	0/360	0/360	N/A	N/A	N/A	N/A
September	0/360	0/360	N/A	N/A	N/A	N/A
October	0/360	0/360	N/A	N/A	N/A	N/A
November	2/360 (0.6 to 3.3)[a]	0/360	N/A	N/A	N/A	N/A
December[g]	0/360	0/360	7/62 (11.3)[c]	26/97 (26.8)[ac]	1/1[c]	N/A
January	0/360	0/360	N/A	N/A	N/A	N/A
Total	3/2880 (0.1 to 0.6)[a]	0/2880	53/159 (33.3)[a]	72/195 (36.9)[c]	1/4[d]	1/3[a]

Data presented as number of six-egg batches positive for *S*. Enteritidis[other serotypes]/number of batches tested (possible range percentage positive for *S*. Enteritidis)[percentage positive range for other serovars]. N/A, not applicable.
S. Enteritidis Phagetypes: [a] PT4, [b] PT35, [c] RDNC, [d] PT7.
[f] Three-eights of flock vaccinated.
[g] All birds vaccinated.
[h] Hens in one of the eight stacks were vaccinated.

Table 5. *Total summary of prevalence of* Salmonella *in eggs and other samples from vaccinated cage laying flocks*

	Shells (six-egg batches/total number of individual eggs)	Contents (six-egg batches/total number of individual eggs)
Total	24 [54]/13 652 (0.18 to 1.05)[ijlm][0.4 to 2.37][acde]	6 [3]/13 640 (0.04 to 0.26)[l][0.02 to 0.13][cd]

	Spent hens	Bulked faeces	Environmental samples	Post C & D samples	Mouse droppings	Rat droppings	Flies
Total	67[3]/699 (9.6)[ij][0.4][ac]	64[81]/562 (11.4)[ijl][14.4][adefh]	246[183]/961 (25.6)[ijlm][19.0][ade]	231[338]/1365 (16.9)[ilm][24.8][adeg]	16[7]/26 (61.5)[il][26.9][ade]	1/1[4]	4[1]/10 (40.0)[ilo][10.0][a]

Data presented as number of six-egg batches positive for *S.* Enteritidis[other serotypes]/number of batches tested (possible range percentage positive for *S.* Enteritidis)[percentage positive range for other serovars].
S. Enteritidis phagetypes: [i] PT4, [j] PT6, [k] PT7, [l] PT21B, [m] PT35, [n] RDNC, [o] PT5a.
Other serotypes: [a] *S.* Typhimurium DT104, [b] *S.* Typhimurium DT204B, [c] *S.* Newport, [d] *S.* Infantis, [e] *S.* Livingstone, [f] *S.* Anatum, [g] *S.* Tennessee, [h] *S.* Yoruba.

Miyamoto *et al.*, 1997; Okamura *et al.*, 2001). One Spanish study found 1.1% of 372 egg surfaces from flocks implicated in food poisoning outbreaks to be contaminated compared with 0.5% in other flocks (Perales & Audicana, 1989). In one UK flock involved in an outbreak 5.2% of 194 egg surfaces were contaminated with *S.* Enteritidis PT4 (Humphrey *et al.*, 1989a). In contrast, no shell contamination was found in eggs from a free-range flock where 7.4% of the contents of 68 eggs were contaminated (Humphrey *et al.*, 1989b; Mawer *et al.*, 1989). In the current study the level of shell contamination varied between flocks but was considerably higher than the level of contamination of contents. The level of shell contamination

Table 6. *Seasonal variation of the prevalence of* Salmonella *in eggs*

	Shells	Contents
January 2002	1[1]/426 (0.23 to 1.4)[e][0.23 to 1.4][c]	0/426
February	5[1]/690 (0.7 to 4.3)[eh][0.1–0.9][d]	0[1]/690 [0.14 to 0.87][a]
March	1[13]/726 (0.14 to 0.8)[f][1.8 to 10.7][a]	0/726
April	3[10]/708 (0.4 to 2.5)[gh][1.4 to 8.5][a]	0/708
May	1/450 (0.2 to 1.3)[f]	0/450
June	1[3]/966 (0.1 to 0.6)[e][0.3 to 1.9][a]	0/966
July	0[4]/966 (0)[0.4 to 2.5][ab]	0/966
August	0[4]/966 (0)[0.4 to 2.5][ab]	0/966
September	0[11]/1026 (0)[1.1 to 6.4][ab]	0/1026
October	0[7]/1116 (0)[0.6 to 3.8][ab]	0/1116
November	2/936(0.2 to 1.3)[e]	0/936
December	0/756	0/756
January 2003	0[1]/1206(0)[0.08–0.5] [a]	0[1]/1194 [0.08 to 0.5] [a]
Total	14 to 84[55 to 330]/10938 (0.13 to 0.8)[efgh][0.5 to 3.0][efgh]	0[2 to 12]/10926 (0)[0.02 to 0.11][a]

Data presented as number of six-egg batches positive for *S.* Enteritidis[other serotypes]/number of batches tested (possible range percentage positive for *S.* Enteritidis)[percentage positive range for other serovars].
S. Enteritidis phagetypes: [e] PT4, [f] PT21B, [g] PT35, [h] PT6.
Other serovars: [a] *S.* Infantis, [b] *S.* Livingstone, [c] *S.* Typhimurium DT104, [d] *S.* Typhimurium DT204B.

usually correlates with visible faecal contamination of shells and with the degree of excretion of *Salmonella* in faeces (Gast & Beard, 1990; de Louvois, 1993) but *S.* Enteritidis originating from the oviduct can be found on shells even when no *Salmonella* is present in faeces (Humphrey *et al.*, 1991a). *Salmonella* on egg shell shows a rapid natural reduction in ambient conditions but survival may be prolonged in more humid or cold conditions (Baker, 1990) and some strains of *S.* Enteritidis show more prolonged survival than others (de Louvois, 1994). It is possible for egg contents to be contaminated via the shell, especially if contamination occurs before the cuticle has dried (Sparks & Board, 1985; Padron, 1990) or when shell quality is poor in older birds (Jones *et al.*, 2002). Contaminated shell may also cause cross-contamination in the kitchen or fragments may become included in bulked liquid egg products (Humphrey *et al.*, 1989a).

