Emergence of *Salmonella* serotype Enteritidis phage type 4 in Hawaii traced to locally-produced eggs

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Received 9 February 2004; received in revised form 13 September 2004; accepted 5 October 2004

**Corresponding Editor:** Marguerite Neill, Pawtucket, USA

**KEYWORDS**

Egg farm; Egg refrigeration; Hawaii; Human SE illness; Phage type 4; *Salmonella* Enteritidis; SE PT4 outbreak

**Summary**

**Objectives:** In August 1998, the Hawaii Department of Health observed a nine-fold increase in human *Salmonella* Enteritidis (SE) infections. Isolates were phage type 4 (PT4). An investigation was initiated to determine the source of the outbreak.

**Methods:** A matched case-control study enrolled 38 cases. Cases were Hawaii residents with diarrhea and a stool culture yielding SE.

**Results:** Eating eggs was associated with SE illness; 28 cases (74%) ate eggs in the three days before illness compared to 34 (45%) of 76 controls (MOR = 3.0, 95% CI = 1.4–7.4). Eighteen (47%) of 38 case patients ate eggs from Farm A compared to 11 (14%) of 76 controls (MOR = 12.0, 95% CI = 3.1–78.0); the eggs were not properly handled or refrigerated. Cultures from Farm A yielded SE. Human illness subsided following selective flock depopulation.

**Conclusions:** This outbreak highlights the importance of proper handling and refrigeration of eggs. The egg industry must implement quality assurance programs to prevent the spread of SE PT4 and human SE illness.

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Introduction

Salmonellosis is the most common food-borne bacterial illness in the United States. Each year an estimated 1.4 million people are infected with Salmonella. Most infections result in mild to moderate gastroenteric illness, but severe infections also occur. Salmonella infections result in approximately 18,000 hospitalizations each year in the United States. Salmonellosis is a significant economic burden because of the cost of medical treatment and lost wages; the estimated annual costs (in 1998 dollars) of medical care and lost productivity due to food-borne Salmonella infections were between $0.5 billion and $2.3 billion.

Salmonella serotype Enteritidis (SE) is a common serotype of Salmonella in the United States accounting for 5906/33781 or 17% of culture-confirmed Salmonella infections reported in 1998 to the Centers for Disease Control and Prevention (CDC). Although the incidence of other Salmonella infections increased by only 18% between 1972—1996, the incidence of SE increased by 459% during the same period. In recent years, including 1993—1996, Enteritidis was the most frequently-reported serotype in the United States. The increase in SE across the United States first occurred in New England (1978), followed by the mid-Atlantic region (1984) and more recently in the Pacific region (1990). In 1990 only about one tenth of all Salmonella isolates in California were SE compared to approximately one third by 1996. In 1996, approximately one fourth of all SE isolates in the United States were reported by California. A majority of these isolates were phage type 4 (PT4), a phage type not known to be present in the United States prior to a 1993 outbreak in El Paso, Texas. However, since the 1980s, PT4 has been the dominant phage type in Europe, where it has been associated with a dramatic rise in human SE infections. In the United States, phage types 8, 13 and 13a have been the most prevalent, but outbreaks of PT4 have been increasing in the Pacific West. In areas where PT4 has spread through poultry flocks, human SE infections have increased concurrently. In Hawaii, SE (including SE PT4) had been occasionally isolated from ill persons, but illness had never been associated with eating a locally-produced product (Hawaii Department of Health, unpublished data, 1997).

Eggs are the principal source of human SE infections in the United States. Results from more than ten years of outbreak investigations have found that when a source can be identified, four out of five times eggs or an egg-containing food is the source of human SE infection. SE can inhabit the alimentary tract of chickens without causing illness, sometimes reaching the oviduct and ovaries where the bacteria can infect intact eggs. Healthy hens and normal appearing eggs allow the organism to spread silently, and it is not until outbreaks of human illness occur that SE might be traced back to an infected flock.

In August 1998, the Hawaii Department of Health (DOH) detected an increase in culture-confirmed human SE infections. Between 15—29 August, 18 cases of SE were reported compared to only two during the same period in 1997. An investigation was launched to confirm the outbreak, determine its source and develop intervention strategies.

Methods

Case finding

This study attempts to identify all culture-confirmed Salmonella Enteritidis cases in Hawaii. Ascertainment was enhanced by the state requirement that clinical laboratories report all Salmonella isolates from humans to the health department. Furthermore, most human Salmonella isolates are submitted to the Hawaii State Laboratories Division for serotyping.

