

Emergence of *Salmonella enteritidis* Phage Type 4 in the Caribbean: Case-Control Study in Trinidad and Tobago, West Indies

Lisa Indar-Harrinath,^{1,2} Nicholas Daniels,³ Parimi Prabhakar,¹ Clive Brown,¹ Gail Baccus-Taylor,² Edward Comissiong,² and James Hospedales¹

¹Caribbean Epidemiology Centre, Pan American Health Organization/World Health Organization, Port of Spain, and ²Food Technology Unit, Department of Chemical Engineering, University of the West Indies, Trinidad and Tobago; ³Department of Medicine, Division of General Internal Medicine, University of California San Francisco

A prospective case-control study involving 46 case patients and 92 age- and neighborhood-matched control subjects was conducted in Trinidad and Tobago (T&T) between March 1998 and May 1999 to determine the etiology, sources, and risk factors for *Salmonella enteritidis* (SE) infection. SE infection in T&T was found to be associated with the consumption of shell eggs, and in particular raw or undercooked eggs. SE isolates from 30 (88%) of 34 patients and from 9 implicated egg or egg-containing food samples were phage type 4. Homemade eggnog and ice cream, cake batter, and egg-containing beverages were the main raw egg-containing foods, reflecting the cultural practices of the people of T&T. Public health education on the risks of eating raw or undercooked eggs, thorough cooking of all egg dishes, and refrigeration of shell eggs and egg dishes; studies tracing infected eggs to their sources; and testing of flocks of layer chickens for SE are needed to reduce the incidence of this infection.

Salmonella enteritidis (SE) is now the most common cause of human salmonellosis in most countries [1–3]. Salmonellosis, an infectious zoonotic disease caused by consuming food contaminated with the *Salmonella* bacteria, is the second leading cause of all food-borne diarrheal illnesses [1]. Since 1985, the incidence of salmonellosis has rapidly increased worldwide, doubling in the United States and the United Kingdom. Much of this increase has been attributed to SE [3–5]. Investigations of SE outbreaks and sporadic cases have identified

shell eggs as the dominant source of SE and the consumption of raw or undercooked eggs as a major risk factor for infection. Additionally, SE phage type 4 (PT4) was found to be more virulent than other SE phage types, causing marked increases in illnesses [6–12]. The global emergence of SE appears to be due to the acquired ability of SE to infect the hen's ovary and contaminate the intact egg without visible sign [3, 13].

In 1989, SE emerged in the Caribbean; it became a pathogen of public health concern when it caused a number of sporadic cases and outbreaks of diarrhea involving local and tourist populations. Isolation of SE steadily increased, and in 1996, it became the most frequent cause of salmonellosis in the Caribbean. The largest increases in the last 5 years have been reported in Trinidad and Tobago (T&T), followed by Jamaica and Barbados [14]. Since 1997, recurrent SE outbreaks have occurred at major tourist hotels in Jamaica and Barbados (Caribbean Epidemiology Centre, unpublished data).

Recent descriptive studies of SE infections in T&T

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This work is in compliance with the guidelines of the Caribbean Epidemiology Centre Ethics Committee. Informed consent was obtained from patients and control subjects or their parents or guardians.

Reprints or correspondence: Lisa Indar-Harrinath, Caribbean Epidemiology Centre, PAHO/WHO, 16-18, Jamaica Blvd., Federation Park, Port of Spain, Trinidad and Tobago, West Indies (north@carib-link.net).

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[15] showed that from 1992 to 1996, the isolation rate of SE markedly increased from 0.08 to 8.5 per 100,000 population and that the percentage of SE increased from 1% to 61%. From 1995 to 1997, children aged <10 years had the highest rate of SE infection (25 per 100,000 population), with children aged 0–4 years being the most susceptible (20–45 per 100,000 population). Forty-one percent of infections occurred in December and January, the Christmas–New Year holiday season. Of 100 prospective SE cases, 72% had eaten foods that contained raw eggs, and 31% reported that they had eaten undercooked eggs in the 3 days before onset of illness, strongly implying that there may be an association between consuming these foods and acquiring SE.

To explore the hypothesized risk factors derived from the descriptive studies, we conducted a case-control study of SE infections in T&T to define the etiology, sources, and risk factors for SE infection and to recommend prevention and control measures to reduce the incidence of infection.

