SALMONELLA ENTERITIDIS PHAGE TYPE 21 OUTBREAK IN AUSTRIA, 2005

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We report an outbreak of gastroenteritis due to *Salmonella* Enteritidis PT 21 associated with attending an annual traditional fair in a small Austrian village on 4 May 2005. The outbreak lasted from 4 to 8 May. Descriptive and analytical epidemiological investigations were conducted in order to determine the extent of the outbreak and to identify outbreak risk factors. Of the 115 persons who visited the fair, 85 persons fulfilled the criteria of an outbreak case (attack rate = 73.9%). Stool specimens from 52 patients, including two kitchen staff, were tested for salmonella, and 20 specimens were positive for *Salmonella* Enteritidis PT 21. The cohort study revealed mixed salad (which included potatoes) as the likely cause of the outbreak (RR: 10.4, 95%CI 2.8 – 39.1; P=<0.001). The causative agent of the outbreak was cultured from the stock of eggs used at the fair and from all three drag swabs used for faecal samples and one barn dust sample collected from the responsible egg laying flock. Molecular subtyping by pulsed-field gel electrophoresis of genomic DNA after *Xba*I digestion showed that isolates from eggs, from the flock and from humans were indistinguishable. We hypothesise that cross contamination from eggs to boiled potatoes occurred in the kitchen area, where raw eggs were handled by village residents preparing a traditional Viennese egg dressing for the meat dishes. Unrefrigerated storage of peeled potatoes may have favoured bacterial growth. Eggs from small rural flocks of laying hens kept in a traditional 'natural' way should not be assumed to be salmonella-free.

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Introduction

Foodborne zoonoses may cause human suffering, as well as economic losses to food production and the food industry. The European zoonoses directive 2003/99/EC specifies that competent authorities should investigate foodborne outbreaks and that the investigation should provide data on the epidemiological profile, the potentially implicated foodstuffs and the potential causes of the outbreak [1]. According to the European Commission, thorough investigation of zoonotic foodborne outbreaks provides the opportunity to improve prevention and control of foodborne diseases. We describe the investigation of an outbreak of salmonellosis associated with attending a traditional annual village fair.

Materials and Methods

Outbreak background

A time-space cluster of approximately twenty cases of gastroenteritis due to *Salmonella* Enteritidis PT 21 was reported to the Österreichische Agentur für Gesundheit und Ernährungssicherheit (Austrian Agency for Health and Food Safety) by provincial health authorities on 12 May 2005. On 9 May, local general practitioners had alerted the local district health office to a cluster of gastroenteritis cases that started on 4 May and was restricted to a small district in southern Austria. On 11 May, a clinical microbiology laboratory reported a cluster of stool samples positive for *Salmonella* Enteritidis. The case series investigation revealed that was a relationship between the cases and visiting an annual fair in a small village on 4 May. An outbreak investigation team was set up by the provincial health authorities on 13 May, and it was decided that a full investigation should be carried out to determine the extent of the outbreak and to identify the outbreak cause by using a retrospective cohort design.

Outbreak case definitions

Probable case: A probable case was defined as a person who (1) visited the particular fair in village X on 4 May, (2) consumed dishes served at the fair, (3) subsequently fell ill with symptoms of diarrhoea and (4) had no bacteriologically confirmed infection with *S.* Enteritidis PT 21.

Confirmed case: A confirmed case was defined as a person who (1) visited the fair in village X on 4 May, (2) consumed dishes served at the fair, (3) subsequently fell ill with symptoms of diarrhoea and (4) had a bacteriologically confirmed infection with *S.* Enteritidis PT 21.

Recruitment of the cohort

The municipality provided a list of all residents of village X. Of 95 households , 87 (91.6%) were contacted and asked whether household members or relatives and friends not residing in village X had visited the fair. Of the 294 village residents contacted, 76 people (in 31 households) had taken part in the fair. An additional 39 people (village residents' relatives and friends who lived outside village X) were reported to have visited the fair. A total of 115 people were considered for the cohort analyses.

Exposure

An exposure was defined as consumption of a food item prepared and served by the staff of the inn where the fair was held.

