Food Research International 45 (2012) 745–754

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Salmonella serovars isolated from table eggs: An overview

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ARTICLE INFO

Article history:
Received 7 February 2011
Accepted 31 March 2011

Keywords:
Egg
Salmonella Enteritidis
Salmonella Typhimurium
Salmonella serovars

ABSTRACT

Salmonellosis can be acquired through consumption of infected raw or undercooked eggs. The European Commission has set criteria to control *Salmonella* Enteritidis (SE) and *Salmonella* Typhimurium (ST) infections in laying flocks, to reduce the risk of contaminated eggs entering the food chain. SE is considered the serovar mostly implicated in *Salmonella* egg related food poisoning, for its peculiar ability to contaminate the egg contents through vertical transmission. Other *Salmonella* serovars (SO) and ST generally contaminate eggs externally, and are found in the egg contents following penetration through the eggshell. A review of the information available on egg contamination by SE, ST and SO is presented, followed by a collation of the surveys investigating table egg contamination at retail. Where available, information on the serovars identified in these surveys and *Salmonella* contamination of the egg (shell, contents or both) is detailed. SE appears to play a major role in egg contamination. Isolation of ST from eggs is not frequent, and appears to be mostly on the eggshells. In the majority of the studies, more samples were positive for SO than for ST. In some studies, one individual serovar exceeded ST. The data presented in this article shows how ST is not often isolated from table eggs and that contamination of table eggs with SE and SO is more frequent.

1. Introduction

Salmonellosis is an important foodborne disease worldwide, characterized by acute gastroenteritis with short incubation period, and is caused by Gram negative bacteria belonging to the genus *Salmonella* (OIE, 2004). This is divided into two species: *Salmonella enterica* and *Salmonella bongori*. *S. enterica* is further divided into six sub-species and most *Salmonella* belong to the subspecies *S. enterica* subsp. *enterica* (EFSA, 2010a). The subspecies are further divided in serotypes or serovars (e.g. *S. enterica* subsp. *enterica* serovar Typhimurium) (Heyndrickx, Pasmans, Ducatelle, Decostere, & Haesebrouck, 2005). Several classification schemes have been developed to further divide some serovars into phage types (PTs) on the basis of their affinity with specific bacteriophages (Jones, 2000). Studying the distribution of specific PTs of *Salmonella* provides important epidemiological information for outbreak-investigation (Ward, de Sa, & Rowe, 1987).

The host range of different serovars varies significantly. Host restricted *Salmonella* serovars are associated with only one particular host species (e.g., *Salmonella typhi* with humans and *Salmonella gallinarum* with poultry). Host adapted *Salmonella* serovars (e.g., *S.* Dublin and *S.* Choleraesuis) are primarily associated with a particular host, but they can also cause disease in other animal species. Un-restricted serovars, e.g., *S.* Enteritidis (SE) and *S.* Typhimurium (ST), usually induce self limiting gastroenteritis or an asymptomatic carrier state in a broad range of animal species (Uzzau et al., 2000). The un-restricted *Salmonella* serovars are also characterized by a wider geographical spread, as they can be carried by a range of animal vectors. Almost 2400 serovars are able to cause disease in humans, but the epidemiology of human disease is dominated by a relatively small number of serovars (Cogan & Humphrey, 2003).

The European Commission (EC) has set the following criteria to define *Salmonella* serovars of public health significance (EC, 2003): (1) the most frequent *Salmonella* serovars in human salmonellosis, (2) prevalence of the serovar in the animal population or feed, (3) serovars that show rapid and recent ability to spread and (4) serovars with increased virulence or resistance to important therapeutic antimicrobials. The most frequent *Salmonella* serovars in human salmonellosis in the European Union (EU) in 2008 were SE (58%), ST (21.9%), *S.* Infantis (1.1%), *S.* Virchow (0.7%) and *S.* Newport (0.7%). The remaining 17.6% of cases were associated with other serovars, each contributing less than 0.7% (EFSA, 2010b). The current national control plans (NCPs) for laying hens in the EU cover SE and ST, but more serovars could be targeted in accordance with the criteria listed above (EC, 2003).

Human infections with SE originate mainly from eggs and egg products (when consumed raw or undercooked), while ST infections originate predominantly from pigs, cattle and poultry meat (EFSA, 2010a), as well as from environmental contamination (e.g., sand boxes) (Doorduyn, Van Den Brandhof, Van Duynhoven, Wannet, & Van Pelt, 2006) contact with companion animals (Anonymous, 2010a; Harker, Lane, E., & Adak, 2010; Leonard et al., 2010) and wild bird related infections (Hughes et al., 2008; Taylor & Philbey, 2010).

Abbreviations: SE, *Salmonella* Enteritidis; ST, *Salmonella* Typhimurium; SO, *Salmonella* serovars other than SE or ST.

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0963-9969/$ – see front matter.
doi:10.1016/j.foodres.2011.03.054

In Europe, initiatives to control *Salmonella* infection in commercial flocks of laying hens started in some Member States between the end of the 1980s and the beginning of the 1990s (AFSSA, 2009; Defra, 2007). An EU-wide baseline study to determine the prevalence of *Salmonella* was conducted on commercial large scale laying hen holdings in 2004–2005. The Community weighted observed *Salmonella* spp. holding prevalence resulted 30.8% (95% CI = 29.8–31.8). The Community weighted SE/ST observed holding prevalence was 20.4% (95% CI = 19.5–21.3) with a range from 0% (Ireland, Luxembourg, Latvia, and Sweden) to 62.5% (Czech Republic) (EFSA, 2007). In the United Kingdom (UK), during this survey, the estimated holding level prevalence of *Salmonella* on layer farms was 11.9% (CI 95% 9.9–14.7%) and 7.9% (CI 95% 6.2–10.1%) were positive for SE and/or ST (Defra, 2007; EFSA, 2007). EC regulation 1168/2006 has set a minimum 10% yearly reduction of SE and ST prevalence in holdings producing eggs in the EC Member States. This has resulted in a dramatic decrease in the reported prevalence of SE and ST in laying flocks (EFSA, 2010a). In Europe in 2008 an average of 5.9% of flocks of laying hens was reported as being positive for any *Salmonella* serovar following routine monitoring tests during the production phase. Of the positive flocks, 52.5% were identified as SE, 8.5% as ST and 39% composed the group of other serovars, nontypeable and unspecified (EFSA, 2010a). The estimated prevalence of *Salmonella*-positive adult commercial egg laying flocks for all serovars derived from testing within the NCP in Great Britain in 2008 was 1.25%, and for SE and/or ST specifically was 1% (Anonymous, 2008). This was further reduced in 2009 (Anonymous, 2010b). In a recent EFSA report (EFSA, 2010b) an average prevalence of 0.5% of eggs contaminated with *Salmonella* was reported across the Member States of the EC. Positive eggs were found in surveys conducted in Europe during the 1990s and in more recent surveys contaminated eggs are still found, but to a progressively lower extent.