There are a large number of publications referring to studies of *Salmonella* contamination of eggs. In these studies eggs have usually been batched and shell and contents contamination not differentiated. In Great Britain a survey carried out in 1991 found 0.15% of eggs collected at retail outlets to be contaminated with *Salmonella* and 0.12% were contaminated with *S.* Enteritidis. The survey was repeated in 1995/1996 when a higher level of contamination was found (0.18% *Salmonella*, 0.15% *S.* Enteritidis) (ACMSF, 2001). In an earlier survey in 1989, 0.007% of eggs were found to be internally contaminated (Duguid & North, 1991). The results of the current study of eggs from known infected flocks suggested an overall minimum contamination rate of shells and contents combined of 0.24%. This is not much higher than the results of randomized surveys shown earlier, which would have included eggs from non-infected flocks. It is also quite likely that levels of *Salmonella* on egg shells in the current survey would have been greater than those found in the same eggs at retail since numbers of organisms are likely to have fallen during distribution and storage in the latter.

In the US *Salmonella* was found in 0.149 to 0.191% of free-range eggs and 0.015% and 0.041%

of eggs from cage laying flocks (mean, 0.0264%; including a range of 0 to 62.5/10 000 eggs) (Kinde et al., 1996; Henzler et al., 1998) and a prevalence of 0.027% was found in another US study (Schlosser et al., 1995). A survey in Northern Ireland found an overall contamination rate of 0.43% of batches of six eggs (Wilson et al., 1998). Sampling of sources of eggs that are suspected of being involved in food-poisoning outbreaks has also yielded variable results, 0 to 35% of batches of six eggs (overall 0.4%) involved in outbreaks in the UK in 2002 were found to harbour Salmonella but no contamination was found in 321 batches of eggs from vaccinated flocks operating under the Lion Code (Anonymous, 2002a,b).

Eggs from known infected flocks could be expected to have higher levels of Salmonella contamination. One per cent of 2412 eggs from a naturally infected free-range flock and 0.4% of 2489 from cage layer units were found to be contaminated in one study (Humphrey, 1999) and 0.6% in another (Humphrey et al., 1991b; Humphrey, 1994). Whiting & Buchanan (1997) found less than 1% contamination (range 0 to 19%) of individual eggs from known infected birds, whereas Morris (1990) found only 0.1% overall from infected flocks and 0.5% from known infected individual birds. Poppe et al. (1992) found a prevalence of less than 0.06% of contaminated eggs from two infected flocks. A modelling approach has predicted 0.005% contamination of individual eggs (Ebel & Schlosser, 2000).

It is difficult to interpret the results of these studies, however, as there can be marked clustering of contamination, with up to 35% of some batches being contaminated (Paul & Batchellor, 1988; Humphrey et al., 1989b; Vugia et al., 1993; Anonymous, 2002a). Sample preparation and culture techniques reported are also extremely variable. A source of iron and prolonged culture has been recommended to overcome the iron-depleted and inhibitory characteristics of albumen (Reissbrodt & Rabsch, 1993; Chen et al., 2001). It is advantageous to use sufficient pre-enrichment broth to liquify the egg and dilute inhibitory factors in the albumen (Hara-kudo et al., 2001b) and vigorous mechanical mixing or blending of egg contents is to be avoided (Seo et al., 2002). These factors have been taken into account in the current study. It is difficult, however, to directly compare the results of the current study with previous work but the methods used are likely to have been among the most sensitive. The minimum prevalence of S. Enteritidis-contaminated egg contents in the current study was only 0.04%, or one in 2500 eggs. This would be the situation if only one of the six eggs in each positive batch was contaminated, which is the most probable when the prevalence is so low. This is less than the prevalence found in non-vaccinated flocks by the author and others

quoted earlier ($P < 0.005$; Fisher's Exact Test). These results suggest that vaccination has had a beneficial effect on egg contamination but that there is still some contamination risk associated with the presence of S. Enteritidis in infected vaccinated flocks. It is also apparent from the current study that when Salmonella serovars other than S. Enteritidis are present concurrently in flocks vaccinated for S. Enteritidis, then considerably more shell contamination with these may occur, although content contamination was relatively less common. This finding also suggests a partially protective effect of S. Enteritidis vaccine against the homologous serovar under the same housing and husbandry conditions. In the UK the vast majority of commercial laying birds are now vaccinated against S. Enteritidis so it is difficult to carry out controlled comparative studies. Large nationwide randomized studies are needed to accurately assess the current prevalence of contaminated eggs.