PATIENTS AND METHODS

Case-control study. A matched case-control study to explore hypothesized risk factors for SE infections was conducted in T&T during March 1998–May 1999 using a standard written questionnaire administered via face-to-face interviews. Hypothesized risk factors used to design the questionnaire for this study were identified from previous descriptive studies [15]. A case patient with SE was defined as a person with diarrhea (3 or more loose stools in a 24-h period) and with SE isolated from stool or blood specimens during the study period. When >1 person in the same household, cluster, or outbreak had culture-confirmed infections, only the person with the earliest diarrheal illness onset date was included in the case-control study. People whose primary residence was not T&T or who had visited T&T for <1 month were excluded from the study.

Case patients were identified through enhanced laboratory surveillance at 3 major hospitals, private laboratories, and the Trinidad Public Health Laboratory. Laboratories were contacted daily to minimize the time between the date of identification of a patient with SE and the interview date. Two age- and neighborhood-matched control subjects with no diarrheal illness in the previous 4 weeks were matched to each case patient. Age-group matching criteria were as follows: <1 year, 1–4 years, 5–9 years, 10–19 years, 20–49 years, and >50 years of age. Case patients were interviewed about foods and beverages they had consumed, recent travel, and food-handling practices in the 3 days before onset of illness; control subjects were asked about the same exposure risks during the same 3 days as the matched patient. For case patients and control subjects who were <15 years old, a parent or guardian was also interviewed.

Laboratory. All *Salmonella* group D isolates identified at

hospitals or private laboratories were confirmed by API 20 E test kits (Analytab Products) [16]. This was followed by serological analysis using standard methods [17] and antimicrobial susceptibility testing [18]. Suspect food samples (e.g., egg-containing foods or eggs that were used to prepare the egg dishes) were collected from ill patients, from the point of purchase, or both; refrigerated; promptly transported to the laboratory; and cultured for *Salmonella* by the standard US Food and Drug Administration method [19], followed by serotyping. Shell eggs were sterilized [19] and pooled [20] before cultures were performed. Selected clinical and food SE isolates were phage typed [21] at the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia.

Statistical analysis. Data were entered and analyzed by Epi Info version 6.04b software (CDC). Matched ORs (mORs) and 95% CIs were calculated for food exposures. A mOR value >1 with 2-sided $P < .05$ was considered to demonstrate a significant association between consuming a particular food and acquiring SE infection. Risk factors that were significant and had defined ORs were analyzed further by conditional logistic-regression models through stepwise deletion of variables using SAS software version 6.12 (SAS).

RESULTS

Case-control study. We identified a total of 46 case patients and 92 age- and neighborhood-matched control subjects. Case patients and control subjects were statistically similar to each other in terms of age, sex, ethnic distribution, and place of residence (table 1). Dates of onset of illness ranged from 15 March 1998 to 24 April 1999. The median age of case patients was 8 years (range, 4 months to 67 years). Twenty-eight (61%) patients were <10 years of age; 24 (52%) were girls or women. The 46 case patients included people who were of African descent (52%), of Indian descent (26%), of mixed race (20%), and white (2%). A similar ethnic distribution was observed among the control subjects (table 1). However, the ethnic background of the case patients and control subjects was slightly different from that of the general population of T&T (Africans, 39%; Indians, 40%; and mixed race, 19% [22]).

All 46 patients sought medical care at general hospitals (85%), health centers (8%), or private doctors (7%). Reported symptoms included diarrhea (100%), fever (96%), abdominal cramps (94%), vomiting (91%), bloody diarrhea (61%), nausea (52%), myalgias (50%), and headache (41%). The median duration of illness was 7 days (range, 2–17 days). Thirty (65%) patients were hospitalized for a median of 4 days (range, 1–21 days); 2 (4%) died. Treatments administered included oral rehydration fluids (96%), iv fluids (67%), antibiotics (72%), and antidiarrheal (59%) medications. Before the onset of SE diarrheal illness, 8 (17%) patients reported an underlying illness

Table 1. Characteristics of 46 case patients infected with *Salmonella enteritidis* and 92 control subjects, matched case-control study, Trinidad and Tobago, March 1998–May 1999.