The aim of this article is to provide a summary of the information available on *Salmonella* contamination of table eggs, with particular focus on the role of different serovars in egg infection. A detailed report of surveys describing the prevalence of *Salmonella* in retail eggs will be presented to provide an overview of the public health risk related to egg consumption of the different serovars of *Salmonella*.

2. *Salmonella* infection and eggs

Eggs can be infected by *Salmonella* via two major routes, vertical and horizontal. Vertical transmission (transovarian infection) occurs when the egg contents are contaminated with *Salmonella* during the formation of the egg, before this is covered with the shell (Messens, 2005). Horizontal transmission includes trans shell infection of the contents of the egg during transit through the cloaca or after oviposition and fecal contamination of the external surface of the shell (EFSA, 2005).

Vertical transmission is common in host restricted *Salmonella* serovars, such as *S.* Gallinarum and *S.* Pullorum, but has also been demonstrated in un-restricted *Salmonella*, such as SE, ST and *S.* Heidelberg (Poppe, Duncan, & Mazzocco, 1998). Transmission via this route is directly related to the affinity of certain serovars for the reproductive tract of the hens (EFSA, 2010a). Individual *Salmonella* strains (within and across serotypes) can show a different ability in colonizing the hen's reproductive tract. This can depend both on genotypic and phenotypic characteristics of the strain, which can influence its virulence, ability to evade the hen's immune response and persistence in the reproductive tract (Gantois et al., 2009).

Various *Salmonella* serovars can also be found in the egg contents following penetration through the eggshell (trans shell transmission). This is more likely to happen in the first minutes after oviposition, when the egg's cuticle is immature and offers less protection against the penetration of bacteria into the eggs. Furthermore the positive temperature differential (the egg just laid is warmer than the environment) creates a negative pressure that aids the entrance of bacteria inside the egg if there is a moist environment at the shell surface (Messens, 2005). Trans-shell contamination of the contents is more likely when the shell quality is poorer for older birds or when there are nutritional problems or certain viral infections (Jones, Anderson, Curtis, & Jones, 2002). Fecal contamination of the eggshell is normally considerably higher than the contamination of contents, and usually correlates with visible eggshell contamination and with the degree of excretion of *Salmonella* in feces (Davies & Breslin, 2004). Externally contaminated eggs represent a risk in the processing phase, as they could cross-contaminate the egg contents or other foodstuffs (Humphrey, Baskerville, Mawer, Rowe, & Hopper, 1989).

In a recent study conducted in France, 150 eggs were collected from the one day production of each of 28 randomly selected *Salmonella* positive flocks. Eleven of the 28 flocks (39.3%) had at least one positive eggshell. Of the total of eggs tested, the prevalence of *Salmonella* in the eggshells was 1.05% (Chemaly et al., 2009).

Inside the egg, the growth of *Salmonella* is eased by storage temperature, suggesting that eggs should be stored at a constant temperature that should not exceed 20 °C (temperatures below 10 °C are preferable) (ACMSF, 1993). In the egg albumen, *Salmonella* can grow at 20 °C, while it is unable to grow at temperatures < 10 °C. If *Salmonella* reaches the egg yolk, it can grow rapidly, even at room temperature (25 °C) (Gantois et al., 2009). The age of the egg represents a further risk factor, because the yolk releases iron and nutrients over time. The deterioration of the vitelline membrane leads to the leakage of these nutrients into the albumen and attracts the bacteria towards the yolk, therefore easing the growth of *Salmonella* (Gantois et al., 2009). Furthermore the permeability of the yolk membrane increases over time at temperatures above 10 °C (Humphrey, 1994). Rapid cooling of eggs can be used to reduce the opportunity for bacterial multiplication but lower temperatures can enhance the survival of *Salmonella* on the shells and lead to condensation associated problems (Davies & Breslin, 2004). It was shown that condensation can encourage bacterial penetration of the eggshell, but seems to have a smaller impact on whole egg contamination (De Reu et al., 2006). Cooling eggs rapidly can also lead to damage of the egg shells, with an increase of cracked eggs (D. R. Jones et al., 2002).

The information available on egg contamination by SE, ST and other *Salmonella* serovars (SO) is discussed below.

2.1. *Salmonella* Enteritidis and eggs

SE is the serovar most frequently associated with egg infection (EFSA, 2010a). This is due to two main factors: its unique ability to colonize the ovary and the oviduct of laying hens long term, and its spread and persistence in the parental breeder flock population in most of the world (Thorns, 2000).

An intravenous infection model demonstrated that the ovary and the preovulatory follicules were colonized significantly more frequently by SE than by the five other serovars used (including ST). SE was the only serovar found in egg contents (Okamura, Kamijima, et al., 2001). The success of SE in the transovarian transmission can be associated with the presence in this serovar of the SEF14 fimbriae (which might be involved in the colonization of the reproductive organs) and of the *yaf*D gene (which is essential for resistance in the albumen) (Messens, 2005). The enhanced survival of SE at 42 °C and the production of lipopolysaccharides that specifically helps persistence in the egg, confer to SE a higher ability to efficiently infect the eggs (EFSA, 2010a).

SE prevalence in chickens, and in the human population, rose abruptly during the 1980s, quickly becoming a pandemic. There is evidence that SE became endemic in the parental breeder flocks. The contamination with SE of flocks at the top of the breeding pyramid, has led to a rapid spread of the infection in most parts of the world, possibly through contaminated embryos (Thorns, 2000). This is supported by the observation that SE never became endemic in Australian laying flocks, most likely because of their strict rules on the importation of animal products (Fullerton, 2008).

Despite the high occurrence of SE in laying flocks, the frequency of egg contamination by SE is normally relatively low and depends on the level of contamination of the flock and the time of the production period in which the eggs are laid. Eggs produced soon after the flock was infected with SE, and especially around the onset of lay, are more likely to become internally contaminated (Braden, 2006).