Although fresh contaminated eggs typically harbour low numbers of Salmonella (Mawer et al., 1989; Humphrey et al., 1991b; Gast & Beard, 1992; Gast et al., 2002a), occasional individual eggs do contain thousands of organisms (Humphrey et al., 1991b). At ambient temperatures multiplication can occur so in some eggs very high numbers of organisms are reached without changing the appearance or smell of the egg (Humphrey & Whitehead, 1993; de Louvois, 1994; Gast & Holt, 2001). The level of inhibitory factors in the albumen and shell strength in eggs laid in hot weather or stored at higher temperatures may be reduced (Hara-Kudo et al., 2001a; Al-Saffar & Rose, 2002; Latimer et al., 2002). S. Enteritidis deposited on the yolk in infected birds can multiply within 72 h at 15°C and this temperature has been recommended as a maximum for a 30-day shelf life (Almonacid et al., 2002). Rapid cooling by forced air, especially after washing, which increases the heat retention of stored eggs, can be used to reduce the opportunity for bacterial multiplication (Thompson et al., 2000) but lower temperatures can enhance survival of Salmonella on shells (Radkowski, 2002).

Since it is not economically or aesthetically acceptable to heat-treat all eggs to eliminate Salmonella, the primary control must be at farm level. Theoretically it would be expected that it would be easier to control Salmonella in larger enclosed cage layer houses. In reality, multistage production, large flock size, linkage of houses by egg and droppings belts, spread of contaminated dust between closely located houses, and the difficulty in controlling farm pests and effectively disinfecting housing and equipment has resulted in greater problems of persistent S. Enteritidis in such sites despite vaccination (Davies & Breslin, 2001; Matsumoto et al., 2001). There is also a constant

risk of introduction of *S.* Enteritidis via materials or wildlife contaminated with faecal waste from infected humans or imported materials from parts of the world where the organism is more common (Shirota *et al.*, 2001). Vaccination is therefore an important aid to reduction or possible elimination of *S.* Enteritidis from laying farms, and several publications have demonstrated a reduction in infection following the use of killed vaccines incorporating various adjuvants (Timms *et al.*, 1990, 1994; Gast *et al.*, 1992, 1993; Barbour *et al.*, 1993; Davison *et al.*, 1999; Feberwee *et al.*, 2001). The extent of the reduction of infection is variable, however (Zhang-Barber *et al.*, 1999) and studies have shown a reduction in shedding of *S.* Enteritidis and the number of contaminated eggs (Yamane *et al.*, 2000; Woodward *et al.*, 2002), but tissues may still be colonized in a proportion of the flock. Recurrences of excretion and egg contamination may occur during periods of stress such as the onset of lay, overheating or the final stages of lay (Humphrey *et al.*, 1991a; Nakamura *et al.*, 1998; Tenk *et al.*, 2000; Clifton-Hadley *et al.*, 2002). In the current study, based on work carried out prior to that described in this paper, it was expected that there would be an increase in egg contamination during the hotter summer months but this proved not to be the case. Although infection in breeding and pullet rearing flocks is uncommon (Anonymous, 2001), there is also a possibility that birds could encounter infection before vaccination is complete and exhibit a poor response (Holt *et al.*, 1999). This risk may be reduced by the use of live vaccines that can be given at 1 day old (Springer *et al.*, 2000).

Although vaccination of laying flocks for *S.* Enteritidis is to be recommended for all situations where risk cannot be fully controlled by other means, the current study has confirmed that flock infection and production of contaminated eggs may still occur, albeit at a lower frequency than would be expected in unvaccinated flocks. Currently the majority of the UK laying flock has changed to a live *S.* Enteritidis vaccine so the effect of this change on the incidence of infection in chickens and people will be interesting to follow. It is essential, however, to combine vaccination with good husbandry, which should include all in–all out production, effective C&D between flocks and a high standard of pest control. It is also important to adequately monitor laying flocks so that persistently infected farms can be identified and *Salmonella* eliminated as houses are depopulated (Davies & Breslin, 2001; Gast *et al.*, 2002b). More molecular genetic work is also required to quantify the sources of *S.* Enteritidis for humans since the relationship between *S.* Enteritidis and other serovars from humans and the various food animal species is still not fully clear (Icgen *et al.*, 2002). Improved multiserotype or live vaccines may offer

greater control of contamination in future (Springer *et al.*, 2000; Clifton-Hadley *et al.*, 2002) and this may be combined with selection of birds that are able to respond better to vaccines (Kaiser *et al.*, 2002). In the shorter term, improved control over international trade in contaminated eggs is required since the cost and effort of measures taken by conscientious domestic producers may be jeopardized by exposure of consumers to *Salmonella* from countries where control is currently less effective (Anonymous, 2002b,c).

References

ACMSF (2001). *Advisory Committee on the Microbiological Safety of Food; Second Report on Salmonella in eggs* (pp. 5–7). London: The Stationery Office.

Almonacid, S., Gutierrez, J., Jaques, A. & Simpson, R. (2002). *Salmonella Enteritidis* risk assessment: a kinetic analysis. *Journal of Food Science*, *67*, 1115–1120.

Al-Saffar, A.A. & Rose, S.P. (2002). Ambient temperature and the egg laying characteristics of laying fowl. *Worlds Poultry Science Journal*, *58*, 317–331.

Anonymous (2001). *The Report of the Chief Veterinary Officer: Animal Health 2001*. London: DEFRA Publications.

Anonymous (2002a). Public health investigation of *Salmonella* Enteritidis in raw shell eggs. *CDR Weekly*, *12*, 3–5.

Anonymous (2002b). London public health investigation of *Salmonella* Enteritidis in raw shell egg. *CDR Weekly*, *12*, 3–4.

Anonymous (2002c). *Salmonella enteritidis* in eggs imported to Norway. *Eurosurveillance Weekly*, *6*, 2.

