

## Surveillance and outbreak reports

# OUTBREAK OF *SALMONELLA* SEROVAR STANLEY INFECTIONS IN SWITZERLAND LINKED TO LOCALLY PRODUCED SOFT CHEESE, SEPTEMBER 2006 – FEBRUARY 2007

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*Salmonella* serovar Stanley is rare in Europe. In Switzerland, the number of reported isolates has increased from 2 in 2000 to 25 in 2005. A nationwide outbreak of gastrointestinal illness due to *S. Stanley* occurred from September 2006 through February 2007. Eighty-two cases were documented. Males were 56%; mean age of the cases was 45.7 years (range 0-92). Forty-seven cases (57%) occurred in three western cantons: Vaud, Bern, and Geneva. Twenty-three cases (28%) were hospitalised. In the case-control study conducted to find the source of the outbreak, cases were more likely than controls to have eaten local soft cheese (OR 11.4,  $p=0.008$ ). One clone of *S. Stanley* strain was isolated from soft cheese and from 77 cases (94%) who reported no history of having travelled abroad. The outbreak ended after the withdrawal of the cheese from the market. This is the first *S. Stanley* outbreak in Switzerland and the first in Europe unrelated to imported products, suggesting an increased local circulation of this previously rare serotype.

### Introduction

*Salmonella enterica* subspecies *enterica* serovar Stanley (*S. Stanley*) is common in Asia, but rare in Europe, America and Australia. Most of the cases reported in Europe have a history of travelling in Asia or consumption of food products imported from Asia [1,2,3,4,5]. Contaminated peanut shells produced in China and alfalfa sprouts of unknown country of origin imported from Italy, Hungary and Pakistan were the source of two large international *S. Stanley* outbreaks in Europe and North America [6,7,8]. A high frequency of septicaemia during the sprout-borne outbreak in Finland in 1995 and cases of severe illness associated with *S. Stanley* have been reported in the literature [9,10]. Resistance to aminoglycosides, tetracycline and cotrimoxazol have been documented. In Europe, *S. Stanley* represents on average 27% of all multidrug-resistant salmonellae [2,5].

In Switzerland, the National Centre for Enteropathogenic Bacteria (NENT) is the reference laboratory for typing and molecular analysis of *Salmonella* sp. isolates nationwide. The annual number of *S.*

Stanley isolates reported by the NENT to the Federal Office of Public Health (FOPH) increased from 2 in 2000 to 25 in 2005.

On 20 October 2006, the NENT reported 22 human isolates of *S. Stanley* detected since the beginning of the month and one isolate of this serotype from chicken meat tested during a routine quality control; the meat was imported from Hungary, processed as sliced fresh meat in Switzerland and distributed nationwide. Initially, this chicken meat was considered the most probable source of the human cases. However, although the incriminated meat was no longer on sale, cases continued to occur during the following two weeks. Therefore, the FOPH launched an investigation to identify the source of the outbreak in order to prevent the occurrence of further cases.

### Methods

#### Epidemiological investigation

A case was defined as a resident in Switzerland, presenting with onset of gastrointestinal symptoms after 25 September 2006, and a stool or blood sample testing positive for *S. Stanley*.

Cases were identified by the NENT. In addition, the NENT sent an alert through Enter-net, the international surveillance network for enteric infections [11,12], in order to detect *S. Stanley* cases occurring in the countries participating in the network.

A retrospective case-control study was conducted between 3 and 17 November 2006, including the first 40 cases (onset of illness in weeks 39 - 44, 2006). A sample size of 120 with a ratio of cases/controls of 1/2 was estimated to provide a level of significance of 5%, and statistical power of 80% to detect an OR  $\geq 3$ .

The controls were residents in Switzerland selected in two stages: households were randomly selected from the household database of the Swiss Federal Office of Statistics; in each household the person who celebrated his/her birthday most recently was selected to be interviewed.