SE is typically associated with egg-related outbreaks (EFSA, 2010a). In particular, SE PT4 has been closely associated with the consumption of table eggs (Cogan & Humphrey, 2003). SE has not been always the most prevalent serovar in human infections, for example in the UK during the late 1970s ST was predominant, and *S.* Agona was most common before then (Cogan & Humphrey, 2003). In the UK, a sharp increase of salmonellosis was observed during the 1980s. This was largely due to an epidemic of SE PT4 that, in the UK, commenced in 1982–1983 and reached its peak in 1993, to start declining only in 1997 (Defra, 2007). In the UK some layer farms subscribe to the British Egg Industry Council (BEIC) that provides a code of practice (Lion Code) on farms' hygiene and welfare standards. Vaccination against *Salmonella* started in layer flocks in 1998 for farms that subscribe to the BEIC Lion Code (Cogan & Humphrey, 2003; Ward, Threlfall, Smith, & O'Brien, 2000). These typically larger farms produce more than 80% of retail eggs in the UK. Since the introduction of control measures for *Salmonella* in layers, such as control of the breeding flocks and vaccination, the number of human infections caused by SE, especially PT4, has reduced dramatically (Cogan & Humphrey, 2003). The number of SE PT4 isolates reported to the Health Protection Agency (HPA) in the UK has decreased from 15,564 in 1990 to 581 in 2009 (HPA, 2010a).

In the United States (US) SE infections in humans rose during 1980s, and replaced ST as predominant serovar (Altekruse, Koehler, Hickman-Brenner, Tauxe, & Ferris, 1993). The SE PTs initially predominant in the US were PT8 and PT14b (Altekruse et al., 1993), but subsequently a marked increase of PT4 was observed (Thorns, 2000). During 1991–1995 SE was isolated from 35% of US laying flocks and in 1999 from 7%, probably as a result of improvements in the on-farm biosecurity measures (Braden, 2006).

2.2. Salmonella Typhimurium and eggs

ST is often indicated as the prototype un-restricted *Salmonella*, even though it has a number of distinct sub-types (or PTs) that vary in their degree of host adaptation. There are more than 80 PTs involved in foodborne disease outbreaks, and these are normally characterized by having a broad host range (Rabsch et al., 2002).

Few egg related outbreaks of salmonellosis caused by ST are reported in humans in the EU (3.5% against 77.2% caused by SE) (EFSA, 2010a). Experimental studies (Gantois et al., 2008; Keller, Schifferli, Benson, Aslam, & Eckroade, 1997) have suggested that SE and ST can be equal in their potential to colonize the reproductive tract of hens and to infect forming eggs after a high level artificial challenge. However only SE was isolated from eggs after laying. A similar result was obtained in an oral and intravenous inoculation of laying hens with ST that did not cause contamination of the inner nor outer surface of the eggs laid (Baker, Goff, & Mulnix, 1980). Okamura et al. (2001) reported that after intravenous infection of hens with ST, all the eggs laid were negative for ST. The same authors reported a vaginal inoculation that produced ST positive eggs (Okamura, Miyamoto, et al., 2001). It was also demonstrated that ST can persist in the egg albumen during egg formation, and that it could resist lysozyme in the albumen better than SE (Gantois et al., 2008).

Limited reports are available on vertical transmission of ST. Cox, Davis, Watts, and Colmer (1973) reported a low level of egg contamination of chickens infected with ST. One hundred percent of shells and membranes of fertile eggs inoculated with ST by immersion in a concentrated bacterial broth were *Salmonella* positive 30 min after inoculation, but only 38% were still positive after 17 to 21 days post inoculation after incubation at 42 °C. The majority of chicks hatching from eggs with positive shells and membranes were *Salmonella* negative (Cason, Bailey, & Cox, 1993). During experimental infection of hatching eggs with ST, it was demonstrated that the ST strain was able to penetrate the egg shell and membranes and infect the embryos (Cason, Cox, & Bailey, 1994).

During the 1990s ST definitive phage type (DT) 104 spread worldwide and is now common in the animal population, including poultry, of many countries. In the UK ST DT104 peaked in 1996 and has since declined (Helms, Ethelberg, & Molbak, 2005). ST DT104 does not appear to frequently infect laying flocks and even when they are infected contamination of eggs or egg handling equipment is very rare (Carrique-Mas et al., 2009). In experimental conditions ST DT 104 was shown to be able to infect the contents of intact shell eggs (Williams et al., 1998). Okamura et al. (2010) reported a low capability of ST DT104 to cause egg contamination. An increased risk of egg contamination was however observed if the hens were infected at point of lay (Okamura et al., 2010).

Certain phage types of ST, such as DT2 and DT99, are host-adapted to wild birds (Rabsch et al., 2002) and infection in laying flocks with these strains is normally short-lived. ST of wild-bird origin may be found in free-range flocks, or occasionally in enclosed flocks as a result of feed contamination by bird droppings (during the final stages of growth in the field or during storage) (EFSA, 2010a).

Results from voluntary surveillance and NCP egg samples in the UK in the period between 2003 and 2010 show that ST was isolated only four times, three times from the contents of duck eggs and once from the shell of chicken eggs. SE was isolated from chicken eggs 6 times, 4 from the shells and 2 from the contents, SO were isolated from chicken eggs 5 times, 3 from the contents (one *S.* Pullorum, one *S.* Liverpool and *S.* Montevideo) and 2 from the shells ('O' Rough:Z:1,6, *S.* Senftenberg) (Veterinary Laboratories Agency data). As information on the source of these eggs is not available it is possible to hypothesize that *S.* Pullorum is likely to have been isolated from an embryonated hatching egg.

ST is the predominant serovar in ducks in the UK. This has been attributed to the fact that ST in ducks is effectively transmitted through the vertical route. In experimental studies ducks infected orally or intravenously with ST did not produce contamination of egg shells or contents. Contamination of hatching eggs from infected parental flocks is likely, but does not always occur (Henry, 2000). *Salmonella* isolates from two studies targeting infected ducklings were mostly ST (93% and 61% respectively) (Price & Bruner, 1962; Simko, 1988).

