Lessons Learned from a *Salmonella* Enteritidis Phage Type 4 Outbreak in Austria, 2005

**DANIELA SCHMID,1 ANITA LUCKNER-HORNISCHER,2 GERDA HOLZHAMMER,2 DIETMAR ROKITA,2 MARTIN FEDERSPIEL,2 HEIMO LASSNIG,2 ANNA-MARGARETA PICHLER,1 INGEBORG LEDERER,1 ANDREAS BERANEK,1 CHRISTIAN KORSCHOBER,1 CHRISTIAN BERGHOLD,1 AND FRANZ ALLERBERGER1**

1Austrian Agency for Health and Food Safety, Vienna, Austria; and 2Amt der Tiroler Landesregierung, Innsbruck, Austria

**ABSTRACT**

An outbreak of gastroenteritis due to *Salmonella* Enteritidis phage type 4 occurred in people who attended a traditional hunting festivity in a small village in western Austria 6 through 11 November 2005. Of approximately 250 attendees, 227 had consumed dishes offered at the festival, and of these consumers 35 persons fulfilled the outbreak case definition (attack rate of 15.4%). Spätzle (traditional pastalike side dish) was most likely the contaminated part of the incriminated main course (relative risk of 18.9, 95% confidence interval of 4.6 to 76.7; \( P < 0.001 \)). Thirteen eggs that remained from the preparation of the spätzle were negative for *Salmonella* when tested individually without shell disinfection, as were 1,200 eggs collected at the egg production plant and examined with shell disinfection. The back-traced egg production farm had been initially certified as *Salmonella* free by a voluntary quality control program. However, an intensified environmental investigation of the incriminated egg production farm performed in the first quarter of 2006 and based on an appropriate method of sampling revealed *Salmonella* Enteritidis phage type 4 in 4 of 13 flocks. Although a combination of epidemiological and microbiological investigations allowed elucidation of the mode of spread, no restrictions were placed on the incriminated flocks of laying hens. These flocks were kept in production until they were stalled out due to age in August 2006. In June 2006, a cluster of 23 cases of *Salmonella* Enteritidis phage type 6 infection was again associated with this egg production farm. Evidence provided by epidemiological analyses is often disregarded by decision makers. However, negative results from microbiological testing of food involved in an outbreak are often weighted as strong evidence against a causal association between that food and the outbreak.

Foodborne zoonoses may cause human suffering and economic losses to the food production industry. Zoonoses directive 2003/99/EC specifies that competent authorities must investigate foodborne outbreaks and that the investigation must produce data on the epidemiological profile, the foodstuffs potentially implicated, and the potential causes of the outbreak (1). According to the European Commission, thorough investigation of zoonotic foodborne outbreaks provides the opportunity to improve methods of prevention and control of foodborne diseases. We describe here the investigation of an outbreak of salmonellosis due to *Salmonella enterica* subspecies *enterica* serovar *Enteritidis* phage type (PT) 4.

From 12 through 29 November 2005, 10 cases of gastroenteritis due to *Salmonella* Enteritidis PT 4 were reported to a local public health officer in western Austria. The time and place clustering of these 10 cases initiated an investigation of the history of exposure. The onset of clinical signs occurred between 6 and 10 November, and all affected persons had attended an annual festival hosted by a local hunting society on 5 November. An investigation of this cluster of acute gastroenteritis cases was conducted to identify the source of infection, the reservoir of the causative agent, and the mode of spread.

**MATERIALS AND METHODS**

**Outbreak case definitions.** A confirmed case was defined as a person who (i) visited the festival in village X on 5 November 2005, (ii) consumed food that was served there, (iii) subsequently fell ill with symptoms of gastroenteritis (diarrhea or vomiting) between 5 and 12 November, and (iv) had a bacteriologically confirmed *Salmonella* Enteritidis PT 4 infection. A probable case was defined as a person who (i) visited the festival in village X on 5 November 2005, (ii) consumed food that was served there, (iii) subsequently fell ill with symptoms of gastroenteritis (diarrhea or vomiting) between 5 and 12 November, and (iv) had no microbiological confirmation of a *Salmonella* Enteritidis PT 4 infection.