Clinical data on cases were collected through interviews with treating physicians. For each case fulfilling the inclusion criteria, permission to contact the patient was obtained from the physician. Demographic data and information on food consumption, recent travel history and cooking hygiene were collected through telephone interviews with cases and controls. Cases were interviewed on food-borne exposures during the three days preceding the onset of illness whereas controls were asked about the food items they had consumed during the last week of October.

The association between investigated exposures and illness was estimated using crude odds ratios (OR) and ORs corrected for canton of residence and age (ORMH) and respective 95% confidence intervals (95% CI). Chi-square and Mantel-Haenszel tests were performed to assess whether observations differed from what would be expected by chance. A multivariate analysis through a logistic regression model was performed including variables with  $p < 0.1$  in bivariate analysis; the final model was build with STATA v9.1 using the backward method and looking at interactions.

Interviews with cases were continued after the end of the case-control study. Therefore, information on food consumption and other possible risk factors are available for more cases than included in the study (58 cases).

#### Analysis of food and environmental samples

The Food Safety Division of the FOPH coordinated the environmental investigations. The Federal Research Station responsible for testing food products of animal origin (ALP) conducted bacteriological testing of suspected food and environmental samples at the place of production.

#### Microbiological investigations

The NENT serotyped *Salmonella* sp. isolates collected nationwide from clinical, food and environmental specimens using commercial antisera according to standard protocols for slide agglutination. The NENT performed the molecular analysis of all isolates positive for *S. Stanley* using Pulsed Field Gel Electrophoresis (PFGE). PFGE profiles from extracted total DNA, restricted with XbaI,

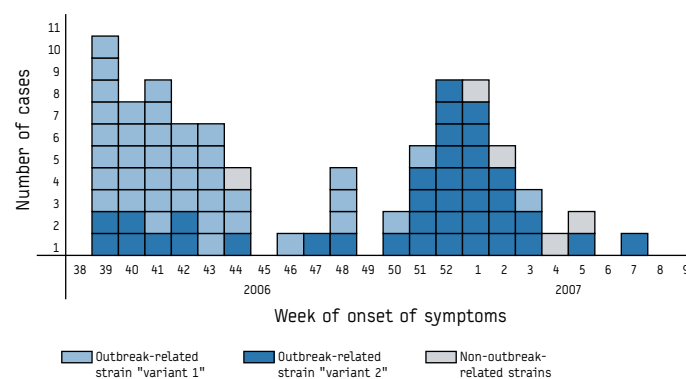
were generated using a harmonized protocol, and *S. Braenderup* (H9812) was used as the standard size marker [13].

## Results

### Description of the outbreak

Between 25 September 2006 (week 39) and 11 February 2007 (week 7), a total of 91 human isolates of *S. Stanley* were identified in Switzerland. Nine of these isolates were from patients not meeting the case definition: two were asymptomatic patients with stool samples (*S. Stanley* was an occasional finding) and seven had positive urine samples only. A total of 82 cases complied with the case definition. No other cases were notified by countries participating in Enter-net during this period.

**FIGURE 1**  
Distribution of *Salmonella Stanley* cases (n=82) by week of onset of symptoms and by strain, Switzerland, September 2006 – February 2007



**TABLE 2**  
Numbers of cases of *Salmonella Stanley* and incidences per 100,000 inhabitants in the cantons of residence of the patients, Switzerland, September 2006 – February 2007

Canton	number of cases	population	incidence
Vaud	21	662,145	3.2
Bern	19	958,897	2.0
Geneva	7	433,235	1.6
Zurich	6	1,284,052	0.5
Fribourg	5	258,252	1.9
Aargau	4	574,813	0.7
Basel-Stadt	3	187,920	1.6
Basel-Land	3	168,912	1.8
Grisons	3	267,166	1.1
Neuchatel	3	184,822	1.6
Valais	3	294,608	1.0
Jura	1	107,171	0.9
Lucerne	1	69,292	1.4
Nidwalden	1	359,110	0.3
St. Gallen	1	40,012	2.5
Zug	1	461,810	0.2
Total	82		

