Eurosurveillance

Volume 13, Issue 37 - 11 September 2008

Rapid communications

Surveillance and outbreak reports

Letters

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EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

Two severe cases of botulism associated with industrially produced chicken enchiladas, France, August 2008

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Two severe familial cases of botulism were reported to the health authorities in Brittany, north-west France, on 11 August 2008. An investigation was undertaken to identify additional cases, the vehicle of transmission, and to put in place adapted control measures.

Methods

Following notification of the cases, health authorities issued a communication to French hospitals, anti-poison and toxin centres and general practitioners to alert health professionals. No specific case definition was used for the investigation; the health professionals were requested to immediately report all clinical suspicions of botulism to the local health authorities using the routine mandatory notification system for the disease.

Serum samples from the cases and samples recovered from the food investigation were analysed by the National reference laboratory (NRL) for anaerobic bacteria and botulism at the Pasteur Institute, Paris. The presence of botulinum toxin was confirmed by intraperiteonal administration of patient serum to mice, and the toxin type was ascertained by the specific neutralisation technique.

The food history of the cases in the three to four days before onset of symptoms was documented.

Results

The two cases, a mother (in her 60s) and daughter (in her 20s), presented with gastrointestinal symptoms accompanied by dysphagia, blurred vision and facial paralysis on 9 August 2008. Both patients were hospitalised the day of symptom onset with a rapid evolution towards generalised and complete paralysis. The two women required intubation and mechanical ventilation. They remain in this condition in intensive care as of 3 September, with minor early signs of improvement. A trivalent antitoxin (toxin types A, B, E) was administered to the patients on 13 August. This antitoxin was imported from a commercial laboratory in Germany as botulism antitoxins are not commercially available in France. An authorisation for temporary usage of the product was issued by the French Health Products Safety Agency (Afssapsf).

The diagnosis of botulism (toxin type A) was confirmed for both cases by the NRL, by detection of botulinum toxin in blood samples of the patients. No other botulism cases associated with this episode were identified

The investigation of the food history for both women revealed that they had consumed an industrially produced pre-cooked Mexican-style "Tex-Mex" dish, chicken enchiladas, the day before onset of symptoms. These chicken enchiladas are sold as a pre-prepared kit consisting of several sachets containing a cheddar cheese sauce, a pre-cooked chicken and vegetable mix and two wheat tortillas. The product is consumed after reheating in a microwave oven. Microbiologic testing of the remaining chicken and vegetable mix revealed the presence of *Clostridium botulinum* and a high level botulinum toxin type A contamination (2.8×10^5 mouse lethal doses/g). The remaining cheese sauce was negative for botulinum toxin.

The epidemiological investigation of the two cases suggested that the contaminated enchiladas had been mistakenly stored at room temperature for two weeks between purchase and consumption, contrary to the producer's recommendation of refrigerated storage. They were consumed one day after the use-by date. However, the recommended storage conditions on the packaging are not easily visible to the consumer.

Risk analysis

The chicken enchiladas had been produced in France. The incriminated batch of enchiladas had a 'use-by' date of 7 August 2008. This batch was distributed only in France. Other batches of the enchiladas as well as pre-cooked chicken fajitas are also distributed in Belgium, Switzerland and Spain.

Stored production samples from the contaminated batch of enchiladas as well as other batches of enchiladas and fajitas and other products produced by the company around the same time were analysed and tested negative for botulinum toxin and *C. botulinum*.

A risk-analysis carried out on 14 August at the production plant concluded that the plant conforms to hygiene and safety regulations. An investigation of the fabrication protocols showed that the fabrication process includes a pasteurisation step of heating the product to 85°C for two hours.

Public health measures

The company issued a recall of the implicated batch of enchiladas on August 12. As a precautionary measure, this recall was then widened to include all enchiladas and fajitas produced by the firm. The population was informed of this outbreak through national

inter-ministerial press releases and posters placed in supermarket chains. European countries were informed via the 'Early Warning and Response System' and an alert in the 'Rapid Alert System for Food and Feed'.

In light of the potential role of incorrect product conservation in facilitating the multiplication of *C. botulinum* and toxin production in the contaminated enchiladas, a generalised reminder about respecting the storage conditions of such products was communicated by the French authorities. The producer of the enchiladas agreed to change the packaging of this and similar products to make the recommended storage conditions more visible for the consumer.