Baker, R.C. (1990). Survival of *Salmonella enteritidis* on and in shelled eggs, liquid eggs and cooked egg products. *Dairy, Food, and Environmental Sanitation*, *10*, 273–275.

Baker, R.C., Goff, J.P. & Mulnix, E.J. (1980). Salmonellae recovery following oral and intravenous inoculation of laying hens. *Poultry Science*, *59*, 1067–1072.

Barbour, E.K., Wayne, W.M., Nabbut, N.H., Poss, P.E. & Brinton, M.K. (1993). Evaluation of bacterins containing three predominant phage types of *Salmonella enteritidis* for prevention of infection in egg-laying chickens. *American Journal of Veterinary Research*, *54*, 1306–1309.

Berchieri, A., Jr., Wigley, P., Page, K., Murphy, C.K. & Barrow, P.A. (2001). Further studies on vertical transmission and persistence of *Salmonella enterica* serovar Enteritidis phage type 4 in chickens. *Avian Pathology*, *30*, 297–310.

Bichler, L.A., Nagaraja, K.V. & Halvorson, D.A. (1996). *Salmonella* Enteritidis in eggs, cloacal swab specimens and internal organs of experimentally infected white Leghorn chickens. *American Journal of Veterinary Research*, *57*, 489–495.

British Egg Industry Council (2002). *Lion Quality: Code of Practice for Lion Eggs*. London: BEIC Publications.

Chen, H.Q., Anantheswaran, R.C. & Knabel, S.J. (2001). Optimisation of iron supplementation for enhanced detection of *Salmonella enteritidis* in eggs. *Journal of Food Protection*, *64*, 1279–1285.

Clifton-Hadley, F.A., Breslin, M., Venables, L.M., Sprigings, K.A., Cooles, S.W., Houghton, S. & Woodward, M.J. (2002). A laboratory study of an inactivated bivalent iron restricted *Salmonella enterica*

serovar Enteritidis and Typhimurium dual vaccine against Typhimurium challenge in chickens. *Veterinary Microbiology*, *89*, 167–179.

Davies, R.H. & Breslin, M. (2001). Environmental contamination and detection of *Salmonella enterica* serovar Enteritidis in laying flocks. *Veterinary Record*, *149*, 699–704.

Davison, S., Benson, C.E., Henzler, D.J. & Eckroade, R.J. (1999). Field observations with *Salmonella enteritidis* bacterins. *Avian Diseases*, *43*, 664–669.

de Buck, J., van Immerseel, F., Meulemans, G., Mast, J., Haesebrouck, F. & Ducatelle, R. (2002). Adhesion of *Salmonella* Enteritidis strains to chicken istmal glandular secretions. *Proceedings of the 3rd International Symposium on Salmonella and Salmonellosis* (pp. 157–162). St Brieuc, France.

de Louvois, J. (1993). *Salmonella* contamination of eggs: a potential source of human salmonellosis: a report of the Public Health Laboratory Service survey of imported and home produced egg. *PHLS Microbiology Digest*, *10*, 158–162.

de Louvois, J. (1994). *Salmonella* contamination of stored hens eggs. *PHLS Microbiology Digest*, *11*, 203–205.

Duguid, J.P. & North, R.A.E. (1991). Eggs and *Salmonella* food poisoning: an evaluation. *Journal of Medical Microbiology*, *34*, 65–72.

Ebel, E. & Schlosser, W. (2000). Estimating the annual fractions of eggs contaminated with *Salmonella* Enteritidis in the United States. *International Journal of Food Microbiology*, *61*, 51–62.

Feberwee, A., de Vries, T.S., Elbers, A.R.W. & de Jong, W.A. (2000). Results of a *Salmonella enteritidis* vaccination field trial in broiler-breeder flocks in the Netherlands. *Avian Diseases*, *44*, 249–255.

Feberwee, A., de Vries, T.S., Hartman, E.G., de Wit, J.J., Elbers, A.R.W. & de Jong, W.A. (2001). Vaccination against *Salmonella enteritidis* in Dutch commercial layer flocks with a vaccine based on a live *Salmonella* Gallinarum 9R strain: evaluation of efficacy, safety and performance of serologic *Salmonella* tests. *Avian Diseases*, *45*, 83–91.

Gast, R.K. & Beard, C.W. (1990). Isolation of *Salmonella enteritidis* from internal organs of experimentally infected hens. *Avian Diseases*, *34*, 991–993.

Gast, R.K. & Beard, C.W. (1992). Detection and enumeration of *Salmonella enteritidis* in fresh and stored eggs laid by experimentally infected hens. *Journal of Food Protection*, *55*, 152–156.

Gast, R.K. & Holt, P.S. (1998). Persistence of *Salmonella enteritidis* from one day of age until maturity in experimentally infected layer chickens. *Poultry Science*, *77*, 1759–1762.

Gast, R.K. & Holt, P.S. (2001). Assessing the frequency and consequences of *Salmonella enteritidis* deposition on the egg yolk membrane. *Poultry Science*, *80*, 997–1002.

Gast, R.K., Stone, H.D., Holt, P.S. & Beard, C.W. (1992). Evaluation of the efficacy of oil–emulsion bacterin for protecting chickens against *Salmonella enteritidis*. *Avian Diseases*, *36*, 992–999.

Gast, R.K., Stone, H.D. & Holt, P.S. (1993). Evaluation of the efficacy of oil–emulsion bacterin for reducing faecal shedding of *Salmonella enteritidis* in laying hens. *Avian Diseases*, *37*, 1085–1091.