**TABLE 1**  
Characteristics and symptoms of *Salmonella Stanley* infection in outbreak-related cases (n=82) as reported by their treating physicians, Switzerland, September 2006 – February 2007

Characteristics of the disease	Value
Signs and symptoms (%)	
Diarrhoea	98
Fever	49
Abdominal cramps	35
Vomiting	18
Severe dehydration	9
Nausea	7
Muscle and joint pain	5
Asthenia	4
Other	16
Positive isolate from (%)	
Stools	96
Blood	4
Hospitalisation (%)	28
Mean duration of illness, in days (range)	9.4 (2-35)

Of the 82 cases, 46 (56%) were male. The average age was 45.7 years (range 0-92 years). Ninety-eight percent of cases were of Swiss nationality. Twenty-three cases (28%) were hospitalised: 19 for acute severe gastroenteritis or resulting complications and four for underlying diseases worsening due to salmonellosis. One case died for reasons not directly related to the infection (invasive cancer). In seven cases (9%) the disease outcome was unknown, the remaining patients recovered. Forty-five cases (57%) were treated with antibiotics, most of them (36 cases) with ciprofloxacin. Reported symptoms are summarized in Table 1.

The distribution of cases by week of onset of symptoms shows a first peak in week 39/2006 and a second in weeks 52/2006 – 1/2007 (Figure 1). Cases were distributed in 16 of the 26 Swiss cantons; 47 cases (57%) were reported from three western cantons: Vaud, Bern, and Geneva. (Table 2).

Four cases occurred among two couples of siblings aged four months and three years, and two and five years, respectively. Four cases referred having a total of five relatives or contact persons who had developed similar symptoms in the same time period. None of those contacts was laboratory tested.

#### Case-control study

The study included 40 cases and 82 controls. The response rate among cases was 98% and among controls it was 62%. The proportion of people aged less than 35 years was higher among cases than among controls (43% versus 19% of controls; OR 3.5,  $p=0.005$ ), as was the proportion of those living in French-speaking cantons (53% versus 24%; OR 3.4,  $p<0.0001$ ) and reporting buying food in small dairies (28% versus 11%; OR 3.1,  $p=0.03$ ) (Table 3).

As for food consumption, cases were more likely than controls to have eaten "raclette", a melted semi-hard cheese (13% of cases and 2% of controls; OR 9.8,  $p=0.03$ ), sliced chicken (21% of cases and 4% of controls; OR 7.1,  $p=0.01$ ), and a certain brand

(henceforth referred to as "brand X") of soft cheese (35% of cases and 7% of controls; OR 7.4,  $p=0.0001$ ) (Table 3).

The association between soft cheese of "brand X" and illness was higher among cases living in German-speaking cantons (OR 21.7, 95% CI 2.3–203.0) than in French-speaking ones and persisted when adjusting for cantons of residence (ORMH 5.4, 95% CI 1.7–17.2,  $p=0.02$ ). For sliced chicken, the specific ORs for <35 and  $\geq 35$  years old were lower than the crude OR and the OR adjusted by age was not statistically significant (ORMH 4.7, CI95% 0.1 - 26.1).

Consumption of soft cheese "brand X" remained the only exposure associated with the infection after adjusting for the other factors in the multivariate model (adjusted OR 11.4, 95% CI 1.9 – 69.6) (Table 4).

Interviews with cases on food consumption and other risk factors were continued after the end of the case-control study. Of the total of 82 cases, 58 were interviewed about the food they had consumed prior to onset of symptoms, and of these 24 (41.4%) reported having eaten soft cheese "brand X".