Egg related ST outbreaks are reported in the literature. In the period between 1984 and 1995 12 ST egg related outbreaks were reported in Great Britain (HPA — UK data). In France and Italy ST has been associated with egg-borne outbreaks (Carraminana, Humbert, Ermel, & Colin, 1997; Greig & Ravel, 2009; Scuderi, Fantasia, Filetici, & Anastasio, 1996). Aphasic (not expressing any phase of the H flagellar antigen) ST in eggs has caused a large outbreak in France in 2009 (AFSSA, 2009) and ST DT8 contamination of duck eggs has caused significant prolonged outbreaks of salmonellosis in humans in England, Northern Ireland and Eire (HPA, 2010b). In Australia, where SE is not endemic in laying flocks, egg related *Salmonella* outbreaks most often involve ST, usually due to contamination of the shells which is a recognized issue (Fullerton, 2008). In Europe, SE was reported as mostly related with egg associated outbreaks (40.9% of the total), while ST was primarily associated with pork meat related outbreaks (7.1% of the total). In the food borne *Salmonella* outbreaks in 2008, pig meat was the vehicle reported for 3.9% of the verified outbreaks. Particularly, pig meat might have contributed to the recent significant rise in ST cases in humans in most EU countries (EFSA, 2010b).

2.3. Other serovars and eggs

Other *Salmonella* serovars, e.g., *S.* Mbandaka, *S.* Livingstone, *S.* Heidelberg, *S.* Hadar, *S.* Infantis and *S.* Virchow, also occur with low frequency in layers and consequently on egg surfaces (Chemaly

Table 1
Details on surveys of *Salmonella* contamination in table eggs considered in this review. Information on the localization of the isolates is provided when available (S: shell only; C: contents only; S + C: both shells and contents). Where available, the origin of imported eggs is detailed in a footnote.

Country (year) and references	Positives/samples (pool size)	Shell only	Contents only	Both contents and shell	SE	ST	SO	SO list
UK (Jan 1991–Dec 1991) (de Louvois, 1993) 1993 British eggs.	65/7045 (pools of 6 eggs)	48	7	10	47 (30S, 7C, 10S + C)	6	12	*S.* Infantis (1), *S.* Livingstone (8), Others not specified (3).
UK (Jan 1991–Dec 1991) (de Louvois, 1993) 1993 Imported eggs (a).	138/8630 (pools of 6 eggs)	110	2	26	19 (14S, 2C, 3S + C)	9	110	*S.* Infantis (55), *S.* Livingstone (31), *S.* Braenderup (8), Others not specified (16).
UK (Jan–Feb 1991), British eggs. CVL Weybridge Unpublished data	18/2510 (pools of 6 eggs)	13	3	2	8 (6S, 2S + C)	3 (2S, 1S + C)	7	*S.* Livingstone (2S), *S.* Derby (1S), *S.* Isangi (1S), *S.* Untypable (1S), *S.* Senftenberg (2C).
UK (June1991–July1992) Eggs packed in England and Wales (MAFF CVL Weybridge, Unpublished data)	122 (pools of 6 eggs)	97	14	11	65 (51S, 6C, 8S + C)	7 (7S)	50	*S.* Virchow PT26 (6S, 1 S + C), *S.* Livingstone (incomplete data), *S.* Goldcoast (4S + 3C), 0:Z:1,6 (1S + 1C), *S.* Agama (2S, 1S + C, 1C), *S.* Panama (4S), *S.* Braenderup (3S), *S.* Poona (2C, 1S + C), Untypable (incomplete report), *S.* Bredeney (1S), *S.* Derby (1S), *S.* Heidelberg (1S), *S.* Newport (incomplete report), 4,12:–:1 (1S).
UK (1995–1996) (ACMSF, 2001) UK eggs.	138/13970* (pools of 6 eggs)	NA	NA	NA	119	6	19	*S.* Mbandaka (4), *S.* Livingstone (5), *S.* Kimuenza (2), *S.* Indiana (2), *S.* Virchow (2), *S.* Infantis (1), *S.* Braenderup (1), Other serotypes (2).
UK (1996–1997) (ACMSF, 2001) Imported eggs.	29/1433 (pools of 6 eggs)	NA	NA	NA	18	0	11	*S.* Taksony (5), *S.* Livingstone (2), *S.* Braenderup (2), *S.* Virchow PT2 (1), *S.* Infantis (1).
UK (2002) London catering establishments (Little, Surman-Lee, et al., 2007)	7/726 (pools of 6 eggs)	NA	NA	NA	at least 2	0	at least 2	*S.* Cerro, *S.* Livingstone.
UK 2002–2004 Catering England and Wales associated with SE outbreaks (b). (Little, Surman-Lee, et al., 2007)	88/2102* (pools of 6 eggs)	NA	NA	NA	80	0	33	*S.* Infantis (12), *S.* Livingstone (2), *S.* Altona (7), *S.* Bredeney (1), *S.* Ohio (11).
UK (2003) Catering eggs (c) (Elson, Little, & Mitchell, 2005)	17/5686 (pools of 6 eggs)	NA	NA	NA	15	1	1	*S.* Livingstone (1).
UK(2003) UK produced shell eggs on retail sale (FSA, 2004)	9/4753 (pools of 6 eggs)	9	0	0	7 (7S)	0	2	*S.* Infantis (1S), *S.* Livingstone (1S).
UK (2004) Eggs from positive flocks (Davies & Breslin, 2004)	92/13652 (pools of 6 eggs)	78/13652	9/13640	5/13682	33/13682 (24S, 6C, 3S + C)	2/13652 (2S)	57/13682	*S.* Infantis (41S + 2C), *S.* Livingstone (11S), *S.* Newport (2).
UK (2005–2006) Imported eggs (d) (FSA, 2007; Little, Walsh, et al., 2007)	157/1744* (pools of 6 eggs)	147	NA	10	136 (129S, 7S + C)	0	21	*S.* Braenderup (1S), *S.* Infantis (1S), *S.* Mbandaka (11S + 3C), *S.* Panama (1S), *S.* Rissen (2S), *S.* Unnamed (6S), *S.* Weltevreden (1S).
UK (2006) Catering premises (f) (FSA, 2007; Little et al., 2008)	6/1588 (pools of 6 eggs)	5	NA	1	5 (4S, 1S + C)	0	1	*S.* Mbandaka (1S).
UK (2008) Catering establishments (Gormley, Little, Murphy, de Pinna, & McLauchlin, 2010)	1/764 (mixed size pools)	NA	NA	NA	1	0	0	NA
NORTHERN IRELAND (1996–7) (I. G. Wilson, Heaney, & Powell, 1998)	9/2090 (pools of 6 eggs)	8	1	0	39 (2S, 1C)	1(1S)	5	*S.* Mbandaka (1S), *S.* Montevideo (1S), *S.* Infantis (2S), *S.* Kentucky (1S).
REPUBLIC OF IRELAND (2003) (Anonymous, 1993)	0/1169 (pools of 6 eggs)	NA	0	NA	NA	NA	NA	NA
IRELAND (2005–2006) (Murchie et al., 2007)	2/5018 (pools of 6 eggs) (pools of 10 eggs)	2	0	0	0	0	2	*S.* Infantis (1S), *S.* Mondevideo (1S).