Characteristic	Case patients	Control subjects
Age, median (range)	8 y (4 mo–67 y)	7 y (2 mo–70 y)
Age <10 y	28/46 (61)	56/92 (61)
Sex, %, F:M	52:48	53:47
Race, % African:East Indians:mixed	52:26:20	48:31:19
Underlying illness before onset of diarrhea in case patient	8/46 (17)	1/92 (1)
Consumed eggs 3 d before onset of illness in case patient ^a	44/46 (96)	57/92 (62)
Ate raw eggs 3 d before onset of illness in case patient ^{a,b}	35/44 (80)	6/57 (11)
Purchased eggs from unrefrigerated sources ^{a,b}	35/44 (80)	14/57 (25)
Did not refrigerate eggs at home ^{a,b}	33/44 (75)	7/57 (12)
Does not wash eggs before use ^a	45/46 (98)	19/92 (21)

NOTE. Data are no. (%) of patients, unless otherwise indicated.

^a $P < .05$.

^b Percentage calculated from subset of subjects who consumed raw eggs before the onset of illness in the case patient.

(table 1), including sickle cell anemia ($n = 4$), septicemia ($n = 2$), HIV infection ($n = 1$), and multiple myeloma ($n = 1$), and 7 (15%) patients reported taking an antimicrobial agent. Four patients (9%) were hospitalized for other causes at the time they acquired SE infection. None of the patients or control subjects reported traveling to another country during the month before the onset of illness.

In the matched case-control study, illness was associated with eating shell eggs (mOR, 8.8; 95% CI, 2.7–98) and strongly associated with eating dishes containing raw or undercooked eggs (mOR, undefined; table 2). The risk of illness correlated with the frequency of egg consumption (χ^2 for trend, 57; table 3). In univariate analysis, it was found that, among both adults and children, consuming foods or beverages that contained raw or undercooked eggs, such as homemade eggnog, cake batter, homemade ice cream, punch a creme (which is similar to eggnog), and stout and eggs, and having an underlying blood or immunologic disorder were significantly associated with SE illness. Although eating cake was found to be a significant risk factor in the univariate analysis, it was no longer associated with illness when the data were stratified by those for eating cake batter. For children <10 years of age, the consumption of cake batter in particular was strongly associated with illness. Case patients were less likely than control subjects to have bought refrigerated eggs or to have refrigerated their eggs at home (table 2). No association was found between eating chicken and acquiring SE.

The following variables were included in multivariate models:

eating shell eggs, refrigerating eggs at home, using chlorinated water, and the number of times eggs were consumed. In multivariate analysis, the consumption of shell eggs (OR, 7.4) and the number of times shell eggs were consumed (OR, 4.0) remained associated with illness. Refrigeration of eggs at home remained protective (OR, 0.07), but the use of chlorinated water was no longer protective against infection ($P > .05$; table 4).

Laboratory. SE was confirmed either by stool (45 isolates) or blood culture (1 isolate) in all 46 patients in the study. Thirty-four patients' SE isolates were selected for phage typing; of these, 30 (88%) were found to be PT4, and 4 (12%) were phage type 1. SE was isolated from 15 (45%) of 33 food samples implicated by case patients' food histories. These were as follows: 12 (40%) of 30 samples of pooled eggs, 2 homemade eggnog samples, and 1 punch a creme drink. Nine of the 15 food isolates were phage typed, and all 9 were PT4. All isolates were susceptible to ampicillin, chloramphenicol, gentamicin, neomycin, streptomycin, tetracycline, and ciprofloxacin.

DISCUSSION

In this first reported case-control study of SE infection in the Caribbean, we have demonstrated that the consumption of shell eggs, in particular raw or undercooked eggs or foods containing them, is a significant risk factor for sporadic SE infections. These results are similar to international findings [9–12, 23, 24]. The range of raw egg-containing foods associated with SE illness in our study, including cake batter, and

Table 2. Univariate analysis of selected risk factors for sporadic *Salmonella enteritidis* infections for a matched case-control study, Trinidad and Tobago, March 1998–May 1999.

