Articles

Epidemic *Salmonella enteritidis* Infection in Los Angeles County, California
The Predominance of Phage Type 4

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Between April and July 1994, 501 cases of *Salmonella enteritidis* infection were reported in Los Angeles County, California, nearly 5 times the number reported between April and July 1993; of these, 422 (84%) were sporadic (not related to known outbreaks). A case-control study was done to determine risk factors for sporadic illness; the distribution of *S enteritidis* phage types was evaluated. Case-patients (n = 58) were county residents older than 1 year with culture-confirmed *S enteritidis* infection in August 1994. One to two acquaintance controls (n = 98) were matched to each case by age, sex, and race. Two risk factors—eating raw or undercooked eggs (matched odds ratio [MOR] = 6.6; 95% confidence interval [CI] = 1.9, 23.0) and eating in restaurants (MOR = 4.9; 95% CI = 1.2, 19.4) in the 3 days before the onset of illness—remained significant in the conditional logistic regression model. Of 16 randomly selected *S enteritidis* case-isolates, 15 (94%) were phage type 4. The reasons for the regional predominance of phage type 4, an *S enteritidis* subtype recently associated with large and destructive increases in salmonellosis among poultry and humans in Britain and much of Europe, are unclear. To minimize human *S enteritidis* infection, food service workers need frequent training in the proper handling of raw foods, eggs should be kept refrigerated during distribution and storage, and eggs should be cooked until the yolk is firm, particularly for persons at the greatest risk for serious illness: pregnant women, elderly persons, and those with compromised immune systems. Clinicians should obtain stool specimens for culture from patients who present with diarrhea and fever or bloody diarrhea or who are possibly part of an outbreak.

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Food-borne illness due to *Salmonella enteritidis* usually presents as a self-limited diarrhea with abdominal cramping, fever, and occasional vomiting. Pregnant women, elderly persons, and those with immunocompromise are at an increased risk for the development of invasive disease. Human *S enteritidis* infection has become an increasingly important public health problem since the early 1980s.[1,2] Before then, *S enteritidis* was an uncommonly reported *Salmonella* serotype globally. In the past 15 years, however, rates of illness due to this serotype have increased by fivefold to tenfold and more in the United Kingdom and in parts of Europe, Africa, and South America.[1,3,4] In the United States, the incidence of reported human *S enteritidis* infection has been highest in New England and the mid–Atlantic region.[3]

In Los Angeles County, California, 501 cases of *S enteritidis* infection were reported during the period April through July 1994, nearly five times the number of cases reported in the same four months of 1993 and more than twice the yearly average for 1990 to 1993 (Figure 1). Of these 501 cases, 422 (84%) were sporadic (unrelated to known outbreaks). This upsurge involved much of southern California: the number of cases tripled in nine southern California counties, from 261 in April through July 1993 to 799 in April through July 1994. In contrast, northern California counties experienced no overall increase during this period.

Of ten randomly selected Los Angeles County *S enteritidis* isolates collected in April 1994, nine (90%) were phage type 4. *Salmonella enteritidis* phage type 4 was a strain rarely isolated in the United States before 1990, and the first documented *S enteritidis* phage type 4–associated food-borne outbreak in this country was not reported until April 1993, in El Paso, Texas.[5] In May

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ABBREVIATIONS USED IN TEXT

CDC = Centers for Disease Control and Prevention
CI = confidence interval
DHS = Department of Health Services
MOR = matched odds ratio
USDA = United States Department of Agriculture

1994, eggs and hens in a large southern California egg ranch were found to be contaminated with *S enteritidis* phage type 4, the first time this strain had been documented in US poultry.[6]

In August 1994, the Los Angeles County Department of Health Services (DHS) and the California DHS undertook an investigation to identify possible risk factors for sporadic illness due to *S enteritidis* and to evaluate the distribution of *S enteritidis* phage types in this epidemic.

Cases and Methods

A matched case-control design was used to identify possible risk factors (foods and behaviors) associated with sporadic *S enteritidis* infection. A case was defined as an illness associated with culture-confirmed *S enteritidis* identified either in stool or blood specimens collected during August 1994 from a Los Angeles County resident older than 1 year. Only one case was included from each known outbreak or household cluster during the study period. Persons whose date of illness onset was not well defined due to the presence of chronic diarrhea—defined as more than 1 loose or watery stool daily for longer than a month—were excluded from the study. Also excluded were persons submitting specimens for "clearance" culture to return to work. Each case-patient provided one to two acquaintance controls, matched by age group (±1 year for case-patients <7 years old, ±3 years for case-patients 7 to 12 years old, ±5 years for case-patients 13 to 64 years old, and ±15 years for case-patients >64 years). Controls for case-patients older than 12 years were also matched for sex and race.