The food items available at the fair included three different soups (garlic cream soup, liver dumpling soup, and soup with cut pancake pieces), frankfurter sausages, turkey fillets, boiled meat, roast pork, and a variety of food items prepared according to traditional Austrian recipes, breaded chicken, pork, Emmentaler cheese, goat meat (this is food dressed with a mixture of raw egg and white bread crumbs, and then fried),mixed salad (including potato salad, lettuce, green beans, haricot beans, and tomatoes), cooked rice and fried potatoes, and four different cakes.

Disease status

Patients who developed diarrhoea (with or without vomiting), abdominal pain or fever in the four days after visiting the fair were considered to have been ill with acute gastroenteritis.

Analysis

A standard questionnaire was developed at the Austrian Agency for Health and Food Safety using EpiInfo 3.3.2. Interviews were carried out by telephone, and included questions on basic demographic data, symptoms and clinical signs, date and time of clinical onset, duration of illness, admission to hospital, date of admission to hospital and food history, which included using the list of food items available at the fair.

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Epi Info 3.3.2 was used for data collection, and data were analysed with Epi Info v6.04d and the Statistical Analysis System (SAS). We compared the food-specific attack rates (AR) for each food item on the fair list among the exposed and the non-exposed cohort members in the univariate analysis using chi-square test or two-tailed Fisher's exact test. The measure of association was the relative risk (RR). In order to assess simultaneous effects of the exposure variables, a logistic regression model was applied. The variables age and sex, and the food items with a P value ≤0.1 in the univariate analyses were included in the regression model. Variables were removed one by one until only significant (P≤0.05) variables remained in the model.

Microbiology

The National Reference Laboratory for Salmonella at the Austrian Agency for Health and Food Safety receives the majority of all human and non-human salmonella strains isolated in Austria. All salmonella isolates received routinely undergo serotyping (Kauffmann-White method) and all *S.* Enteritidis isolates are phage typed as described elsewhere [2]. Pulsed-field gel electrophoresis of genomic DNA after *Xba*I (New England Biolabs, USA) digestion was performed as described elsewhere [3,4].

Results

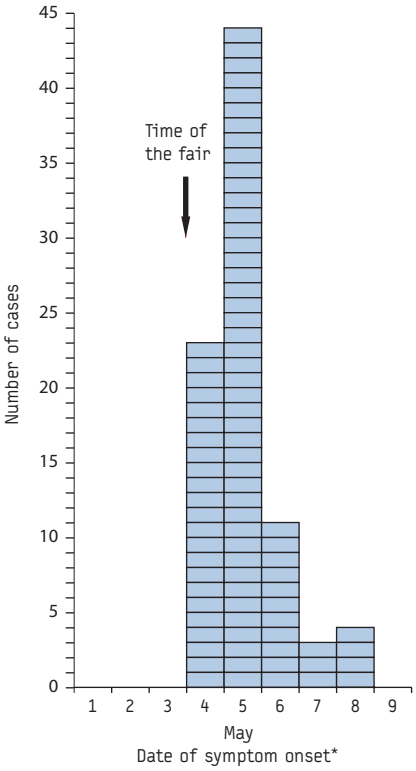
The questionnaires were completed for all cohort members (100% response). Only data on time of clinical onset were incomplete.

Women made up 53 (46.1%) of the 115 cohort members. The median age was 45 years (range 2–84).

The outbreak lasted from 4 to 8 May, peaked on May 5 and indicated a common point source outbreak [FIGURE].

FIGURE 1

Cases of *S.* Enteritidis infection by date of symptom onset (n= 85) after visiting a traditional fair in a small village in southern Austria on 4 May 2005.



*Data on time of onset were not available

Eighty five patients in the cohort fulfilled the case definition of an outbreak case (attack rate, AR=73.9%). Among these 85 cases, there were 39 women and 46 men (54.1%), the median age was 44 years (range 2 - 84). The case distribution by age group (0-9 years, 10-19 years, 20-29 years, 30-39 years, 40-49 years, 50-59 years, 60-69 years, >70 years) and sex is illustrated in table 1.