ALBANIA (1996–1997) Imported eggs (g) (Telo et al., 1999)	1/79	1	0	0	0	0	1	*Salmonella* group C (no further serotyped).
FRANCE (2008) Eggs collected from positive flocks (Chemaly et al., 2009)	44/4200 (individual eggs)	44	NA	NA	17 (17S)	3 (3S)	24	*S.* Montevideo (2S), *S.* Virchow (18S), *S.* Infantis (4S).
JAPAN (2007–2008) Catering eggs (Sasaki et al., 2010)	5/2030* (pools of 10 eggs)	5	0	0	2 (2S)	0	3	*S.* Derby (2S), *S.* Livingstone (1S), *S.* Cerro (1S).
JAPAN (2004–2006) Soiled eggs (dirty) (Lapuz et al., 2008)	30/1766 (pools of 90 eggs)	NA	NA	30	7 (S + C)	1? (S + C)	22/23	*S.* Infantis (22), 1 no data available.
JAPAN (2004–2006) Processed eggs (clean) (Lapuz et al., 2008)	116/11280 (pools of 40 eggs)	NA	116	NA	112 (C)	0	4	*S.* Infantis (4C).
JAPAN (2004–2006) Packed eggs (supermarket) (Lapuz et al., 2008)	3/9010 (pools of 10 eggs)	NA	3	NA	2 (C)	0	1	*S.* Infantis (1C).
URUGUAY (2000–2002) (Betancor et al., 2010)	58/620 (pools of 20 eggs)	NA	58	NA	8 (C)	0	50	*S.* Derby (39C), *S.* Panama (2C), *S.* Gallinarum (9C).
USA — ARKANSAS (1994) (Schutze et al., 1996)	1/100 (pools of 12 eggs)	1	0	0	0	0	1	*S.* Heidelberg (1S).
USA (1993–1994) Eggs at washing plants (1) (F. T. Jones et al., 1995)	8/180 (individual samples)	8	NA	NA	0	0	8	*S.* Heidelberg and *S.* Montevideo.
USA (1993–1994) Eggs at washing plants (2) (F. T. Jones et al., 1995)	0/180 (individual samples)	NA	0	NA	0	0	0	NA
USA (2006) Restricted eggs (D. R. Jones & Musgrove, 2007)	2/180 (pools of 6 eggs)	1	0	0	0	0	2	*S.* Heidelberg (2S).
HAWAII (1989) (Ching-Lee et al., 1991)	10/106* (pools of 12 eggs)	10/106	0	0	0	0	10	*S.* Braenderup (2S), *S.* Oranienburg (4S), *S.* Mbandaka (1S), *S.* Ohio (1S), *S.* Havana (1S), *S.* Montevideo (2S), *S.* Livingstone (1S).
CANADA (1996) Eggs from washing and grading stations. (Poppe et al., 1998)	1/252	NA	NA	1	0	0	1	*S.* Agona (1).
NEW ZEALAND (2005–2006) (Wilson, 2007)	9/514 (pools of mixed sizes)	9/514	0	0	0	0	9	*S.* Infantis (9S).
AUSTRALIA (2009) (Chousalkar et al., 2010)	0/500 (individual eggs)	0/500	0	0	0	0	0	NA
SOUTH INDIA (1997–1998) (Suresh, Hatha, Sreenivasan, Sangeetha, & Lashmanaperumalsamy, 2006)	39/492* (individual eggs)	30	0	9	35 (26S, 9S + C)	0	4	*S.* Cerro (2S), *S.* Molade (1S), *S.* Mbandaka (1S).
NORTH INDIA (2006–2007) Eggs from poultry farms (Singh, Yadav, Singh, & Barthy, 2010)	10/260 (individual eggs)	2	7	1	0	9 (9S)	1	*S.* Africana (1).
NORTH INDIA (2006–2007) Eggs from marketing channels (Singh et al., 2010)	17/300 (individual eggs)	10	5	2	0	6 (S)	11	*S.* Lagos (6), *S.* Rough Strain (4), S II (1).
IRAN (June–August 2008) Retail outlets (Jamshidi et al., 2010)	4/250 (individual eggs)	4	0	0	0	4 (S)	0	NA

SE: *Salmonella* Enteritidis.
ST: *Salmonella* Typhimurium.
SO: *Salmonella* serovars other than SE or ST.
NA: Not available.
Countries of origin of the eggs and number of pools of eggs analyzed for each country:
(a) Belgium (550), Denmark (830), France (350), Germany (750), The Netherlands (6130), Italy (20).
(b) UK (528), Germany (2), Portugal (50), USA (60), Spain (1100) and Not Known (362).
(c) UK (4987), Spain (22), Germany (10), Portugal (7), Republic of Ireland (3), Holland (3), Italy (3), Not Known (651).
(d) Belgium (13), France (348), Germany (45), Poland (4), Portugal (25), Republic of Ireland (23), Spain (1157), The Netherlands (129).
(f) UK (1413), Spain (48), Germany (38), The Netherlands (33), Frances (27), Portugal (8), Republic of Ireland (1), Poland (1), Mixed origin UK and Spain (2), Not Known (17).
(g) Bulgaria (60), Italy (6), Greece (6), Turkey (2), Rumania (2) Macedonia (2), Hungary (1).
* More than one serovar was found in one or more samples.

et al., 2009). Their occurrence varies greatly between countries (Poppe, Johnson, Forsberg, & Irwin, 1992; Snow et al., 2007).

In surveys on *Salmonella* prevalence in eggs conducted worldwide, a number of *Salmonella* serovars other than SE and ST have been isolated (see Table 1). These serovars were isolated mainly from eggshells, but also from egg contents (e.g., *S.* Senftenberg, *S.* Livingstone, *S.* Infantis). Several experimental studies have been conducted to compare the potential of egg invasiveness of SE to that of other serovars (Gantois et al., 2008; Gast, Guraya, Guard-Bouldin, Holt, & Moore, 2007; Gast, Holt, & Murase, 2005; Lublin & Sela, 2008; Okamura, Kamijima, et al., 2001; Okamura, Miyamoto, et al., 2001).