Gast, R.K., Guard-Petter, J. & Holt, P.S. (2002a). Characteristics of *Salmonella enteritidis* contamination in eggs after oral, aerosol and intravenous inoculation of laying hens. *Avian Diseases*, *46*, 629–635.

Gast, R.K., Nasir, M.S., Jolley, M.E., Holt, P.S. & Stone, H.D. (2002b). Detection of experimental *Salmonella enteritidis* and *S. Typhimurium* infections in laying hens by fluorescence polarisation assay for egg yolk antibodies. *Poultry Science*, *81*, 1128–1131.

Guard-Petter, J. (2001). The chicken, the egg and *Salmonella enteritidis*. *Environmental Microbiology*, *3*, 421–430.

Hara-Kudo, Y., Sakakibara, Y., Konuma, H., Sawada, T. & Kumagai, S. (2001a). Laying season and egg shell cracks on the growth of *Salmonella* Enteritidis in the egg albumen during storage. *Journal of Food Protection*, *64*, 1134–1137.

Hara-Kudo, Y., Kumagai, S., Masuda, T., Goto, K., Ohtsuka, K., Masaki, H., Tanaka, H., Tanno, K., Miyahara, M. & Konuma, H. (2001b). Detection of *Salmonella enteritidis* in shell and liquid eggs using enrichment and plating. *International Journal of Food Microbiology*, *64*, 395–399.

Hayes, S., Nylen, G., Smith, R., Salmon, R.L. & Palmer, S.R. (1999). Undercooked hens eggs remain a risk factor for sporadic *Salmonella enteritidis* infection. *Communicable Disease and Public Health*, *2*, 66–67.

Henzler, D.J., Kradel, D.C. & Sischo, W.M. (1998). Management and environmental risk factors for *Salmonella enteritidis* contamination of eggs. *American Journal of Veterinary Research*, *59*, 824–829.

Holt, P.S., Gast, R.K., Porter, R.E. & Stone, H.D. (1999). Hyporesponsiveness of the systemic and mucosal humoral immune systems in chickens infected with *Salmonella enterica* serovar *enteritidis* at one day of age. *Poultry Science*, *78*, 1510–1517.

Hoop, R.K. & Pospischil, A. (1993). Bacteriological, serological, histological and immuno-histochemical findings in laying hens with naturally acquired *Salmonella enteritidis* phage type 4 infection. *Veterinary Record*, *133*, 391–393.

Humphrey, T.J. (1994). Contamination of shell eggs and contents with *Salmonella enteritidis*: a review. *International Journal of Food Microbiology*, *21*, 31–40.

Humphrey, T.J. (1999). Contamination of eggs and poultry meat with *Salmonella enterica* serovar Enteritidis. In A.M. Saeed (Ed.), *Salmonella enterica serovar Enteritidis in Humans and Animals, Epidemiology, Pathogenesis and Control* (pp. 183–192). Ames, IA: Iowa State University Press.

Humphrey, T.J. & Whitehead, A. (1993). Egg age and the growth of *Salmonella* Enteritidis in egg contents. *Epidemiology and Infection*, *111*, 209–219.

Humphrey, T.J., Cruikshank, J.G. & Rowe, B. (1989a). *Salmonella enteritidis* PT4 and hens' eggs. *Lancet*, *1*, 281.

Humphrey, T.J., Baskerville, A., Mawer, S.L., Rowe, B. & Hopper, S. (1989b). *Salmonella enteritidis* PT4 from the contents of intact eggs: a study involving naturally infected hens. *Epidemiology and Infection*, *103*, 415–423.

Humphrey, T.J., Chart, H., Baskerville, A. & Rowe, B. (1991a). The influence of age on the response of SPF hens to infection with *Salmonella enteritidis* PT4. *Epidemiology and Infection*, *106*, 33–43.

Humphrey, T.J., Whitehead, A., Gawler, A.H.L., Henley, A. & Rowe, B. (1991b). Numbers of *Salmonella enteritidis* in the contents of naturally contaminated hens' eggs. *Epidemiology and Infection*, *106*, 489–496.

Icgen, B., Gurakan, G.C. & Ozcengiz, G. (2002). Characterisation of *Salmonella enteritidis* isolates of chicken, egg and human origin from Turkey. *Food Microbiology*, *19*, 375–382.

Jones, D.R., Anderson, K.E., Curtis, P.A. & Jones, F.T. (2002). Microbial contamination in inoculated shell eggs. 1. Effects of layer strain and hen age. *Poultry Science*, *81*, 715–720.

Kaiser, M.G., Lakshmanan, N., Wing, T. & Lampert, S.J. (2002). *Salmonella enterica* serovar enteritidis burden in broiler breeder chicks genetically associated with vaccine antibody response. *Avian Diseases*, *46*, 25–31.

Keller, L.H., Benson, C.E., Krotec, K. & Eckroade, R.J. (1995). *Salmonella enteritidis* colonisation of the reproductive tract and forming and freshly laid eggs. *Infection and Immunity*, *63*, 2443–2449.

Kinde, H., Read, D.H., Chin, R.P., Bickford, A.A., Walher, R.L., Ardans, A., Little, H.E., Willoughby, D., Berchold, D., Breitmeger, R., Kerr, D. & Gardner, I.A. (1996). *Salmonella enteritidis* phage type 4 in a commercial layer flock in Southern California: bacteriologic and epidemiologic findings. *Avian Diseases*, *40*, 665–671.