TABLE 1

Outbreak cases by age group and sex, southern Austria, 2005

Age group years	Sex		Total
	Male (%)	Female (%)	
0-9	5 (10.9)	1 (2.6)	6
10-19	4 (8.7)	5 (12.8)	9
20-29	5 (10.9)	1 (2.6)	6
30-39	2 (4.3)	6 (15.4)	8
40-49	12 (26.1)	9 (23.1)	21
50-59	5 (10.9)	3 (7.7)	8
60-69	6 (13)	12 (30.8)	18
≥ 70	7 (15.2)	2 (5.1)	9
Total	**46**	**39**	**85**

Stool specimens from 52 patients, including two kitchen staff, were tested for salmonella, and 20 specimens (38%) were positive for *Salmonella* Enteritidis PT 21 and fulfilled the definition criteria of a confirmed outbreak case.

In addition to diarrhoea, 78 patients (91.8%) had nausea, 33 patients (38.8%) had vomiting, and 39 patients (45.9%) had fever. The mean duration of illness was 5 days (interquartile range 4 to 7 days). Fourteen patients (16.5%) were admitted to hospital. All 85 patients recovered.

On 12 May, food inspectors collected 10 table eggs from the inn's kitchen, where the meals for the fair had been prepared. No other food item served at the fair was available for microbiological examination at this point in time. The eggs came from a flock of 17 laying hens kept by the innkeeper. The flock had not been vaccinated against salmonella and was kept in a barn with access to a small fenced-in outside area. *Salmonella* Enteritidis PT21 was isolated from the pooled eggs and was cultured from all three drag swabs (pooled faecal samples) and one barn dust sample collected on 18 May.

It was not possible to ascertain the exact time when food exposure occurred, because food was available from 11.00 am until the late afternoon, and visitors were not able to recall the exact time of food consumption or the time of clinical onset.

Molecular subtyping by pulsed-field gel electrophoresis of genomic DNA after *Xba*I digestion revealed an isolate obtained from the eggs and four isolates from the flock to be indistinguishable from four human isolates.

The univariate analyses of food exposures revealed that consumption of soups, frankfurter sausages, fried potatoes, cakes, pork, boiled meat, fried chicken and fried Emmentaler cheese had no effect on the disease risk.

An association with disease risk at a 5% significance level was found for fried goat meat (RR: 1.4, 95%CI 1.3-1.6; P= 0.007), rice (RR: 1.4, 95%CI 1.0 -1.6; P=0.02), and for the mixed salad (RR: 10.4, 95%CI 2.8 – 39.1; P=<0.001). Consumption of turkey was found to have a protective effect on disease risk (RR 0.4; 95%CI: 0.19 – 1.2; P= 0.03) [TABLE 2]. No difference in the sex-specific attack rates was found.

The multivariate analysis found the mixed salad to be the only food exposure with a significant independent effect on the disease risk.

Discussion

In 2004, a total of 7320 laboratory confirmed salmonella infections in humans were documented in Austria. *S.* Enteritidis accounted for 83.0% of all human isolates, with 82.9% of all *S.* Enteritidis isolates belonging to three phage types: PT 4, PT 8 and PT 21 [5]. Berghold et al recently showed that Austrian chicken meat is nowadays only a minor source of human *S.* Enteritidis infections, regardless of phage type [6]. This applies to chicken meat as direct source of infection as well as infections from secondary contamination [6]. The main focus of preventive measures should be directed at reducing the risk of infection caused by table eggs [7-9]. The PT 21 outbreak described in this paper further underlines the importance of eggs as a vehicle for salmonella infection.

Table 2

Food-specific attack rates for *Salmonella* Enteritidis phagetype 21 infections associated with attending a fair, May 2005