Case-control study

A case was defined as a Hawaii resident with a stool culture collected between 15 August—2 November 1998 that yielded SE. To determine the source of the outbreak, a case-control study was performed among enrolled cases. Patients were excluded if they traveled outside Hawaii in the three days before illness onset, if they did not report diarrhea (three or more loose stools in a 24-hour period), or if they could not be contacted within three weeks after illness onset. Secondary cases (other persons in the same household with a stool culture yielding SE within four weeks after the primary case) were excluded.

Each enrolled patient was matched with two age- and sex-matched healthy controls. All patients were interviewed within three weeks of their illness onset, while control interviews were conducted within 7—10 days of the patient interview (except one interview that was 11 days later). Controls were selected using a progressive sequential digit dialing procedure starting with the telephone number of the patient. Controls were excluded if during the previous month they had a history of (a) travel outside the state, or (b) diarrhea. Following verbal consent, patients and controls were interviewed by telephone. Question-
naires for persons ≤15 years of age were answered by a consenting parent or guardian.

The questionnaire inquired about exposures the patients might have had in the three days preceding their illness, including contact with ill persons, contact with animals, drinking water source, and food consumption history both in and outside the home. Food questions focused on eggs and egg-containing dishes. Patients were asked where they purchased their eggs, the egg brand name and whether the eggs had a USDA stamp identifying eggs produced outside Hawaii. Whenever a patient reported eating eggs or an egg-containing dish at a restaurant, the brand of eggs served there was determined by contacting the restaurant. With the exception of questions about the patient’s illness, controls were asked the same questions. However, controls were asked about the most recent three-day period that coincided with the same three days of the week as his or her matched patient. Respondents who could not remember whether they had eaten a specific food item were considered to have not eaten it for the purposes of this investigation.

Statistical analysis

Univariate matched analysis was performed using Epi Info 6.04(c).

Environmental investigation

The implicated egg farm was visited in October 1998. Approximately 250 cloacal swabs for SE culture were collected from 1000 hens (one swab/four hens) randomly selected from each of 17 henhouses. Ovaries from 39 euthanized hens were cultured for SE. An additional 152 specimens were collected from the egg-belt machinery and manure pits inside henhouses using the recommended sampling protocol and cultured according to standardized methods.17,18 To examine the surrounding wildlife for SE infection, approximately 250 rats, mice, mongooses, geckos, cats and birds were trapped near the farm and tested for SE.

Laboratory investigation

Salmonella isolates were serotyped at the Hawaii State Laboratories Division. SE isolates were phage typed at the Centers for Disease Control and Prevention (CDC).19

Results

Outbreak investigation

In 1998, 70 human Salmonella Enteritidis isolates were identified at the Hawaii State Laboratories Division; 20 occurred outside the outbreak period (15 August—2 November 1998) (Figure 1). Of the 50 cases that occurred during the outbreak period, 12 cases were excluded for the following reasons: (a) five traveled outside Hawaii in the three days before illness onset, (b) three did not report having diarrhea, (c) two could not be reached within three weeks after illness onset, and (d) two were secondary cases. The remaining 38 cases were enrolled in the case-control study.

The age range of the 38 patients was 1–72 years (median age 23 years); 21 (55%) were male. Three of the 38 (8%) were hospitalized; there were no deaths. Although a majority of Oahu’s population (approximately 60%) lives on the south-eastern side of the island, predominately in Honolulu, Kaneohe and Kailua, 34 (89%) of 38 patients lived on the...
north-central part of the island, about 50 kilometers from Honolulu. 20 (Figure 2).

Consumption of eggs or egg-containing foods was significantly associated with SE infection; 28 (74%) of 38 patients recalled eating eggs in the three days prior to illness onset, compared to 34 (45%) of 76 controls (MOR = 3.0, 95% CI = 1.4—7.4) (Table 1). No other food item was associated with illness, and none of the patients recalled eating eggs or egg dishes that were undercooked. In addition to eggs being the only identified risk factor, a single source of eggs accounted for many cases. Eighteen (47%) of 38 patients reported eating eggs from Farm A compared to 11 (14%) of 76 controls (MOR = 12.0, 95% CI = 3.1—78.0). Eight of the 18 patients purchased unrefrigerated eggs directly from Farm A, the other ten ate Farm A eggs at restaurants that had purchased them from the farm.

Environmental investigation

At the time of the outbreak, 60% of eggs consumed in the state were produced locally in Hawaii; the remaining eggs were shipped to Hawaii from the continental United States (Hawaii Department of Agriculture, written communication, 1998).

The implicated egg farm was located on two sites approximately eight kilometers apart in north-central Oahu, 40 kilometers from Honolulu. The first site had 12 henhouses; 11 houses had a capacity of 5000 hens each and one had a capacity of 20,000 hens. The second site had five henhouses with a capacity of 9000 hens each. All houses were opened-sided without screens.