**Analytical study design.** A retrospective cohort study was performed because a clearly identifiable risk group for which information could be obtained was available: the attendees of the traditional festival. The hypothesis to be tested was generated by results of initial interviews of the first 10 cases. Consumption of spätzle (a special kind of homemade pasta typical of southern Germany and western Austria) was causally associated with the risk of falling ill with gastroenteritis in the week following attendance at the hunting festival.

**Recruitment of the cohort.** According to the organizer, approximately 250 persons attended the festival. Of those persons, 243 were identified based on the following sources of information: list of guests provided by the village mayor, list of guests of honor provided by the head of the local hunting society, official registry.
of hunting card owners, and personal interviews of guests providing data on individuals that shared tables at the festival.

**Definition of exposure.** Exposure was defined as consumption of at least one dish served at the festival. The dishes offered included two different main courses. Main course I was pork tenderloin, mushroom (cep) and cream gravy, Brussels sprouts, and spaetzle. Main course II was roast of hart (venison), dumplings, and red cabbage. A warm dessert of fried apple slices and pancake dough prepared according to a traditional Austrian recipe and four different homemade cakes (made with eggs provided by the caterer) also were offered. Bavarian veal sausage was offered as a midnight snack. The preliminary interviews in the group of interest revealed that of the 243 persons identified, 227 persons (93%) had consumed at least one of the food items offered. This number was in accordance with the registered count of dishes served; 204 fixed main courses were served (103 venison and 101 pork tenderloin), and 188 portions of a warm dessert and 69 portions of a midnight snack were consumed. Two persons refused the interview; thus, 225 persons remained for the cohort analysis.

**Analysis.** A standard questionnaire was developed at the Department of Infection Epidemiology at the Austrian Agency for Health and Food Safety (Vienna) using Epidata software (EpiInfo 3.3.2 for Windows, EpiData Association, Odense, Denmark). Interviews were performed by telephone by three trained interviewers. Information obtained included demographic data, gastrointestinal disease status in the 7 days following attendance at the festival (active case finding), clinical onset, kind of symptoms (fever, nausea, vomiting, diarrhea), duration of illness, hospitalization, work lost, and food consumed at the festival. Food-specific attack rates (ARs), relative risks (RRs), and 95% confidence intervals (CIs) were calculated for the two main courses (I and II), the warm dessert, the four cakes, and the Bavarian sausage. The food-specific ARs among the exposed and nonexposed cohort members were compared in a univariate analysis using the chi-square test or the two-tailed Fisher’s exact test. The measure of association was the RR.

**Environmental investigation and microbiological analysis.** Sampling and microbiological workup of specimens obtained from laying hen holdings during the outbreak investigation was performed as described by the Standing Committee on the Food Chain and Animal Health (2). Individual eggs whose shells had not been disinfected and batched eggs whose shells had been disinfected were examined as described elsewhere (9). The *Salmonella* isolates from the current outbreak investigation were sent to the National Reference Laboratory for *Salmonella*, which receives the majority of all human and nonhuman *Salmonella* isolates found in Austria. The *Salmonella* isolates routinely undergo se-rotyping (Kauffmann-White method), and all the *Salmonella* Enteritidis isolates are phage typed as described elsewhere (12). Pulsed-field gel electrophoresis of genomic DNA after *Xba*I (New England Biolabs, Beverly, Mass.) digestion was performed as described elsewhere (8).

**RESULTS**

Among the 225 study participants, 81 were women (36%). The median age was 49 years (interquartile range, 40 to 60 years; total range, 5 to 76 years).

Thirty-five persons in the study cohort fulfilled the case definition (AR, 15.4%) (Table 1). Among these 35 persons, there were 12 women and 23 men; the median age was 53 years (interquartile range, 38 to 69 years; total range, 16 to 69 years). Thirty-two patients (91.4%) had diarrhea, 16 (45.7%) had nausea, 7 (20%) had vomiting, and 10 (28.6%) had fever. The median duration of illness was 6 days (interquartile range: 2 to 8 days), and two patients (5.7%) were hospitalized. All 35 patients recovered.