	Exposure Yes			Exposure No				
Dishes available	Ill	Total	AR%	Ill	Total	AR%	RR (95% CI)	p
Soup with pancake pieces	6	7	85.7	79	108	73.1	1.2 (0.8 1.6)	0.4*
Liver dumpling soup	5	6	83.3	80	109	73.4	1.1 (0.8 1.7)	0.5*
Garlic cream soup	2	3	66.7	83	112	74.1	1.2 (0.8 1.6)	0.5*
Frankfurter sausages	0	0	0	85	115	73.9	Incalculable	-
Boiled meat soup	0	0	0	85	115	73.9	Incalculable	-
Fried chicken	21	29	72.2	64	86	74.4	1.0 (0.8 1.3)	0.9
Fried pork	32	46	69.6	53	69	76.8	0.9 (0.7 1.1)	0.5
Fried Emmentaler cheese	0	1	0	85	114	74.6	Incalculable	0.3*
Turkey fillet	3	8	37.5	82	107	76.6	0.4 (0.2 1.2)	0.03*
Goat meat	15	15	100	70	100	70	1.4 (1.3 1.6)	0.007*
Roast pork	11	12	91.7	74	103	71.8	1.3 (1.0 1.6)	0.1*
Cooked rice	26	28	92.9	59	87	67.8	1.4 (1.1 1.6)	0.02
Fried potatoes	29	41	70.7	56	74	75.7	0.9 (0.7 1.2)	0.7
Mixed salad°	83	92	90.2	2	23	8.7	10.4 (2.8 39.1)	< 0.001
Cake I	6	8	75	79	107	73.8	1.0 (0.6 1.5)	0.7*
Cake II	5	5	100	80	110	72.7	1.4 (1.2 1.5)	0.2*
Cake III	8	8	100	77	107	72	1.4 (1.2 1.6)	0.08*
Cake IV	2	2	100	83	113	73.5	1.4 (1.2 1.5)	0.5*

* Fisher's exact test was used

° Mixed salad including potato salad, lettuce, green beans, haricot beans, tomatoes

The epidemic curve indicated a common exposure of the cases at one point in time. The maximum and minimum incubation periods were traced back, using the clinical onset dates of the first and last cases, and 4 May was found to be the date of common exposure. A foodborne outbreak caused by dishes served at the fair was assumed, as there was no information about any other mass gathering as a potential common exposure prior to the fair. As this rural fair is usually visited exclusively by village residents and by their friends and relatives, a cohort study was chosen to elucidate the cause of the outbreak.

The likelihood that a selection bias was introduced is reasonably small, as contact was made with over 90% of the village households. The capture of outside visitors named by village community members appeared to be reliable, and all identified attendees responded to the questionnaire.

The analytical epidemiological investigation revealed the mixed salad as the highly likely cause of the outbreak. The causative agent of the outbreak could be cultured from the remainder of the stock of eggs used for the fair and taken from the kitchen. The preparation of the potato salad (part of the mixed salad) began during the night of 3-4 May, when the potatoes were boiled, peeled, and later stored, unrefrigerated for about 8 hours in another room. We hypothesise that cross contamination of the boiled potatoes occurred in a kitchen where raw eggs were used to prepare the traditional Viennese breading for the meat dishes. Storage of the peeled potatoes in inappropriate conditions may have favoured bacterial growth. Furthermore, meal preparation was carried out in the kitchen of an inn that had not been not used for commercial food preparation for several years. The yearly fair was the only occasion when large scale cooking and food serving was done in this location, and was carried out with the help of village residents. Lack of risk consciousness by untrained staff is a well- known risk factor for hygiene failure [10].

Bacteriological examination of the egg producing flock revealed that the innkeeper's hens were infected with PT 21. In rural Austria, many households still use eggs from their own hens. The fact that eggs are from a small flock of laying hens, kept in the traditional 'natural' way, is often misinterpreted as a guarantee against salmonella contamination. The flock owner had purchased his hens two years earlier from an industrial laying hen operation. We assume that the hens were already latently infected at this time.

The voluntary culling of the herd on 2 June led to the elimination of the reservoir of this circumscribed outbreak. This is an impressive example how a foodborne outbreak was traced from cases to the outbreak causative food item, the food-vehicle of contamination, and thence to the food producing animal that was the reservoir of the causative agent. Despite the fact that the causative phage type is widely distributed in Austrian chicken flocks, it was possible to elucidate and interrupt the chain of infection. Close cooperation between health authorities and the Austrian Agency for Health and Food Safety, bridging gaps between the fields of human medicine, food laboratories, and veterinary medicine and providing epidemiological expertise, are the essential bases for the success of such an endeavour.

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