Egg storage and refrigeration

About 25% of the eggs from the implicated farm were sold directly to the public. After being collected, washed and sorted, eggs were typically left at room temperature on the day of collection until they were sold. It was reported that customers preferred to purchase warm eggs, believing that they were more recently laid and thus fresher than chilled eggs. Some of these eggs were delivered to local restaurants as well. Unsold eggs were stored overnight in a walk-in cooler with an ambient temperature between 13—16°C. According to the farm owner, eggs were usually sold within three days of collection. Highest-grade eggs were stored in a refrigerated truck until being delivered to a processor where they were packaged for grocery stores. The temperature inside the truck was not recorded.

Both environmental and avian specimens collected from multiple sources on Farm A yielded

![Figure 2](https://example.com/figure2.png)  
**Figure 2** Map of Oahu, Hawaii, showing the location of culture-confirmed cases. (●) One culture-confirmed case, N = 38 (two cases from Kauai not shown) and (■) Location of Farm A.

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**Table 1** Exposure among cases and controls, Hawaii 1998.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>No. of cases (N = 38); (%)</th>
<th>No. of controls (N = 76); (%)</th>
<th>MOR (95% mid-P limits)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs or egg-containing dish</td>
<td>28 (74%)</td>
<td>34 (45%)</td>
<td>3.0 (1.4—7.4)</td>
<td>0.0049</td>
</tr>
<tr>
<td>Eggs or egg-containing dish from Farm A</td>
<td>18 (47%)</td>
<td>11 (14.5%)</td>
<td>12.0 (3.1—78.0)</td>
<td>0.00005</td>
</tr>
<tr>
<td>Other household member w/diarrhea during prior 4 weeks</td>
<td>6 (16%)</td>
<td>0 (0%)</td>
<td>Undefined</td>
<td></td>
</tr>
<tr>
<td>Chickens at home</td>
<td>0 (0%)</td>
<td>1 (1.3%)</td>
<td>Undefined</td>
<td></td>
</tr>
<tr>
<td>Touched reptile</td>
<td>1 (3%)</td>
<td>2 (3%)</td>
<td>1.00 (0.03—13.1)</td>
<td>0.70</td>
</tr>
<tr>
<td>Had pet in home</td>
<td>14 (37%)</td>
<td>34 (45%)</td>
<td>0.73 (0.3—1.6)</td>
<td>0.28</td>
</tr>
<tr>
<td>Ate chicken</td>
<td>24 (63%)</td>
<td>45 (59%)</td>
<td>1.2 (0.5—2.9)</td>
<td>0.41</td>
</tr>
<tr>
<td>Ate lasagna b</td>
<td>2 (5%)</td>
<td>1 (1.3%)</td>
<td>4.0 (0.3—118.0)</td>
<td>0.26</td>
</tr>
<tr>
<td>Ate pancakes b</td>
<td>9 (24%)</td>
<td>17 (22%)</td>
<td>1.1 (0.4—2.7)</td>
<td>0.52</td>
</tr>
<tr>
<td>Municipal water source</td>
<td>31 (82%)</td>
<td>61/75* (81%)</td>
<td>1.0 (0.4—3.1)</td>
<td>0.61</td>
</tr>
</tbody>
</table>

MOR = Matched Odds Ratio; Univariate matched analysis was performed using Epi Info 6.04(c).

* Responses were obtained for only 75 of the 76 controls regarding water source.

* Lasagna and pancakes are included in egg-containing dishes— their individual associations with SE illness are included to indicate whether a single food item might be more associated with SE illness than others.
SE. Five (3%) of 152 samples collected from egg-belt machinery and manure pits yielded SE. Cloacal swabs (three [1.2%] of 250) and ovarian cultures (three [8%] of 39) obtained from hens at Farm A also yielded SE. The 11 specimens yielding SE had been collected from four henhouses. SE was also isolated from three animals (one mongoose, one mouse, and one feral cat) on Farm A premises. None of the 75 samples from wild birds yielded SE.

Phage typing

Isolates from 35 (92%) of 38 enrolled cases were PT4; one isolate was phage type 8 and two were phage type 1. None of the patients with non-PT4 isolates had eaten Farm A eggs. From the implicated farm, five egg-belt and manure pit SE isolates collected from henhouses and three chicken isolates (two cloacal and one ovarian) were phage typed; all were PT4. Furthermore, all three SE isolates from wildlife trapped near the farm were PT4.

Control measures

At the end of October 1998, the Hawaii Department of Health intervened to prevent further human SE infections. Farm A was prohibited from selling eggs from henhouses where SE had been isolated. Birds from contaminated houses were destroyed, followed by cleaning and disinfecting the contaminated houses. The Department of Health made a number of recommendations to consumers regarding safe egg handling practices and began enhanced surveillance for human culture-confirmed SE infections. Following these control measures, the number of cases of culture-confirmed human SE infections returned to historical baseline.