**TABLE 4**  
Multivariate analysis of risk exposure for *Salmonella* Stanley infection, Switzerland, September 2006 - November 2006

Risk factor/exposure	Adjusted OR*	95% CI	p value
Age <35 years	1.0	0.9-1.1	0.06
Resident in French-speaking canton	1.9	0.5-7.1	0.32
Buying food in small dairy	1.5	0.2-8.9	0.68
Sliced chicken	7.5	0.7-84.4	0.10
Raclette	4.8	0.3-71.6	0.25
Soft cheese "brand X"	11.4	1.9-69.6	0.008

**TABLE 3**  
Demographic characteristics and food exposures of cases of *Salmonella* Stanley infection (n=40) and controls (n=82) included in the analytic study, Switzerland, September 2006 - November 2006

Risk factor/exposure	Cases exposed; number/total (%)	Controls exposed; number/total (%)	Crude OR	95% CI	p value
Age <35 years	17/40 (43)	15/81 (19)	3.5	1.4-7.5	0.005
Resident in French-speaking canton	21/40 (53)	20/82 (24)	3.4	1.5-7.6	0.002
Sex (male)	20/40 (50)	37/81 (46)	1.2	0.6-2.5	0.65
Buying food in small dairy	9/32 (28)	9/80 (11)	3.1	1.1-8.7	0.03
Peanuts	7/35 (20)	11/79 (14)	1.6	0.5-4.4	0.41
Raw vegetables	21/35 (60)	47/74 (64)	0.9	0.4-2.0	0.72
Beef meat	22/32 (69)	46/76 (61)	1.4	0.6-3.5	0.42
Chicken meat	18/34 (53)	44/77 (57)	0.8	0.4-1.9	0.68
Sliced chicken	7/34 (21)	2/57 (4)	7.1	1.4-36.7	0.01
Pork meat	13/31 (42)	44/77 (57)	0.5	0.2-1.3	0.15
Eggs	11/33 (33)	64/76 (84)	0.1	0.04-0.2	<0.001
Mayonnaise	4/34 (12)	41/79 (52)	0.1	0.04-0.4	<0.001
Hard cheese (any)	21/35 (60)	72/80 (90)	0.2	0.1-0.5	<0.001
Raclette	4/31 (13)	1/67 (2)	9.8	1.0-91.5	0.03
Soft cheese (any)	20/35 (57)	43/79 (54)	1.1	0.5-2.5	0.79
Soft cheese "brand X"	12/34 (35)	5/73 (7)	7.4	2.4-23.4	0.0002

### Microbiological analysis

Within the outbreak period, NENT identified 91 isolates of *S. Stanley* from human samples, one from chicken imported from Hungary and two from soft cheese "brand X". Two variants of an outbreak related clone were identified by molecular analysis. Comparing the PFGE patterns, these variants differed in one single deviating band (Figure 3A). Both variants were distinctly different from *S. Stanley* strains isolated from human and environmental isolates collected during the weeks before the beginning of the outbreak (data not shown).

Of the 82 cases included in the outbreak, 77 (94% of all) carried either one of the two outbreak-related variants. "Variant 1" was identified in chicken meat, in soft cheese "brand X" and in 38 cases (46% of all cases), 28 of whom experienced onset of symptoms after week 49. No food isolates were available for "variant 2".

Of five cases carrying non-outbreak related strains, four reported having travelled in Thailand and Malaysia during the incubation period (Figure 3B). The PFGE pattern of the "variant 1" of the outbreak related strains was compared with the PFGE pattern of the peanut-related outbreak strain from United Kingdom [6]. They were closely related and differed by only two bands: one additional band of 550 Kb in the pattern of the peanut strain and one additional band of 260 Kb in the pattern of "variant 1" (Figure 3C).

### Analysis of food and environmental samples

Two series of cheese samples covering the entire production were collected in week 51/2006 in all 15 factories producing the soft cheese "brand X" in Switzerland. In total, 55 pools of scratch-samples were taken from the smeared surfaces of cheeses.