Risk factor	No. (%) of subjects		Matched OR (95% CI)	P
	Case (n = 46)	Control (n = 92)		
Ingested eggs				
Shell eggs	44 (96)	57 (62)	8.8 (2.7–98)	<.001
Raw or undercooked eggs ^a	35 (80)	6 (11)	Undefined	.001
Homemade eggnog	7 (15)	0 (0)	Undefined	<.001
Raw egg in drink	5 (11)	0 (0)	Undefined	<.01
Cake batter	11 (24)	1 (1)	Undefined	<.001
Soft-boiled eggs	4 (9)	3 (3)	3.50 (0.45–38)	.1
Hard-boiled eggs	8 (17)	29 (32)	0.41 (0.13–1.1)	.04
Fried, runny yolk	3 (7)	0 (0)	Undefined	.01
Fried, hard yolk	6 (13)	21 (23)	0.50 (0.13–1.4)	.1
Ingested egg-containing dishes				
Macaroni pie	13 (28)	18 (20)	1.7 (0.65–4.3)	.2
Macaroni and raw eggs	3 (7)	1 (1)	6.0 (0.48–314)	.1
Cakes	18 (39)	16 (17)	3.4 (1.32–9.2)	<.01
Pastries	13 (28)	11 (12)	1.1 (0.29–3.7)	.05
Ingested egg-containing salads and desserts				
Tuna salad	2 (4)	0 (0)	Undefined	.04
Homemade ice cream	5 (11)	1 (1)	10.0 (1.1–472)	<.01
Mayonnaise	6 (13)	4 (4)	5.0 (0.8–52)	.03
Trifles	1 (2)	0 (0)	Undefined	.2
Ingested chicken				
Any chicken	29 (63)	72 (78)	0.5 (0.19–1.10)	.04
Raw or undercooked chicken ^b	1 (3)	1 (1)	1.5 (0.02–117.6)	.7
Ingested other meats, fish, and poultry				
Ground beef	6 (13)	10 (11)	1.3 (0.33–4.58)	.6
Beef	3 (7)	8 (9)	0.70 (0.1–3.55)	.5
Pork	3 (7)	16 (17)	0.3 (0.03–1.24)	.06
Fish	10 (22)	35 (38)	0.5 (0.19–1.10)	.04
Ingested dairy products				
Powdered milk	31 (67)	56 (61)	1.5 (0.6–4.8)	.2
Creme milk	15 (33)	30 (33)	1.0 (0.4–2.7)	.6
Exposed to animals				
Any	6 (13)	5 (5)	4.50 (0.7–42.7)	.08
Birds/parrots	1 (2)	3 (3)	0.07 (0.01–8.3)	.7
Chickens	5 (11)	8 (9)	1.33 (0.3–5.4)	.4
Food handling				
Bought refrigerated eggs ^a	9 (20)	43 (75)	Undefined	<.001
Refrigerated eggs at home ^a	11 (25)	50 (88)	0.03 (0.0–0.2)	<.001
Handled raw chicken ^b	21 (72)	51 (71)	1.08 (0.38–3.1)	.8
Handled raw eggs	23 (50)	21 (23)	6.00 (1.88–19)	<.001
Prepared dish with >3 eggs	20 (43)	3 (3)	38.0 (5.9–1539)	<.001
Other				
Use of chlorinated water	34 (74)	82 (89)	0.07 (0.00–0.52)	<.01
Underlying illness	8 (17)	1 (1)	16.0 (2.1–710)	<.001

^a Percentage calculated from subset of subjects who consumed raw eggs; n = 44 (case patients) and n = 57 (control subjects).

^b Percentage calculated from subset of subjects who consumed chicken; n = 29 (case patients) and n = 72 (control subjects).

Table 3. Dose response for the number of times eggs or egg-containing dishes were consumed by case and control subjects in the 3 days before onset of *Salmonella enteritidis* illness in the case patient, Trinidad and Tobago, March 1998–May 1999.

No. of times eggs/egg dishes were consumed	No. (%) of subjects		Matched OR (95% CI)	P
	Case (n = 46)	Control (n = 92)		
0	2 (4)	35 (38)	1.0 (reference)	—
1	7 (15)	40 (43)	3.0 (0.6–16.1)	.20
2	15 (33)	15 (16)	9.5 (2.0–46.2)	.005
≥3	22 (48)	2 (2)	68.7 (9.4–500)	.0001

NOTE. Data are no. (%) of patients.

homemade eggnog and ice cream, and raw eggs in drinks (e.g., punch a creme, peanut punch, orange juice, aloes, and stout), reflects the cultural and traditional customs of inhabitants of T&T. The undefined mOR for these foods indicates that the risk of acquiring SE infections from these specialty foods is exceptionally high; in most instances, only the case patients with SE had consumed them. Homemade ice cream and eggnog have previously been implicated as vehicles of SE infection [6, 7, 11, 24]. However, to our knowledge, this is the first study to implicate cake batter. We also observed a greater risk of illness associated with eating undercooked eggs (runny and soft-boiled eggs and dishes such as macaroni and raw eggs), although it was not statistically significant. Undercooked eggs are known vehicles for SE [2–11, 24], and SE has been found to survive in eggs cooked by various methods [25].