A questionnaire was administered by telephone to each study participant or, if younger than 12 years, to his or her parent. The questionnaire included questions about 135 specific food items eaten in the three days before the onset of illness and about activities (for instance, camping, drinking open-source water, or traveling outside Los Angeles County) in the week before onset (a longer time period was used for assessing behavioral exposures because of the ongoing or intermittent nature of many of those exposures). Control participants were asked about the same exposures during the same reference period as the case-patients with whom they were matched. If recall was a problem, the controls were instead asked about the same three days of the week from the week following the reference period for food exposures and the entire following week for other activities. If a participant was uncertain whether a given exposure occurred, a "not exposed" response was coded.

An "egg dish" was defined as scrambled, boiled, fried, poached, or uncooked eggs, omelets, quiches, eggs Benedict, or other foods that contain eggs as a primary ingredient. "Undercooked egg" referred to an egg that was cooked so that any of the yolk remained liquid, regardless of the preparation method. "Seafood" included shrimp, lobster, crawfish, clams, oysters, mussels, squid, and octopus, but not finned fish. "Soft cheese" included rancher's cheese (*queso ranchero*), Brie, or other spreadable cheese, but not cream cheese or cottage cheese. A "restaurant" was any commercial food preparation establishment, including fast-food outlets and street vendors.

All clinical and reference laboratories in Los Angeles County forwarded *Salmonella* group D isolates to the county's Public Health Laboratory or the California DHS Microbial Diseases Laboratory for serotype confirmation and identification. A random sample of 16 (28%) of 58 case-isolates from August 1994 was sent to the

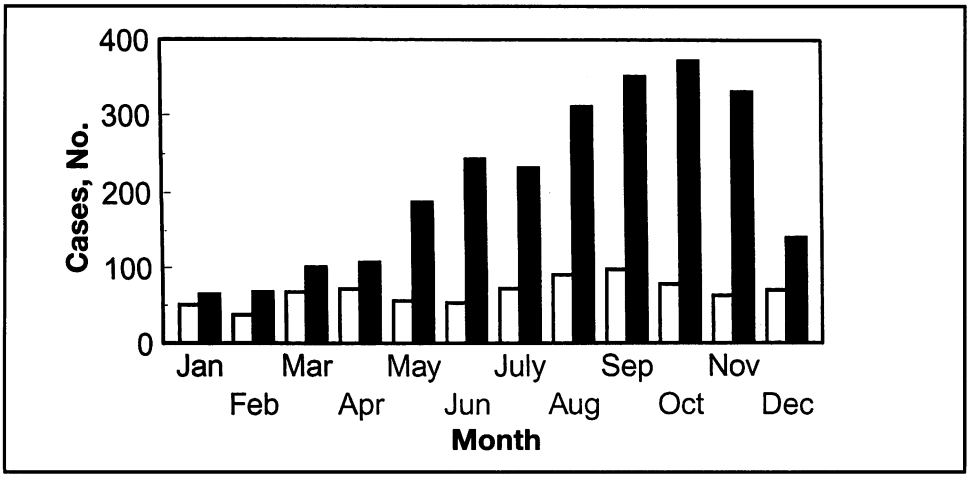

Figure 1.—California State Department of Health Laboratory–confirmed isolates of *Salmonella enteritidis* are shown by month. ❑ = 1990 to 1993 cases (mean), ■ = 1994 cases

TABLE 1.—*Association Between Sporadic Illnes Due to* Salmonella enteritidis *and Selected Foods and Activities Before Onset of Illness, Los Angeles County, August 1994 (matched bivariate analyses only)*