In week 1/2007 the analysis of the first series revealed *Salmonella Agona* in two specimens from one single producer. No other contamination was detected in any of the other production sites. The concerned producer blocked the release of new lots of cheese until they were completely checked for contamination with salmonellae and withdrew cheeses belonging to five different lots on sale. To trace the origin of *Salmonella* contamination in the concerned factory, 14 environmental samples from the production site, 10 environmental samples from ripening cellars and 14 samples of pooled milk from the suppliers of the dairy were collected. None tested positive.

At the end of January 2007, *S. Stanley* "variant 1" was isolated from several cheese samples of the second series taken in week 51/2006 in the same factory and of one of the five lots recalled in January.

Stool samples from workers of the incriminated dairy factory were collected by the concerned producer in the context of self control measures. All samples were negative and no employee declared having had diarrhoea or other gastrointestinal symptoms during the previous three months.

### Discussion and conclusion

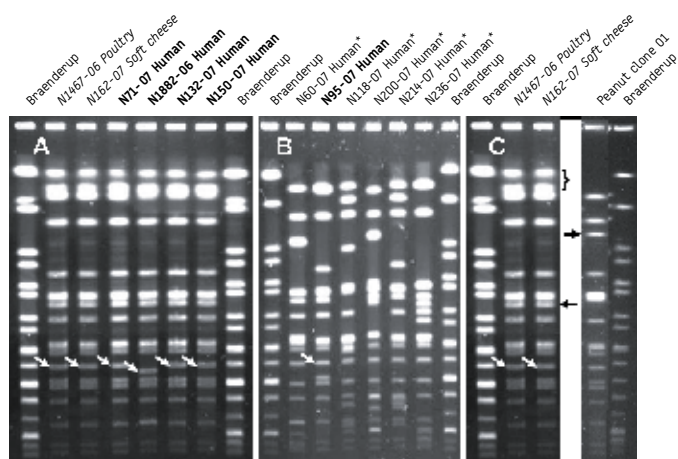
We described a nationwide outbreak involving 82 cases of *S. Stanley* infection in Switzerland. The overall number of cases was probably underestimated because only laboratory-confirmed cases were reported. The distribution of cases by date of onset of symptoms suggested a continuing common source disseminated in Switzerland in two successive periods.

Although chicken meat imported from Hungary was initially suspected on the basis of microbiological findings, our results suggested that this was not the source of the outbreak. Few cases were exposed to sliced chicken. The statistical association between chicken consumption and infection identified in the bivariate analysis was most likely confounded by age. Chicken meat was distributed all over the country whereas cases occurred mainly in the south-western part of Switzerland. Cases continued to occur when the chicken was no longer on sale.

The results of the case-control study indicated that soft cheese "brand X" was the most likely source of the outbreak. Having eaten soft cheese "brand X" was reported by at least 41% of cases. This relatively low percentage might be at least in part due to recall bias. No more cases were identified after the recall of suspected cheese and the strengthening of microbiological controls on new lots. This hypothesis was strongly supported by the microbiological confirmation of the contamination of cheese specimens from one cheese factory. The PFGE analysis of the *S. Stanley* isolates from cases and from cheese samples further confirms the link between the outbreak and soft cheese. The two outbreak-related variants were very closely related, differing only by one slightly deviating band, and were most likely two variants of the same clone [14]. Therefore, it is possible to exclude two parallel unrelated outbreaks; in total, more than 90% of cases carried the same clone as the contaminated cheese.