Our findings highlight the importance of purchasing refrigerated eggs and of storing eggs under refrigeration in homes. Experiments have shown that SE can rapidly multiply to infective levels in eggs that are not properly refrigerated at <7°C (<45°F) [26]. More than 80% of case patients bought eggs from unrefrigerated sources, including the local open street markets, roadside shops, stalls, and egg farms, and 65% did not refrigerate eggs at home, whereas >80% of the control subjects used eggs that were purchased from refrigerated sources and kept refrigerated at home. The occurrence of SE infections correlates with the holiday season, in particular Christmas (December) and New Year (January) in T&T, suggesting that during these times, foods and beverages that contain raw eggs are more frequently consumed. All study patients acquired SE infection in T&T, and most infections (82%) came from foods prepared in the home. Therefore, intervention strategies to prevent SE infections in T&T should include educational messages to household food purchasers, food handlers, and consumers that emphasize the need to purchase refrigerated eggs, to keep eggs refrigerated (<7°C), and to thoroughly cook all eggs and egg-containing dishes (>70°C). The tourist season in the Caribbean peaks during the winter months, at the same time that SE

infections are most common; thus, SE prevention messages should also be directed to hotels and restaurants that cater to the tourist trade.

SE PT4 was the most common phage type isolated from case patients as well as from implicated eggs and egg-containing foods. PT4 is more virulent than other SE phage types and is remarkable for its ability, once it is introduced into poultry, to cause marked increases in human illness [3, 13, 27]. SE PT4 has been dominant in Europe since the 1980s and recently (1994) emerged in the United States [1, 5, 28]. The high prevalence of PT4 in T&T suggests that SE might have been introduced through imported breeder flocks, chicks for layer flocks, or hatching eggs. No regulations currently exist for the importation or microbial monitoring of layer flocks or shell eggs in T&T. Thus, there is a possibility that SE may have been introduced through the importation of SE-infected flocks, eggs, or contaminated animal feed from the United Kingdom or the United States. This is supported by the fact that no cases of SE were reported in T&T before 1992 [15] (figure 1). In addition, a recent study in T&T of 750 fresh eggs from 10 farms (which supply ~75% of the country's eggs) found SE both on the shells (0.7%) and in the egg contents (0.8%) [29]. This demonstrated for the first time in T&T that transovarian SE transmission is occurring on farms and that normal-appearing eggs that harbor SE are being locally produced and marketed.

Several other epidemiologic features of our study are noteworthy. The high incidence rate of SE infection in children, which is similar to that seen in the United States [4], suggests that they are at higher risk for acquiring infection. Young children are susceptible to *Salmonella* infection at lower inocula than are adults [30]. Children aged <5 years were also recently shown to be at greater risk for SE infection from the consumption of raw or undercooked eggs [12]. In T&T, the predominant mode of SE transmission varied by age group. Among children, SE infection was mainly related to exposure to raw eggs in cake batter or homemade ice cream. It is a common practice in T&T to give children cake batter to eat. Interestingly, in adults, SE infection was mainly related to exposure to raw egg-containing foods and beverages, such as homemade eggnog, peanut punch, stout, and orange juice.

Table 4. Multivariate analysis of risk factors for sporadic *Salmonella enteritidis* infections, Trinidad and Tobago, March 1998–May 1999.

Significant variable	Matched OR (95% CI)	P
Egg consumed 1 time	3.1 (0.3–3.3)	.4
Egg consumed 2 times	14.0 (1.4–135)	.02
Egg consumed 3 times	176.0 (8.0–3897)	.001
Underlying illness	1162 (5.0–271,729)	.01
Use of chlorinated water	0.024 (0.001–0.40)	.01