Food* or Activity†	Exposed Cases, (n = 58), No. (%)		Exposed Controls (n = 98), No. (%)		Matched Odds Ratio (95% Confidence Interval)
Egg dishes	37	(64)	45	(46)	3.0 (1.2, 7.9)
Raw or undercooked egg dishes	20	(34)	11	(11)	10.0 (2.5, 46.4)
Raw eggs	6	(10)	1	(1)	11.0 (1.2, 473.1)
Seafood	8	(14)	4	(4)	4.0 (1.0, 26.5)
Soft cheese	8	(14)	5	(5.1)	2.6 (0.7, 13.1)
Chicken	43	(74)	54	(55)	1.5 (0.7, 3.5)
Hamburger	20	(34)	49	(50)	0.3 (0.2, 1.1)
Turkey	6	(10)	27	(28)	0.2 (0.1, 0.8)
Apples	9	(16)	34	(35)	0.1 (0.1, 0.6)
Eating in restaurants	51	(88)	68	(69)	5.6 (1.6, 29.1)
Working in restaurants	1	(2)	0	(0)	Undefined
Travel out of county	11	(19)	9	(9)	3.6 (1.0, 12.7)
Exposure to pets	34	(59)	52	(53)	1.3 (0.6, 2.6)
Drinking from open water source	2	(3)	0	(0)	Undefined

* During the 3 days before the onset of illness.
† During the 7 days before the onset of illness.

Centers for Disease Control and Prevention (CDC) for phage typing.[7]

Crude comparative (bivariate) analysis was done by applying Mantel-Haenszel matched χ^2 analysis to dichotomous variables. Adjusted (multivariable) analysis was done using backward-elimination conditional logistic regression, which was applied to all risk factors associated with a *P* value of less than .20 in the crude comparative analysis.

Results

Of 143 Los Angeles County residents in whom *Salmonella enteritidis* was identified from August 1 through August 31, 1994, 13 persons were determined to have *S enteritidis* infection related to a known outbreak, 7 were younger than 1 year at the time of specimen collection, and 3 were household contacts of persons with documented *S enteritidis* infection. Of the 120 persons who otherwise met the case definition, 32 could not be located despite numerous attempts, and 5 refused or were too sick to provide a food history. Of the remaining 83 persons, 25 did not provide appropriate controls. The 58 remaining persons provided 98 matched controls: 40 provided two matched controls, and 18 provided one matched control. The median age of case-patients was 25 years (range, 1 to 93 years). Males and females were equally represented (29 each). The racial or ethnic distribution of the 58 cases was 34 (59%) non–Hispanic white, 12 (21%) Hispanic white, 8 (14%) African American, and 4 (7%) Asian or Pacific Islander.

Foods eaten more commonly by case-patients than by controls included egg dishes and seafood (Table 1). Notably, of the 37 case-patients who ate eggs, 20 (54%) reported eating them either undercooked or raw.

Behaviors that were significantly more common among case-patients than among controls included eating in any restaurant during the three days before becoming ill and traveling outside of Los Angeles County in the week before the illness (see Table 1). Of 20 study participants who traveled, 18 (90%) also ate in restaurants. No individual restaurants were associated with sporadic *S enteritidis* infection.

In the conditional logistic regression model, eating in restaurants, eating egg dishes, eating seafood, and eating soft cheese in the three days before illness were associated with *S enteritidis* infection. Because *Salmonella* species in food is destroyed by thorough heating, we categorized exposure to "eggs dishes" by how completely they had been cooked before being eaten. When the multivariable analysis was repeated using the subgroups "raw or undercooked eggs" and "well-cooked eggs" in place of "egg dishes (all)," only two exposures—eating raw or undercooked eggs (matched odds ratio [MOR] = 6.6; 95% confidence interval [CI] =1.9, 23.0) and eating in restaurants (MOR = 4.9; 95% CI = 1.2, 19.4)—were associated with an increased risk of *S enteritidis* infection.

One case-isolate was from blood; all other isolates were from stool. Of 16 case-isolates from August 1994 sent to the CDC for phage typing, 15 (94%) were phage type 4, and 1 was phage type 1. Therefore, a total of 24 (95%) of 26 *S enteritidis* isolates from Los Angeles County phage typed by the CDC in 1994 were phage type 4.