Ten of the 23 patients (43%) that provided stool samples for microbiological testing fulfilled the criteria of a laboratory-confirmed case, i.e., stool samples yielding *Salmonella* Enteritidis PT 4. Stool samples obtained from the complete kitchen staff (four persons) were negative for *Salmonella*.

The outbreak extended from 6 to 11 November with a peak on 7 November. The pattern of the epidemic curve indicated a punctiform source (Fig. 1).

On 15 November, a food inspector collected 13 consumable eggs that remained in the caterer’s kitchen after the preparation of the festival dishes. The eggs were from the same batch of eggs that were used for preparing the dumplings, the spätzle, the warm dessert, and the homemade cakes. No other food item offered at the festival was available for microbiological testing at that time. None of the 13 eggs yielded *Salmonella*. Through egg production number, the eggs were traced back to an egg producer in eastern Austria, which controls 13 cage flocks (10 with 10,000 to 12,000 hens per flock, 1 with 55,000 hens, and

### TABLE 1. Food-specific attack rates among 225 attendees of a traditional hunting festival in western Austria on 5 November 2005

<table>
<thead>
<tr>
<th>Food item</th>
<th>Exposed</th>
<th>Unexposed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of cases</td>
<td>Total no. of consumers</td>
</tr>
<tr>
<td>Main course I</td>
<td>33</td>
<td>105</td>
</tr>
<tr>
<td>Main course II</td>
<td>5</td>
<td>101</td>
</tr>
<tr>
<td>Apple slices in pancake dough</td>
<td>27</td>
<td>147</td>
</tr>
<tr>
<td>Homemade cake 1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Homemade cake 2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Homemade cake 3</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Homemade cake 4</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>Bavarian sausage</td>
<td>4</td>
<td>76</td>
</tr>
</tbody>
</table>

* Fisher’s exact test.
2 with 75,000 to 80,000 hens per flock) in four holdings. The flocks were not vaccinated against Salmonella.

The univariate analyses of food exposures revealed that consumption of the dessert and the four cakes had no effect on disease risk. A positive association with disease risk at a 5% significance level was found for consumption of main course I (RR, 18.9; 95% CI, 4.6 to 76.7; \( P < 0.001 \)). A negative association was indicated for consumption of main course II (RR, 0.2; 95% CI, 0.1 to 0.5; \( P < 0.001 \)) and Bavarian sausage (RR, 0.3; 95% CI, 0.1 to 0.7; \( P < 0.001 \)). There was no difference in sex- and age-specific ARs (data not shown).

Because the Bavarian sausage appeared to have no relevance as a vehicle of Salmonella Enteritidis PT 4, persons that consumed only this sausage (\( n = 18 \)) were excluded from the second analysis. This second study cohort (\( n = 207 \)) was comparable to the initial study cohort in frequency distribution of sex, age, and food exposure (except for the Bavarian sausage). The association between consumption of main course I and disease risk remained significant (RR, 16.0; 95% CI, 4.0 to 65.1; \( P < 0.001 \)).

Strictly assuming a maximum incubation period for Salmonella of no longer than 72 h and postulating that person-to-person transmission was relevant in this outbreak, only those persons from the second cohort with a date of onset from 6 through 8 November (\( n = 24 \)) were designated for a third analysis. The strength of association between disease risk and consumption of main course I decreased only slightly (RR, 11.0; 95% CI, 2.6 to 44.3; \( P < 0.001 \)).

Holding 1 of the outbreak-associated egg production farm was sampled in mid-January 2006. All environmental samples were negative for Salmonella Enteritidis PT 4, but samples from five of eight flocks yielded Salmonella Enteritidis PT 6. Subsequent intensified environmental sampling of the whole egg-production plant, performed between January and March 2006, revealed Salmonella Enteritidis PT 4 in 4 of the 13 flocks from two of the four holdings (Table 2). Molecular subtyping by pulsed-field gel electrophoresis of genomic DNA after XbaI digestion revealed that the five isolates obtained from the two holdings were indistinguishable from each other and from the 10 human isolates (Fig. 2).