FIGURE 2

**Pulsed Field Gel Electrophoresis (PFGE) profiles of DNA from *Salmonella Stanley* isolates: A) selected isolates from patients related to the outbreak that occurred in Switzerland from 25 September 2006 - 11 February 2007, from samples of imported chicken meat and soft cheese "brand X" representing both variants of the outbreak clone; B) comparison of outbreak-related and non outbreak-related *S. Stanley* strains isolated from cases occurring during the outbreak period; C) comparison of the outbreak clone "variant 1" to the "peanut outbreak clone".**



Legend: In bold: some outbreak-related cases; in italics: chicken and soft cheese strains; white arrows indicate single up-shifted band in "variant 1", and white arrowheads indicate single down-shifted band in "variant 2" of the outbreak clone; black arrows indicate differing bands in "variant 1" and peanut-related outbreak strain; parenthesis indicates technically artefactual bands (partial restriction digests); \*: non-outbreak-related clinical isolates (mostly from cases imported from Thailand).

The “brand X” soft cheese is produced in the western (French-speaking) cantons of Switzerland. Even though distributed nationwide, it is more often consumed in the French-speaking cantons. It might appear contradictory that in these cantons, the association between “brand X” and illness was lower than in the German-speaking cantons. A possible explanation may be that in the French-speaking cantons, the population is generally more often exposed to this cheese whereby the probability to find controls who did not eat the cheese is lower than in the German-speaking cantons. “Brand X” is an artisanal cheese, made from thermized milk, produced from the end of September to March and ripened for a few weeks. The release of lots of contaminated cheeses ripened in two subsequent periods might explain the distribution of cases in two waves.

The origin of the contamination of the cheese factory remains unexplained. We hypothesise that the contamination occurred at the local level as two different lots produced by the same factory, distributed by different channels, were tested positive for *S. Stanley* “variant 1”. The contamination of individual cheeses was probably not massive as only two family clusters were identified and there were only five symptomatic persons among contacts who shared a meal with cases during the critical days.

We could not explain why the outbreak-related strain was found in imported chicken meat. No human cases related to this source were reported in other European countries, including Hungary where the product came from. One hypothesis might be that the meat was contaminated by an asymptomatic carrier handling the chicken or that a laboratory contamination occurred during food quality control.

#### Food safety recommendations

Several types of soft cheese are known to be products at risk for outbreaks due to listeria and various *salmonella* serovars [15,16,17,18]. In Switzerland, cheese production is subject to the Hazard Analysis Critical Control Point (HACCP) conditions [19]. For the specific dairy product involved in this outbreak, routine investigations for bacterial contamination are performed in white cheese (early stage of production) whereas in ripened cheeses, at the latest stage of production, only controls for listeria are routinely done. Since bacterial contamination may occur at any stage of the production, in order to prevent further outbreaks linked to soft cheese “brand X” and similar dairy product we concluded that testing for *salmonella* should be systematically performed also in fully ripened cheeses, at the latest stage of production. Therefore, in Switzerland, the HACCP monitoring programme and the clearing procedures for the release of products on the market have been revised to intensify the measures aimed at preventing the risk of *salmonella* infections during production and ripening of cheese.

#### Conclusion

This is the first *S. Stanley* outbreak in Europe not linked to imported food items. However, the PFGE profiles indicated that the Swiss outbreak-related strain might have been derived through minor genetic changes from the peanut outbreak strain imported into Europe [6].

In Switzerland, during the years preceding this outbreak, an increasing number of *S. Stanley* isolates had been reported from human and environmental specimens. Routine testing of river water in February 2007 (cantonal laboratory of Aargau) yielded the isolation of *S. Stanley* in a canton only marginally affected by the outbreak. All these findings suggest an increased local circulation of this rare serotype.

*S. Stanley* is not known to be a particularly virulent serotype, although there are reports of severe cases [9,10]. However, during this outbreak the proportion of cases hospitalised was higher than in other salmonellosis outbreaks in Switzerland. In addition, this serotype has already been found to be resistant to some antibiotics [5]. The emergence of this serotype in Switzerland suggests the need to strengthen surveillance of salmonellosis, investigate outbreaks and implement preventive and control measures in order to avoid future outbreaks and prevent new serotypes from establishing in the country.

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