Discussion

Eating in restaurants and eating eggs (particularly undercooked or raw eggs) were significant risk factors for sporadic *S enteritidis* infection in Los Angeles County in August 1994 and may be major contributors to the upsurge of *S enteritidis* infections throughout southern California. California now clearly shares the problem of *S enteritidis* as an emerging infection with

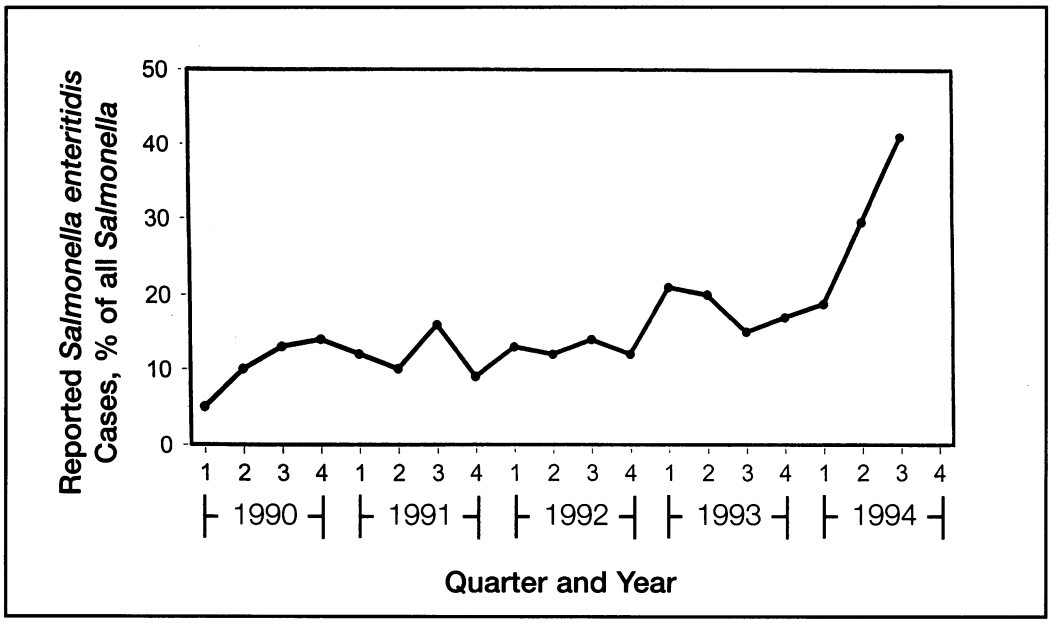

Figure 2.—Cases of *Salmonella enteritidis* as a percentage of all California *Salmonella* cases are shown, by quarter, for 1990 to 1994.

the eastern United States, although it is not clear why northern California has been spared to date.

This upsurge is unlikely to be an artifact of increased reporting. Although the total number of cases of human salmonellosis in California has risen only slightly in the past five years, *S enteritidis* accounts for an increasingly large proportion of these cases: in 1994, *S enteritidis* accounted for 35% of isolates statewide, compared with 18% in 1993 and only 12% in 1990 through 1992 (Figure 2).

The results of this study are consistent with findings of one British and two US studies that reported that eggs are an important risk factor for sporadic *S enteritidis* infection.[8-10] Similarly, eggs have been the most commonly implicated food item in *S enteritidis* outbreaks in the United States (1985 to 1993) for which a source has been identified (193 [86%] of 223 outbreaks).[1]

Eating in restaurants was the other important risk factor for *S enteritidis* infection, and this, too, has previously been noted[9]; undercooked eggs may be an unrecognized ingredient in many dishes, and imperfect food service sanitation practices may result in the cross-contamination or undercooking of foods.[11]

This study has several limitations. One was the possibility of bias in the selection of case-patients: a substantial proportion (31%) of residents who met the case definition were not included in our study because we could not contact them by telephone or they refused to be interviewed. Another limitation is that our study sample does not accurately reflect the racial or ethnic distribution of Los Angeles County. In particular, persons of Hispanic descent were underrepresented: whereas 40% of the county's population is Hispanic, Hispanics comprised only 21% of our case-patients. This occurred largely because Hispanic case-patients were disproportionately unable or unwilling to give out the telephone numbers of possible controls to investigators. Of 32 possible Hispanic case-patients, 20 (63%) were excluded from the study for failing to provide appropriate controls, the highest percentage of any racial or ethnic group.

In May 1994, chicken flocks were found to be infected with *S enteritidis* phage type 4 at an egg ranch in southern California, and eggs from those flocks were diverted to pasteurization. Preventing unpasteurized eggs from these flocks from reaching the consumer market, however, has not prevented the upsurge of *S enteritidis* infection in southern California. This suggests that other egg and nonegg sources of *S enteritidis* continue to contribute to the epidemic of salmonellosis in southern California. Although *S enteritidis* phage type 4 is the epidemic strain of *S enteritidis* in much of the world, including the United Kingdom, western Europe, and parts of South America,[3,5] this is the first time that this strain has been found to predominate among human *S enteritidis* isolates in a region of the United States, and it is the first time that it has been found in a US producer flock. The biologic mechanisms enabling *S enteritidis* phage type 4 to become a predominant phage type causing human illness remain unknown.