Latimer, H.K., Jaykus, L.A., Morales, R.A., Cowen, P. & Crawford-Brown, D. (2002). Sensitivity analysis of *Salmonella enteritidis* levels in contaminated eggs using a biphasic growth model. *International Journal of Food Microbiology*, *75*, 71–87.

Matsumoto, A., Miyama, M. & Murakami, S. (2001). Comparison of *Salmonella* isolation rates in different types of egg-layer houses in Chiba, Japan. *Avian Diseases*, *45*, 195–200.

Mawer, S.L., Spain, G.E. & Rowe, B. (1989). *Salmonella enteritidis* phage type 4 and hens eggs. *Lancet*, *1*, 280–281.

Miyamoto, T., Baba, E., Tanaka, T., Sasai, K., Fukata, T. & Arakawa, A. (1997). *Salmonella* Enteritidis contamination of eggs from hens

inoculated by vaginal, cloacal and intravenous routes. *Avian Diseases*, *41*, 296–303.

Molbak, K. & Neimann, J. (2002). Risk factors for sporadic infection with *Salmonella* Enteritidis, Denmark, 1997–1999. *American Journal of Epidemiology*, *156*, 654–661.

Morris, G.K. (1990). *Salmonella enteritidis* and eggs: assessment of risk. *Dairy Food Environmental Sanitation*, *10*, 279–281.

Nakamura, M., Nagamine, N., Takahashi, T., Suzuki, S. & Sato, S. (1998). Evaluation of the efficacy of a bacterin against *Salmonella enteritidis* infection and the effect of stress after vaccination. *Avian Diseases*, *38*, 717–724.

Okamura, M., Kamijima, Y., Miyamoto, T., Tani, H., Sasai, K. & Baba, E. (2001). Differences among six *Salmonella* serovars in abilities to colonise reproductive organs and to contaminate eggs in laying hens. *Avian Diseases*, *45*, 61–69.

Padron, M. (1990). *Salmonella* typhimurium penetration through the eggshell of hatching eggs. *Avian Diseases*, *34*, 463–465.

Parker, C.T., Liebana, E., Henzler, D.J. & Guard-Petter, J. (2001). Lipopolysaccharide O-antigen microheterogeneity of *Salmonella* Enteritidis and Typhimurium. *Applied and Environmental Microbiology*, *3*, 332–342.

Parker, C.T., Harmon, B. & Guard-Petter, J. (2002). Mitigation of avian reproductive tract function by *Salmonella enteritidis* producing high-molecular-mass lipopolysaccharide. *Environmental Microbiology*, *4*, 538–545.

Paul, J. & Batchellor, B. (1988). *Salmonella enteritidis* phage type 4 and hens' eggs. *Lancet*, *2*, 1421.

Perales, I. & Audicana, A. (1989). The role of hens' eggs in outbreaks of salmonellosis in north Spain. *International Journal of Food Microbiology*, *8*, 175–180.

Petter, J.G. (1993). Detection of two smooth colony phenotypes in a *Salmonella enteritidis* isolate which vary in their ability to contaminate eggs. *Applied and Environmental Microbiology*, *59*, 2884–2890.

Popoff, M.Y. (2001). *Antigenic Formulas of the Salmonella Serovars* 8th edn. Paris: WHO Collaborating Centre for Reference and Research on *Salmonella*, Institut Pasteur.

Poppe, C., Johnson, R.P., Forsbery, C.M. & Irwin, R.J. (1992). *Salmonella enteritidis* and other *Salmonella* in laying hens and eggs with *Salmonella* in their environment. *Canadian Journal of Veterinary Research*, *56*, 226–232.

Rabsch, W., Tschape, H. & Baumler, A.J. (2001). Non-typhoidal salmonellosis: emerging problems. *Microbes and Infection*, *3*, 237–247.

Radkowski, M. (2002). Effect of moisture and temperature on survival of *Salmonella enteritidis* on shell eggs. *Archiv für Geflugelkunde*, *66*, 119–123.

Reissbrodt, R. & Rabsch, W. (1993). Selective pre-enrichment of *Salmonella* from eggs by Siderophore supplements. *Zentralblatt für Bakteriologie*, *279*, 344–353.

Rodrigue, D.C., Tauxe, R.U. & Rowe, B. (1990). International increase in *Salmonella enteritidis*: a new pandemic? *Epidemiology and Infection*, *105*, 21–27.

Schlosser, W., Henzler, D., Mason, J., Hurd, S., Truck, S., Sischo, W., Kradel, D. & Hogue, A. (1995). *Salmonella Enteritidis Pilot Project Progress Report*. Washington, DC: US Government Printing.

Seo, K.H., Brackett, RE., Valentin-Bon, I.E. & Holt, P.S. (2002). Sample preparation methods for rapid and direct detection of *Salmonella* Enteritidis in eggs. *Proceeings of the 89th Annual Meeting of the International Association for Food Protection*, 30 June–3 July (p. 54). San Diego, CA, USA.

Shirota, K., Katoh, H., Murase, T., Ito, T. & Otsuki, K. (2001). Monitoring of layer feed and eggs for *Salmonella* in Eastern Japan between 1993 and 1998. *Journal of Food Protection*, *64*, 734–737.

Sparks, N.H.C. & Board, R.G. (1985). Bacterial penetration of the recently oviposited shell of hens' eggs. *Australian Veterinary Journal*, *62*, 169–170.

Springer, S., Lehman, J., Lindner, T., Thielebein, J., Alber, G. & Selbitz, H.J. (2000). A new live *Salmonella* Enteritidis vaccine for chicken — experimental evidence of its safety and efficacy. *Berliner und Munchener Tierarztliche Wuchenschrift*, *113*, 246–252.