In an experimental study on the colonization of the reproductive organs by different serovars following intravenous infection, only SE and *S.* Hadar were found in the eggs (percentages of contaminated eggs 15.8 and 10 respectively). The other serovars (ST, *S.* Infantis, S. Heidelberg, and *S.* Montevideo) were not found on the eggshell or in the contents of any egg (Okamura, Kamijima, et al., 2001). When the same strains were used to artificially inoculate hens intravaginally with high numbers of *Salmonella* organisms, the percentage of contaminated eggs (either on the outer or inner surface of the eggshells or in the egg contents) was 27.6 for SE, 3.1 for ST, 6 for *S.* Infantis, 9.4 for *S.* Montevideo, 4.5 for *S.* Heidelberg and 4.9 for *S.* Hadar. The egg contents were contaminated only with SE (7.5%) and ST (3.1%) (Okamura, Miyamoto, et al., 2001). In both the experiments the birds were inoculated with 5×10^6 CFU (colony forming unit) (Okamura, Kamijima, et al., 2001; Okamura, Miyamoto, et al., 2001). In field conditions, the birds come in contact with a smaller number of *Salmonella* microorganisms, and there is a positive correlation between the degree of environmental contamination and the level of egg contamination (Wales, Breslin, Carter, Sayers, & Davies, 2007).

In the US, egg-borne outbreaks of *S.* Heidelberg have been reported, in line with the increased prevalence of this serovar in the poultry flocks in recent years (Foley & Lynne, 2008). In orally inoculated laying hens *S.* Heidelberg was able to colonize the reproductive organs and to internally contaminate eggs. The incidence of internal egg contamination during this study was greater for SE than for *S.* Heidelberg (Gast et al., 2007). In an intravenous infection study *S.* Heidelberg was demonstrated to be able to survive in the albumen during egg formation, while *S.* Virchow and *S.* Hadar were eliminated more rapidly (Gantois et al., 2008). In a study on the prevalence of different *Salmonella* serovars in ovaries of spent hens in the US, the most frequently detected serovar was *S.* Heidelberg (56%), followed by *S.* Agona (13%), *S.* Oranienburg (6.1%), *S.* Mbandaka (5.2%), *S.* Kentucky (3.5%), *S.* Montevideo (3.5%) and *S.* London (2.6%), and SE (2.4%) (Barnhart, Dreesen, Bastien, & Pancorbo, 1991).

Experimental studies were conducted to investigate the ability of *S.* Virchow to penetrate through the eggshell and to multiply into the egg contents (Lublin & Sela, 2008; Neill, Campbell, & O'Brien, 1985). The first study confirmed the ability of *S.* Virchow to penetrate eggshells (Neill et al., 1985), while in the second no penetration was observed. During experimental infection of egg contents, *S.* Virchow was able to multiply to large numbers in table eggs stored at room temperature. In cold storage (6 °C), *S.* Virchow survived for 6 weeks, after which the concentration decreased below detection level (Lublin & Sela, 2008).

In some cases the consumption of eggs has been attributed to outbreaks of SO in humans. In a recently published study, from a total of 4093 foodborne outbreaks reported internationally in the period 1996–2005, 46.9% were attributable to *Salmonella*. Of these, 513 were egg related. The number of egg related SO was indicated as 70 (of which 39 in Europe and 23 in the US). ST was linked to 47 egg related outbreaks (of which 31 in Australia and New Zealand) and SE to 396 (of which 326 in Europe). The authors discuss the potential bias that can be generated when attributing food sources from outbreaks reports, nevertheless consider these findings as a reliable order of magnitude when estimating food attribution (Greig & Ravel, 2009). In England and Wales in the period between 1984 and 2009, 6 *Salmonella* egg related outbreaks involving SO were identified (unpublished data HPA — UK).

3. Surveys on *Salmonella* contamination in table eggs

Surveys investigating *Salmonella* contamination of table eggs have been reported in the literature (see Table 1). Data from 27 published and 2 unpublished surveys are included in this article. Some surveys report results from different groups of eggs (e.g., imported or locally produced) and these have been considered as separate studies. Therefore the total number of surveys considered is 36. The data available from these surveys have been collated, to study trends and extrapolate information.

The surveys were conducted in different years, countries, using different bacteriological methods and for different purposes. The surveys were performed in a period ranging from 1991 to 2010 and in several countries: UK (14), Japan (4), USA (5), India (3), Ireland (3), Albania (1), Australia (1), Canada (1), France (1), Iran (1), New Zealand (1), and Uruguay (1).

The way eggs were analyzed varied in the different surveys; most frequently the eggs were pooled in groups of 6, but sometimes pooled in groups of 10 or tested individually. Some of the surveys analyzed the egg content or shell separately, while others mixed them together. Most of the surveys analyzed eggs from packing stations, points of sale or catering establishments. Two studies sampled eggs from *Salmonella* positive flocks (Chemaly et al., 2009; Davies & Breslin, 2004). One study (Little, Surman-Lee, et al., 2007; Little, Walsh, et al., 2007) targeted specifically eggs involved in outbreaks of SE in humans.

Table 1 summarizes this information for each survey. The serovars isolated in the surveys are divided into SE, ST and SO. Where available, a list of the SO is provided in the table.

Fig. 1 shows the proportion of SE, ST and SO in each survey.

At least one *Salmonella* positive sample was found in 33 of the 36 studies, the 3 where no *Salmonella* was isolated are listed in the table (Anonymous, 1993; Chousalkar, Flynn, Sutherland, Roberts, & Cheetham, 2010; Jones, Rives, & Carey, 1995). Two studies specifically targeted eggs coming from *Salmonella* positive flocks, and *Salmonella* were identified (Chemaly et al., 2009; Davies & Breslin, 2004). This can be explained by the fact that eggs from infected flocks could be expected to have a higher frequency of *Salmonella* contamination (Davies & Breslin, 2004). From January 2009 restrictions on human consumption have been applied to eggs produced in flocks infected with SE or ST in order to protect human health in the UK (Defra, 2007).