A trace-back of eggs associated with human *S enteritidis* infection, an important step in identifying the source of this epidemic, could not be performed. In California, as elsewhere in the United States, eggs from various sources are typically mixed by distributors before shipping to retail outlets. Before October 1, 1995, when investigating an outbreak or cluster of *S enteritidis* cases thought to be caused by eggs, the US Department of Agriculture (USDA) attempted to do a trace-back to

the egg ranch(es) of origin. If the trace-back identified two or more ranches as the possible sources, no microbiologic testing of the flock or premise was done. This policy made it difficult to identify the ultimate sources of sporadic egg-associated *S enteritidis* cases. Moreover, starting October 1, 1995, even *S enteritidis* outbreaks with a likely egg source may not be traced back; in the most recent federal budget, the USDA has been prohibited from spending funds on *S enteritidis* activities such as trace-backs and on-ranch testing.

Despite the fact that no trace-back was done as a result of this investigation, the documentation of an *S enteritidis* epidemic in southern California, the recent finding of *S enteritidis* phage type 4 in a local egg ranch concurrent with the epidemic, and the association of Los Angeles County *S enteritidis* cases with egg consumption suggest that this major public health problem may persist or worsen, as experience with *S enteritidis* in the northeastern United States has shown. Indeed, the *S enteritidis* epidemic in California has continued unchecked since this case-control investigation was done: in 1995, 2,495 cases of *S enteritidis* were reported to California DHS, a 300% increase compared with the yearly averages for 1990 to 1993. Furthermore, although no association with egg consumption was shown, ten Los Angeles County residents died after *S enteritidis* infection in 1995, the same number that died with the infection in 1994.

It is disturbing that a high percentage of persons who ate eggs reported eating them raw or undercooked; local, state, and federal agencies, as well as the egg industry, have repeatedly tried to alert the public about the risk of eating undercooked eggs. The USDA and the egg industry also advise consumers to cook their eggs thoroughly. Our findings suggest that the message is not reaching the public or that it is unwilling to change its eating habits.

Unlike most other *Salmonella* serotypes, *S enteritidis* can invade the ovary and oviduct of poultry and lead to vertical transmission: *S enteritidis* that has infected hens by oral, intravenous, or cloacal routes has resulted in the transovarian transmission of *S enteritidis* to eggs and chicks.[12–14] This has profound implications for both the poultry industry and the public: it means that because *S enteritidis* cannot be eliminated from egg-laying flocks using current technology, *S enteritidis*–infected eggs could reach the consumer market despite exemplary sanitation practices from the ranch onward.

Several recommendations can be made to minimize the public health risks associated with *S enteritidis* infection. Persons at greatest risk for serious illness, such as pregnant women and elderly and immunocompromised persons, should be reminded of the importance of properly cooking eggs (until the yolk is firm) to minimize the risk of contracting salmonellosis. Pasteurized eggs should be made widely available to individual consumers and should be used instead of pooled shell eggs in health care institutions and, when possible, in restaurants. Restaurants and institutions should review their recipes containing shell eggs and modify them, where appropriate, to ensure that eggs are adequately cooked. Eggs should be refrigerated during the entire ranch-to-consumer distribution process to assure egg quality and to minimize bacterial counts in previously infected eggs; this has been successfully mandated in California by amendments to the California Uniform Retail Food Facilities Law that were signed January 1, 1996. Together, egg industry and public health officials should develop a system to determine the sources of eggs that are found to be implicated in salmonellosis outbreaks or that are documented to be contaminated. Food workers should receive frequent training in hand-washing techniques and in the proper handling and preparation of all raw food products of animal origin, including shell eggs. Studies should be done to determine the relative virulence of *S enteritidis* phage type 4 for humans and to determine the current prevalence of *S enteritidis* in shell eggs sold to consumers. Clinicians should be alert to the problem of *S enteritidis* infection and should submit stool specimens for culture when their patients present with diarrhea and fever or bloody diarrhea or are suspected to be part of an outbreak.

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