Tenk, I., Gyorvary, I., Erdei, P., Szabo, Z., Kostyak, A. & Matray, D. (2000). Effects on *Salmonella* shedding in breeding turkey flocks of vaccine (Salenvac) against *Salmonella enteritidis*. *Magyar Allatorvosok-Lapja*, *122*, 737–741.

Thiagarajan, D., Saeed, A.M. & Asem, E.K. (1994). Mechanism of transovarian transmission of *Salmonella enteritidis* in laying hens. *Poultry Science*, *73*, 89–98.

Thompson, J.F., Knutson, J., Ernst, R.A., Kuney, D., Riemann, H., Himathongkham, S. & Zeidler, G. (2000). Rapid cooling of shell eggs. *Journal of Applied Poultry Research*, *9*, 258–268.

Timms, L.M., Marshall, R.N. & Breslin, M.F. (1990). Laboratory assessment of protection given by an experimental *Salmonella enteritidis* PT4 inactivated, adjuvant vaccine. *Veterinary Record*, *127*, 811–814.

Timms, L.M., Marshall, R.N. & Breslin, M.F. (1994). Laboratory and field trial assessment of protection given by *Salmonella enteritidis* PT4 inactivated, adjuvant vaccine. *British Veterinary Journal*, *150*, 93–102.

Vugia, D.J., Bishu, B., Smith, M., Tavris, D.R., Hickman-Brenner, F.W. & Tauxe, R.V. (1993). *Salmonella enteritidis* in a restaurant chain: the continuing challenges of prevention. *Epidemiology and Infection*, *110*, 49–61.

Ward, L.R., Threlfall, J., Smith, H.R. & O'Brien, S.J. (2000). *Salmonella enteritidis* epidemic. *Science*, *287*, 1754.

Whiting, R.C. & Buchanan, R.L. (1997). Development of a quantitative risk assessment model for *Salmonella enteritidis* in pasteurised liquid eggs. *International Journal of Food Microbiology*, *36*, 111–125.

Wilson, I.G., Hearney, J.C.N. & Powell, G.C. (1998). Salmonella in raw shell eggs in Northern Ireland 1996–7. *Communicable Disease and Public Health*, *1*, 156–160.

Woodward, M.J., Gettinby, G., Breslin, M.F., Corkish, J.D. & Houghton, S. (2002). The efficacy of Salenvac, a *Salmonella enterica* subsp. Enterica serotype Enteritidis iron-restricted bacterin vaccine, in laying chickens. *Avian Pathology*, *31*, 383–392.

Yamane, Y., Leonard, J.D., Kobatake, R., Awamura, N., Toyota, Y., Ohta, H., Otsuki, K. & Inoue, T. (2000). A case study on *Salmonella enteritidis* (SE) origin at three egg-laying farms and its control with a S. Enteritidis bacterin. *Avian Diseases*, *44*, 519–526.

Zhang-Barber, L., Turner, A.K. & Barrow, P.A. (1999). Vaccination for control of *Salmonella* in poultry. *Vaccine*, *17*, 2538–2545.

RÉSUMÉ

Surveillance de la contamination, par *Salmonella*, des œufs provenant d'un troupeau de pondeuses infectées chez lesquelles la vaccination *Salmonella enterica* serovar Enteritidis a été réalisée

Tous les mois, des œufs ont été collectés dans 12 troupeaux de pondeuses en cage répartis dans quatre élevages, où *Salmonella* Enteritidis a été isolée malgré la vaccination avec un vaccin inactivé S. Enteritidis. En fonction des possibilités, les pondeuses ont fait l'objet de culture à la fin de la période de ponte et des échantillons de l'environnement et des fèces ont été réalisés dans les bâtiments avant et après nettoyage et désinfection. Vingt-quatre lots de six œufs en coquille sur 13 652 testés se sont révélés positifs vis-à-vis de S. Enteritidis (0.18% [0.11–0.26 Cl[95]] équivalent d'un œuf) et 54 pour les autres sérovars (0.40% [0.30–0.52 Cl[95]] équivalent d'un œuf). Six lots de 13 640 contenus d'œuf, vrac d'un mélange de 6 œufs, étaient contaminés par S. Enteritidis (0.04% [0.02–0.10 Cl[95]] équivalent d'un œuf) et trois lots contenant d'autres sérovars. De plus, trois autres lots ont été trouvés contaminés par S. Enteritidis à la fois dans les contenus et dans les œufs en coquille et deux autres lots contenaient d'autres sérovars dans les deux types d'échantillons. Le niveau total de contamination, par S. Enteritidis dans les contenus et à partir des œufs en coquille, trouvé dans les troupeaux vaccinés a été de 33 lots/13 682 œufs (0.24% [0.17–0.34 Cl[95]] équivalent d'un œuf). Le total des contaminations, quel que soit le sérovar de *Salmonella* a été de 92 lots/13 682 œufs (0.68% [0.55–0.84 Cl[95]] équivalent d'un œuf). Ces résultats contrastent avec ceux des testages des œufs de trois troupeaux non vaccinés réalisés avant cette étude où une contamination par S. Enteritidis avait été

144   R. Davies & M. Breslin

observée dans 21 lots d'œufs en coquille sur un total de 2 101 œufs (1.0% [0.63–1.56 CI^{95}] équivalent d'un œuf) et dans six lots de contenus d'œufs sur 2 051 œufs (0.29% [0.11–0.64 CI^{95}] équivalent d'un œuf). *S.* Enteritidis a été trouvé dans 67/699 (9.6%) de pondeuses de réforme vaccinées et dans 64/562 (11.4%) échantillons de fèces prélevés dans les bâtiments. Les caractéristiques communes de ces fermes semblent être l'échec du lavage et de la désinfection des bâtiments ainsi que celui du contrôle des souris.