Microbiological investigation of 1,200 eggs (examined after shell disinfection) collected during 3 weeks (100 eggs per week per positive flock) in May 2006 from the four flocks positive for Salmonella Enteritidis PT 4 yielded no Salmonella.

**DISCUSSION**

The foodborne outbreak of gastroenteritis in western Austria involving 35 persons from an at-risk population of 227 attendees of a hunting festival was successfully elucidated by a descriptive and analytical epidemiological investigation complemented with conventional and molecular microbiological investigations of human and holding environmental specimens (including dust and fecal material). An egg production farm comprising four premises and 13 flocks situated in eastern Austria was identified as the high-ly likely reservoir for the outbreak agent. However, no restrictions were placed on the incriminated flocks of laying hens, which were kept in production until they stopped laying due to age in August 2006. In 2006, the cook of the catering firm that served the food at the hunting festival was convicted of bodily harm caused by negligence.

From 6 through 13 June 2006, a cluster of 23 cases of Salmonella Enteritidis PT 6 infection occurred in Tyrol; it affected six German tourists, eight local adolescents, and nine asymptomatic persons from a hotel. The only common link was food (scrambled eggs and filled eggs) produced by the kitchen at the hotel. The table egg production farm microbiologically and epidemiologically associated with the Salmonella Enteritidis PT 4 outbreak in 2005 was identified as the single egg supplier for this hotel. These 2006 cases could have been prevented by proper action of health authorities after the 2005 outbreak.

Scientific evidence provided by analytical epidemiological studies is often disregarded by decision makers. Health authorities in Austria are predominantly not aware of the significance of epidemiological findings. However,
negative results of microbiologically tested food samples, such as the 13 eggs that remained after preparation of the dishes for the hunting festival, are often misinterpreted as strong evidence against a causal association between the food and the outbreak.

Negative results of microbiological investigations of food cannot by themselves prove the absence of relevance of a particular food item to an outbreak. Bacteriological testing with shell disinfection of the 1,200 eggs sampled in May 2006 from the four flocks positive for Salmonella Enteritidis PT 4 yielded no Salmonella. The probability of yielding such negative results despite a real frequency of ≥1% of eggs with internal Salmonella contamination is ≤5%. The real frequency of internal egg Salmonella Enteritidis contamination is estimated to range from 1 in 100 to 1 in 20,000. Based on epidemiological findings and infection plausibility, in the current outbreak eggs were either carrying the causative agent on the shell surface or were internally contaminated at a frequency of less than 1 in 100.

Both epidemiological and microbiological analyses are subject to type I and type II errors, and a strong association in one is as valid as a strong association in the other. Examples of causes of type II errors are (i) sampling of a flock that produced contaminated eggs for the outbreak but no longer is shedding Salmonella at the time of sampling and (ii) situations in which the frequency of internal egg contamination falls below the sensitivity of either the sampling plan or the analytical method.

The laying hen flocks involved in this outbreak were certified as Salmonella free by a voluntary Salmonella quality control program. Four times each year, one pooled fecal sample consisting of 60 1-g samples was routinely obtained per flock (independent of flock size). To avoid selection bias, the 60 collection locations had to be equally distributed in the flock. A certificate based on such a method of sampling should not preclude further microbiological investigations of the environment when epidemiological findings suggest an association with an outbreak. Therefore, the method of environmental sampling, particularly during investigation of an outbreak, is a crucial factor and should be standardized to allow reliable tracing of an outbreak-associated reservoir. The standing Committee on the Food Chain and Animal Health for the Baseline Study on the Prevalence of Salmonella in Laying Flocks of Gallus gallus in the European Union recommended the following procedure (2). To maximize sampling sensitivity, both fecal material and the holding environment must be sampled. For fecal sampling, five samples of naturally mixed feces from dropping belts should be obtained. The fecal material should be taken from the scraper bars at the end of the belt. A new pair of plastic gloves should be used for each sample, and at least 20 portions from separate belt scrapers should be taken to ensure that all stacks are represented in the final pooled sample. Two 250-ml samples of pooled dusty material should be collected. The dusty material should be taken from the scraper bars at the end of the belt. A new pair of plastic gloves should be used for each sample, and at least 20 portions from separate belt scrapers should be taken to ensure that all stacks are represented in the final pooled sample. Two 250-ml samples of pooled dusty material should be collected. The dusty material should be taken from beneath the cages at 20 separate locations within the holding. This tedious sampling procedure is more reliable and therefore more useful for outbreak investigation than is the sampling procedure applied in the voluntary Salmonella quality control program. Because flocks were sampled months after the outbreak, the hens may have become infected after the outbreak period. How-
ever, we consider this possibility very unlikely because there were no obvious changes in production methods.