SE is the serovar that was most prevalent in the majority of the surveys, but in some of the studies was not isolated at all, or was not the most prevalent serovar (Ching-Lee, Katz, Sasaki, & Minette, 1991; Jones et al., 1995; Jones & Musgrove, 2007; Murchie et al., 2007; Poppe et al., 1998; Schutze, Fawcett, Lewno, Flick, & Kirby, 1996; Telo, Bijo, Sulaj, & Beli, 1999; Wilson, 2007). In two studies (Jamshidi, Kalidari, & Hedayati, 2010; Singh et al., 2010) ST was the most commonly detected serovar. In the first study, this could be explained with the fact that ST is the predominant serovar isolated from poultry farms in the region where the survey was conducted (Singh et al., 2010). In the second study, the authors describe ST as the serovar most commonly isolated from eggs in Iran (Jamshidi et al., 2010).

In some studies SE was not the most frequently isolated serovar. Some of these studies are reported from the US (Jones et al., 1995; Jones & Musgrove, 2007; Schutze et al., 1996). In the US during the 1980s ST was the serovar most frequently isolated from chickens. At the beginning of the 1990s ST prevalence gradually decreased while SE prevalence peaked. Since the mid 1990s the prevalence of a third serovar, *S.* Heidelberg, has been increasing (Foley & Lynne, 2008). In the US studies *S.* Heidelberg was often identified in eggs, even when SE was not found (Jones et al., 1995; Jones & Musgrove, 2007; Schutze et al., 1996). In a study conducted in New Zealand 1.8% of samples were positive for *Salmonella* in the eggshell. All the isolates were *S.*

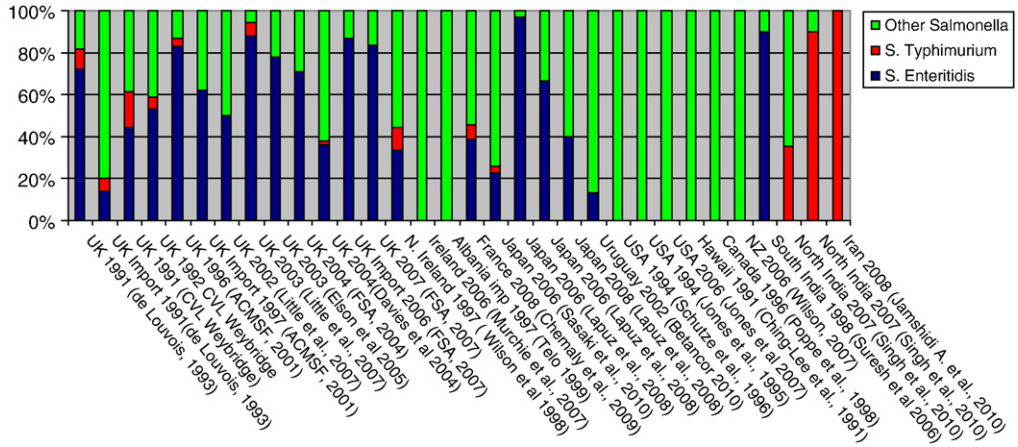

Fig. 1. Proportion of egg samples positive for *Salmonella* Enteritidis, *S.* Typhimurium, and Other *Salmonella* serovars, on the total number of samples tested positive in each survey.

Infantis, which is frequently isolated in New Zealand (Wilson 2007). In Japan *S.* Infantis was also isolated from eggs, both from shells and contents (Lapuz et al., 2008). SE and *S.* Infantis are the two main *Salmonella* serovars associated with human illness in Japan.

In the majority of the studies (29 out of 36), more samples were positive for SO than for ST. In some studies, one individual serovar exceeded ST. For example de Louvois (1993) reported 6 ST and 8 *S.* Livingstone positives in British egg samples (in a total of 65 positives), and 9 ST and 55 *S.* Infantis and 31 *S.* Livingstone positives in UK imported eggs (in a total of 138 positives) (see Table 1). In France (Chemaly et al., 2009) 3 ST and 18 *S.* Virchow samples were identified in a total of 44 positives, in Uruguay (Betancor et al., 2010) no ST was isolated, but 39 *S.* Derby positive samples (in a total of 58 positives) were reported (see Table 1).

The majority of the *Salmonella* were isolated from the eggshells in the studies when shells and contents were analyzed separately. All *Salmonella* serovars were most frequently isolated from the shells, but SE, ST and some SO (e.g., *S.* Infantis, *S.* Senftenberg, *S.* Goldcoast, *S.* Poona, *S.* Mbandaka, *S.* Derby) could be found in the contents also. SE was isolated from egg contents (or both egg contents and shells) in 9 out the 18 surveys in which information on the location of the isolation was given, while SO was found in contents in 4 out of 18 studies and ST in 1 out of 18. ST was isolated from only one sample, positive also on the shell, suggesting the possibility of trans-shell contamination. ST isolates were recovered mainly from the eggshells. This contrasts with findings of artificial infection studies, showing that ST can contaminate egg contents in high dose challenge models or where intravenous or intra-tracheal challenge is used (Keller et al., 1997; Okamura, Miyamoto, et al., 2001).

A number of surveys are available for the UK. Even though the surveys are not part of a homogeneous project, the information can be analyzed in a completive way. Information available from the UK surveys was collated and statistically analyzed. A frequentist approach was used to estimate the prevalence and confidence intervals, assuming a fixed pool size and perfect (100%) test sensitivity and specificity (Method 3 from (Cowling, Gardner, & Johnson, 1999)). Exact confidence limits were calculated based on binomial theory, so that confidence limits were never <0 or >1. Prevalence was estimated by: $p = 1-(1-x/m)^{1/k}$ where (p = estimated prevalence, k = pool size, m = the number of pools tested and x = the number of positive pools). The 95% confidence intervals were estimated by calculating the exact binomial confidence limits for the proportion of positive pools using STATA software (StataCorp, USA) and then transforming these back to individual-level prevalence values using the equation above. If none of the pools tested positive then the prevalence estimate was zero and the confidence intervals were based on a one-sided binomial 95% confidence limit.

Fig. 2 shows the prevalence of SE, ST and SO in different surveys conducted in the UK in the period 1991–2009. Only surveys targeting UK produced and catering eggs are included in the figure.