ZUSAMMENFASSUNG

Beobachtungen zur Salmonellenkontamination bei Eiern aus infizierten kommerziellen Legehennenherden nach Vakzination gegen *Salmonella enterica* Serovar Enteritidis

Von 12 Käfiglegehennenherden auf vier Farmen, bei denen *Salmonella enteritidis* trotz Vakzination mit einem *S. enteritidis*-Bakterin auftrat, wurden monatlich Eier eingesammelt. Wo es möglich war, wurden am Ende der Legeperiode auch Hennen für die Untersuchung entnommen und Umgebungsproben wurden in den Stallgebäuden vor und nach der Reinigung und Desinfektion gezogen. 24 Proben bestehend aus jeweils 6 Eischalen von insgesamt 13,652 getesteten (0.18% (0.11–0.26 CI^{95}) Einzeleiäquivalent) waren positiv für *S. enteritidis* und 54 (0.40% (0.30–0.52 CI^{95}) Einzeleiäquivalent) für andere Serovare. 6 Proben von 13,640 Eiinhalten (0.04% (0.02–0.10 CI^{95}) Einzeleiäquivalent) in Pools von jeweils 6 Eiern beinhalteten *S. enteritidis* und 3 Proben hatten andere Serovare. Außerdem waren bei drei weiteren Proben Inhalt und Schalen positiv für *S. enteritidis* und zwei andere Proben enthielten andere Serovare in beiden Anteilen. Insgesamt waren in den vakzinierten Herden 33 Proben/13,682 Eiern (0.24% (0.17–0.34 CI^{95}) Einzeleiäquivalent) mit *S. enteritidis* kontaminiert. Die totale Kontamination für andere *Salmonella*-Serovare betrug 92 Proben/13,682 (0.68% (0.55–0.84 CI^{95}) Einzeleiäquivalent). Diese Resultate stehen im Gegensatz zu vorherigen Ei-Untersuchungsergebnissen aus drei nicht-vakzinierten Herden, wo 21 Proben aus Eischalen von insgesamt 2.101 Eiern (1,0% (0.63–1.56 CI^{95}) Einzeleiäquivalent) und 6 Eiinhaltsproben von 2.051 Eiern (0.29% (0.11–0.64 CI^{95}) Einzeleiäquivalent) mit *S. enteritidis* kontaminiert waren. *S. enteritidis* wurde in 67/699 (9.6%) untersuchten vakzinierten Schlachthennen und in 64/562 (11.4%) gepoolten frischen Faezesproben aus Legehennenställen gefunden. Misserfolge beim adäquaten Reinigen und Desinfizieren von Legehennenställen und bei der Mäusebekämpfung schienen ein gemeinsames Merkmal auf diesen Farmen zu sein.

RESUMEN

Observaciones sobre la contaminación de huevos por *Salmonella* procedentes de lotes de ponedoras comerciales vacunados frente a *Salmonella enterica* serovar Enteritidis

Se recogieron, mensualmente, huevos de 12 lotes de gallinas de puesta en batería provenientes de 4 granjas en las cuales *Salmonella* Enteritidis estaba presente en los lotes vacunados a pesar de la vacunación con una bacterina de *S.* Enteritidis. También se tomaron gallinas al final del período de puesta para cultivo cuando esto fue posible, y se tomaron muestras del medio ambiente y de heces de las jaulas antes y después de su limpieza y desinfección. 24 lotes de 6 cáscaras de huevo de los 13,652 testados (0.18% [0.11–0.26 CI^{95}] un huevo equivalente) fueron positivos para *S.* Enteritidis y 54 (0.40% [0.30–0.52 CI^{95}] un huevo equivalente) lo fueron para otros serovares. 6 lotes de los 13,640 (0.04% [0.02–0.10 CI^{95} un huevo equivalente) contenidos procedentes de huevos, repartidos en grupos de 6 huevos, contenían *S.* Enteritidis y 3 lotes contenían otros serovares. Además, otros 3 lotes más contenían *S.* Enteritidis tanto en la cáscara como en su contenido y otros 2 lotes contenían otros serovares en ambas partes. Por lo tanto, el nivel total de contaminación por *S.* Enteritidis tanto en la cáscara como en el contenido que se encontró en las manadas vacunadas fue de 33 lotes/13,682 huevos (0.24% [0.17–0.34 CI^{95}] un huevo equivalente). El grado de contaminación total por cualquier serovar de *Salmonella* fue de 92 lotes/13,682 huevos (0.68% [0.55–0.84 CI^{95}] un huevo equivalente). Estos resultados contrastan con los resultados del análisis de huevos procedentes de 3 manadas no vacunadas previos a este estudio en el cual 21 lotes de cáscaras de huevos de un total de 2,101 huevos (1.0% [0.63–1.56 CI^{95}] un huevo equivalente) y 6 lotes de contenido de un total de 2,051 huevos (0.29% [0.11–0.64 CI^{95}] un huevo equivalente) estaban contaminados con *S.* Enteritidis. *S.* Enteritidis se encontró en 67/699 (9.6%) de las gallinas de desvieje y en 64/562 (11.4%) de las muestras de heces frescas que se tomaron de las jaulas. Fallos en la limpieza y desinfección de las jaulas y en el control de ratones parece ser un hecho común en las granjas.