In 2005, a total of 5,615 microbiologically confirmed *Salmonella* infections in humans were documented in Austria. *Salmonella Enteritidis* accounted for 83% of all human isolates, and 30% of all *Salmonella Enteritidis* isolates belonged to PT 4 (3). PT 4 is widely distributed in Austrian chicken flocks. As in the outbreak reported here, health authorities sometimes tend to incorrectly presume that a wide distribution of an outbreak-causing organism makes the identification of the outbreak-associated egg production plant impossible.

This outbreak investigation revealed that the isolates from the human cases were indistinguishable from the environmental isolates obtained from flocks of the suspect egg production plant. Nevertheless, the significance of the molecular data generated by pulsed-field gel electrophoresis (PFGE) of human and environmental isolates to identify clonal concordance among isolates from cases and from the possible source should not be underestimated. In Austria in 2005, more than 90% of the *Salmonella Enteritidis* PT 4 isolates had the same PFGE pattern as found for the isolates from this outbreak. The added value of new typing methods such as fingerprinting based on the variable number of tandem repeats for confirming or discovering epidemiological links associated with *Salmonella Enteritidis* PT 4 still must be elucidated (6).

The limitations of the epidemiological analyses of this outbreak seemed to be marginal. The epidemic curve indicated a point source outbreak. Because the only link among the affected individuals was attendance at the hunting festival, dishes served at that festival were hypothesized to be the outbreak source. The likelihood of having introduced a selection bias for the study cohort was reasonably small. Of a total of approximately 250 attendees at the festival, 243 were identified. Of these, 227 persons have consumed dishes at the festival, in accordance with the registered count of dishes supplied at the festival. For the cohort analyses, 225 persons in the at-risk population were available. The analytical epidemiological analysis revealed that main course I was positively associated with disease risk. The magnitude of the relative risk associated with main course I strongly indicated a causal relationship. To control for factors possibly overestimating the strength of the association, we analyzed the association after excluding persons that had eaten only Bavarian sausage, and in a third analysis we restricted the participant pool to those patients with onset of disease within 3 days after the point exposure, thereby considering cases that could be attributable to person-to-person spread (secondary cases) as nondiseased. Of the dishes in main course I, the spätzle was the most likely source of infection. Spätzle is produced by preparing fresh dough from flour, water, salt, and eggs, forming it into hazelnut-size pieces, and cooking these pieces in boiling water for 12 to 15 min. Eggs are known to be the main vehicle of *Salmonella Enteritidis* infection (4, 5, 7, 10, 11). Various small family outbreaks have been reported in Western Austria, and insufficient boiling time for the spätzle was considered crucial for transmission of *Salmonella*. The negative association of main course II and of Bavarian sausage with the risk of gastroenteritis indicates that under the condition of this outbreak the choice of roast hart, dumplings, and red cabbage or of Bavarian sausage instead of main course I protected cohort members from the contaminated food.

In an outbreak investigation, there is no standard operating procedure for the method of sampling, including determination of sample size and collection procedure for food or environmental samples. When there is epidemiological evidence for or strong suspicion concerning the outbreak source, the vehicle of the causative agent, or the reservoir, microbiological proof often is required by decision makers as justification for an intervention. Appropriate sampling and analytical methods provide valid laboratory results, but even the best laboratory practices sometimes can produce false-negative results for a variety of reasons. Therefore, a regulatory response (e.g., requiring egg pasteurization) should follow a strong epidemiological association from a well-conducted investigation, even in the absence of confirmatory microbiological test results.

**REFERENCES**