In the UK surveys, ST appears to be isolated rarely from eggs when compared to SE and to SO. It is possible to identify a peak of the presence of ST in eggs during the early 1990s. Subsequently, ST prevalence progressively declined and no ST was detected in the two most recent surveys (FSA, 2007; Little, Surman-Lee, et al., 2007). In two of the early studies (CVL Weybridge unpublished data and ACMS, 2001) DT104 was isolated from eggshells. ST DT104 peaked in the UK during the 1990s. ST DT104 was shown experimentally to be able to contaminate eggs, even if sporadically and at a low rate (Okamura et al., 2010; Williams et al., 1998). It is possible to hypothesize that the peak observed in ST egg contamination during the 1990s was due to the DT104 epidemic. In the surveys conducted after the 1990s, isolation of ST from eggs in the UK has been very rare, despite the fact that ST is still detected in flocks of laying hens. In the survey conducted in the UK in 2004 and 2005 ST was detected in 1.8% of the farms tested, predominantly free range holdings and included PT56, that is often associated with wild birds (Snow et al., 2007).

SE showed a peak during the early 1990s as well, and a progressive reduction over time. A higher SE prevalence is reported in the study of Little, Surman-Lee, et al. (2007) which was focused on imported eggs (from Belgium, France, Germany, Poland, Portugal, Republic of Ireland, Spain, and The Netherlands) that were associated with a food-poisoning outbreak. The estimated prevalence of SE positive eggs was particularly high in this study (see Fig. 2), and this could have been due to the link to premises related to SE outbreaks. In this study 52.3% of the pools of 6 eggs analyzed originated from Spain, and 5.5% of these Spanish eggs tested positive for SE. The use of Spanish eggs was identified as a significant risk factor in many non PT4 outbreaks in England and Wales during 2002–2004. The SE prevalence detected in this study does not reflect the prevalence of SE infection in UK eggs. Only 1.1% of the non Lion code quality batches of eggs and 0% of the Lion code quality UK eggs tested positive for *Salmonella*. None of the eggs originating from France, Germany, Portugal or the US were positive for *Salmonella*. A significant contamination rate (6.3%) was found in eggs coming from non UK eggs of unknown provenance (Little, Surman-Lee, et al., 2007).

SO were reported in the UK studies (both from locally produced and from imported eggs). The SO most frequently isolated were *S.* Infantis, *S.* Livingstone, *S.* Braenderup and *S.* Virchow.

4. Conclusions

This article presents a collation of the information available in the literature on contamination of eggs by different *Salmonella* serovars.

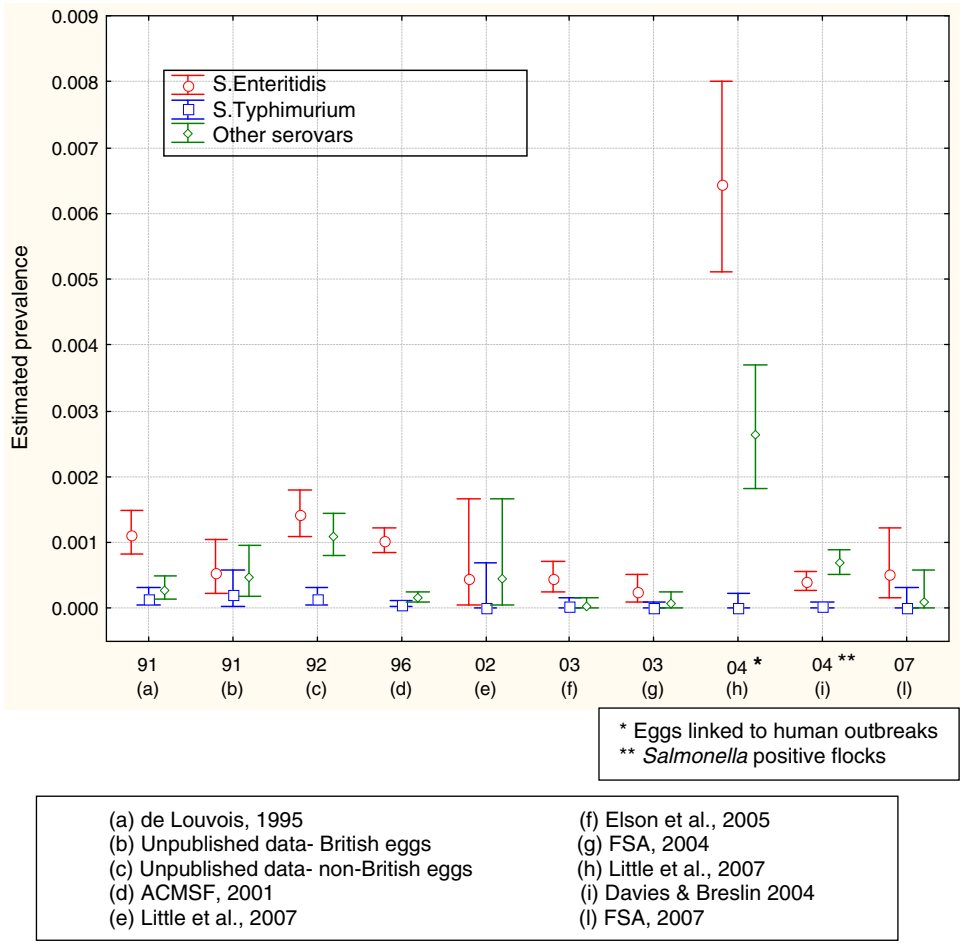

Fig. 2. Surveys of UK produced and catering eggs: estimated SE, ST and SO prevalence in eggs from pooled samples, with relative 95% confidence intervals.

The data available are not homogeneous, representing the distribution of *Salmonella* serovars in different parts of the world and at different time points, but some trends can be identified. SE appears to play a major role in egg contamination. Isolation of this serovar from eggs decreased in the UK after the 1990s, in accordance with the *Salmonella*-control measures adopted in laying flocks. SO are often isolated from eggshells, and they can also occasionally be found in egg contents. Isolation of ST from eggs is not frequent, and appears to be only on the eggshells. ST is currently considered, together with SE, the serovar with major public health relevance in laying hens. The data presented in this review shows how ST is not often isolated from eggs, when compared to SE and with other serovars. Egg-borne outbreaks of ST in humans can occur, but they are not frequent and result from unhygienic conditions in egg production and distribution. This gives rise to a situation that is analogous to passive contamination of meat and is different from the active and persistent ovarian contamination that occurs with SE. Egg contamination with ST does not seem to be frequent, and this underlines the marginal role that ST appears to have in eggborne infections.

Acknowledgments

The authors would like to thank Dr Robin Sayers for the statistical analysis. This work was funded by the Defra project OZ0332 (*Salmonella* Enteritidis and *S.* Typhimurium in egg production: elimination of infection from flocks, and characterization of public health risks).

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