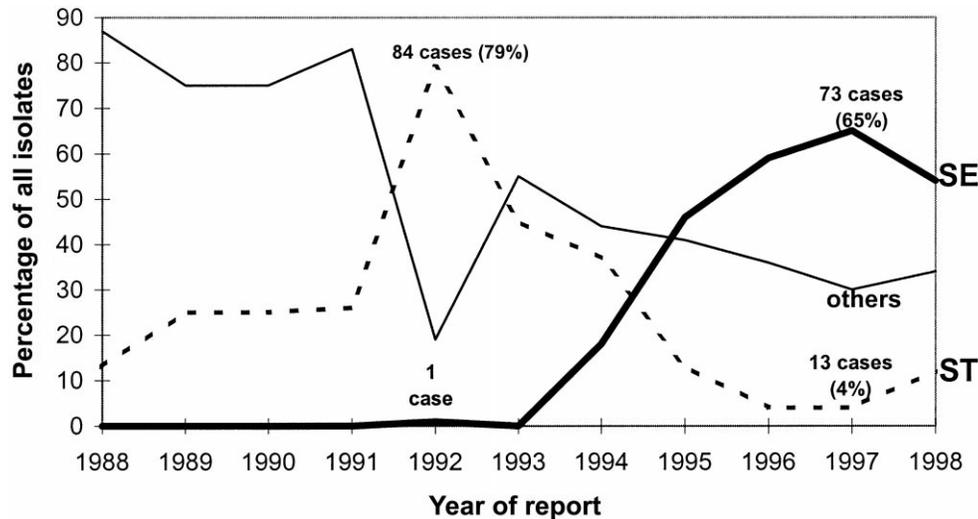


Figure 1. Percentage of infections with *Salmonella enteritidis* (SE), *Salmonella typhimurium* (ST), and other salmonella species by year in Trinidad and Tobago, January 1988–December 1998.

Trinidadians, especially men, view raw eggs as a healthy, invigorating food. This suggests the need for age- and sex-specific health education messages for SE prevention that emphasize the risks associated with raw eggs.

In our study, >60% of patients were hospitalized and 61% reported bloody diarrhea, suggesting that we captured only the most severe infections and that many more SE infections occurred in T&T that were not identified; only culture-confirmed SE cases were included in our study. Four hospitalized patients who acquired SE only ate food prepared in the hospitals, suggesting that these were nosocomial infections. In T&T, hospitals serve boiled eggs and egg dishes made by pooling shell eggs. Although no attempt was made in our study to link affected patients with the foods they consumed, these infections emphasize the importance of using only pasteurized or thoroughly cooked eggs in hospitals. Alternatively, these infections could have resulted from person-to-person transmission [12, 31]. Person-to-person transmission also may have also led to secondary cases in homes, where in many instances >1 person developed diarrhea.

The emergence of SE and the highly virulent SE PT4 in the Caribbean may affect tourism to the region. Because the Caribbean is more dependent on tourism than any other region in the world, continuous assurance of a safe and healthy food supply, particularly in hotels, is critical to sustaining its economy. Recent episodes of gastroenteritis among visitors have resulted in loss of revenue, negative publicity for the region, and litigious actions. It is thus imperative that food service workers in Caribbean hotels receive frequent training in proper handling and storage of shell eggs and preparation of egg-containing foods. Whenever possible, pasteurized eggs should be used for meals that require bulk pooled eggs. Food and

beverages containing raw and undercooked eggs should not be served.

In conclusion, this study confirms that consumption of raw or undercooked shell eggs and egg dishes is the major cause of sporadic SE infections in T&T. It highlights the fact that children are at greater risk than adults for SE infections, the importance of refrigerated storage of eggs, and the dominance of the more virulent SE PT4. These findings have important implications for public health, food safety, agriculture, trade, and tourism in T&T. Prevention and control strategies for SE in T&T require a farm-to-table [32] approach that combines public health education (on the risks associated with eating raw and undercooked eggs and with using unrefrigerated eggs) and strategies for reduction of SE infections among egg-laying flocks (including tracing implicated eggs to source farms and on-farm testing of egg-laying flocks for SE). Specific measures should be targeted at consumers, egg farms, grocers, restaurants, hotels, food service workers, and health institutions. Because raw and undercooked eggs are traditionally consumed in T&T, implementing measures to reduce SE infection at the production stage is important. Such on-farm studies have already begun in T&T. The most effective, but extremely costly, solution would be to replace SE-infected flocks with SE-free flocks. Continuous surveillance of SE is also needed for prompt outbreak recognition and to monitor for changes in phage types and antibiotic resistance.

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