The Department of Health also advised the Hawaiian egg industry of appropriate ‘on-the-farm’ measures to maintain secure and separate biologic environments within henhouses. It is not known how broadly the egg industry has implemented these recommendations in Hawaii. To the authors’ knowledge no microbiological survey for SE has been conducted among Hawaiian farms. Egg pasteurization is not available in Hawaii.

Discussion

An outbreak of human SE PT4 infections is described in Hawaii caused by contaminated eggs produced on a farm in Oahu, along with the first human SE infections in Hawaii shown to be caused by locally-produced eggs.

Eggs from a Hawaiian farm were epidemiologically implicated as the source of Salmonella Enteritidis. Environmental samples from multiple sources on the farm confirmed the presence of SE. This outbreak indicates the furthest western spread of the SE epidemic that began in the north-easter United States in the 1970s. Furthermore, this outbreak demonstrates the continued spread of SE PT4 in the United States.

Anecdotal evidence in this investigation highlights the importance of egg refrigeration in the prevention of human infections. The consumption of eggs (and not the consumption of undercooked eggs) caused the outbreak. Almost half the patients (8 of 18) that had eaten Farm A eggs had purchased them directly from the farm. It was observed that eggs sold on the farm were not refrigerated as well as eggs sold in stores. Furthermore, customers reportedly preferred to purchase warm eggs over chilled eggs because they were believed to be fresher. Studies have shown that increasing the temperature of eggs promotes Salmonella growth in at least two ways: organisms can pass through the egg shell much more easily at higher temperatures, and Salmonella grows much faster when it is present in egg contents at temperatures above 10 °C. Poor refrigeration promotes Salmonella growth to such high levels that eating any eggs — well cooked or not — will make people sick.

To prevent human SE infections, the United States Department of Agriculture (USDA) established regulations in August 1999 requiring eggs destined for consumer use to be shipped and stored at an ambient temperature no higher than 7 °C. Labeling is also required to advise consumers to refrigerate their eggs after purchase. These regulations do not pertain to eggs purchased directly from farms, however. Reducing the risk for similar outbreaks will require further interventions to minimize SE contamination of eggs.

The observation that ten patients also ate eggs from Farm A at restaurants highlights the importance of egg handling in restaurants. Pooling eggs has been identified as a risk factor for SE illness, and this practice has been observed at restaurants in Hawaii. Changes in the food code that ban the practice of egg pooling will be required to improve the safety of restaurant food and reduce the risk of SE.

It is not known how SE PT4 arrived on a chicken farm on the island of Oahu. Human infections with SE PT4 had previously occurred on Hawaii but they were associated with travel or eating eggs produced in the continental United States and shipped to Hawaii (unpublished data, Hawaii DOH, 1997).
Two scenarios seem possible to describe the emergence of SE on a farm in Hawaii: importation of contaminated chicken feed or importation of infected day-old chicks. Farm A routinely received both from the continental United States. Although SE was not identified in feces samples from day-old chicks routinely collected under the National Poultry Improvement Plan (NPIP) or from animal feed collected by the Hawaii State Department of Agriculture, such sampling was very limited, particularly during the outbreak period (unpublished data, Hawaii DOH). Therefore, neither possibility has been sufficiently investigated to discount it. The question is of particular importance because it involves the arrival of an emerging pathogen to a relatively remote island chain in the central Pacific. Understanding this mechanism might be useful in guiding policy to prevent the introduction of pathogens from one part of the globe to another.9

From a public health perspective, the chief concern is that SE PT4 could become endemic in the egg industry in Hawaii. Additional control efforts, including adequate on-the-farm microbiological testing for SE and improvements in biosecurity (the isolation of the biologic environment of a given species from other species that may transmit disease) among the Farm A henhouses may help to ensure that SE does not spread broadly throughout the egg industry in Hawaii. The finding of SE PT4 in wildlife nearby the infected farm indicates the potential for SE PT4 to spread to other farms. It is remarkable that wherever SE PT4 has become endemic in the poultry industry, including parts of Europe and apparently western United States, there has been a consequential rise in human SE infections.10,13,14,24,29–32 Furthermore, once it has become endemic in chickens, control is likely to be difficult.

In conclusion, the first outbreak of human SE infections in Hawaii was traced to a local egg farm. It would be prudent for the egg industry in Hawaii to establish a farm-based surveillance system for Salmonella Enteritidis and to move rapidly to prevent further spread. Enhanced surveillance and investigations of human SE infections is warranted.

Conflict of interest: No conflict of interest to declare.

References