Discussion and conclusion

The two cases represent the clinically most severe cases of botulism reported in France in recent years. Botulism has been mandatorily notifiable in France since 1986, and 96 cases were reported between 2003 and 2006 [1]. Only two cases of botulism due to toxin type A, associated with the more severe form of the disease, were notified during this period, compared to 51 cases of toxin type B (53%) and four of toxin type E (4%) [1,2]. One-third of the cases notified during this period were not confirmed [1].

The epidemiological and environmental investigations support the hypothesis that the two cases ingested the toxin following incorrect storage of the chicken enchiladas which contained a strain of *C. botulinum* after production. Prolonged storage at room temperature could explain the unusually high level of toxin in the chicken and vegetable mix.

Intoxications with *C. botulinum* producing toxin type A are often associated with vegetable-based products that at some point contained soil with *C. botulinum* spores [2,3].

The thermo-resistance of *C. botulinum* spores varies by strain and according to factors such as the lipid and protein content of the food matrix [2]. Exposure to a temperature of 110-120°C for between 0.4 to 6 minutes is necessary to inactivate 90% of a population of *C. botulinum* A spores [2]. It is thus probable that the pasteurisation step during the enchiladas' fabrication process does not prevent the survival of spores present in primary ingredients or potentially introduced during the fabrication process. Thus, correct refrigerated storage of such processed food products is essential to avoid germination of the spores and toxin production.

Certain ingredients used in the production of "Tex-Mex" food products, including industrially produced cheddar-cheese sauce and home-canned jalapeno peppers, have previously been implicated in outbreaks of botulism in the United States [4,5].

This family cluster highlights the potential public health threat of *C. botulinum* spores in incorrectly stored processed food products and underlines the importance of clear labelling of storage conditions for products purchased in the refrigerated sections of supermarkets. In addition, the episode, widely reported in the national media, has served to remind the general population in France that compliance with food storage recommendations is a prerequisite for food safety.

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This article was published on 11 September 2008.

Citation style for this article: King LA, the French multidisciplinary outbreak investigation team. Two severe cases of botulism associated with industrially produced chicken enchiladas, France, August 2008. Euro Surveill. 2008;13(37):pii=18978. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18978

Surveillance of giardiasis in Northwest England 1996-2006: impact of an enzyme immunoassay test

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The incidence of giardiasis in Central Lancashire increased following the introduction of a sensitive enzyme immunoassay diagnostic test in November 2002. We compared the epidemiological trends for 1996-2006 in Central Lancashire with a control area which used a standard wet preparation diagnostic method throughout. Poisson regression modelling was used to investigate trends in giardiasis before and after the introduction of the test. In the control area, incidence of giardiasis was four per 100,000 in 2005. In contrast, in Central Lancashire, the rates increased in temporal association with the introduction of the enzyme immunoassay test from 10.1 per 100,000 population in 2002 to 33.6 per 100,000 in 2006. The increase in giardiasis was unexplained by local factors including travel, outbreaks or sampling trends. The increase in giardiasis occurred in all age groups except for males aged 0-14 years and was most marked in males aged 25-44 years. The relative risk for trend post-test introduction in Central Lancashire was 1.11 (95% CI, 1.01-1.23). This suggests that the increase in giardiasis following the introduction of the sensitive enzyme immunoassay test was at least in part due to improved detection. There appears to be considerable under-diagnosis of giardiasis, particularly in adults. Additional research is required to evaluate the enzyme immunoassay test more widely. The test may assist in standardisation of diagnostic methods for giardiasis and enable more accurate estimation of disease burden and transmission routes.

Introduction

Giardia lamblia is a commonly diagnosed intestinal protozoan infection that causes a significant burden of disease worldwide. Although giardiasis is more prevalent than cryptosporidiosis in the population of England and Wales (33,431 cases of giardiasis were reported between 1995 and 2001 compared with 31,655 cases of cryptosporidiosis [1]), the true incidence and burden of disease attributable to giardiasis and the risk factors for its acquisition have not yet been fully characterised.

Historically the diagnosis of giardiasis has been made by the observation of Giardia cysts or trophozooites in a wet preparation of faeces by microscopy. However, since the early 1990s new antigen detection methods (e.g. enzyme-linked immunosorbant assays [EIA] and immunochromatographic assays) and molecular methods such as polymerase chain reactions (PCR) have been introduced for various infections. In England and Wales laboratory methods for diagnosis of giardiasis are currently not standardised. Most laboratories continue to use the conventional method of wet preparation and microscopy of stool samples. Between laboratories there is variable use of faecal concentration methods and application of selection criteria (e.g. age and travel) to determine which samples are assayed.

In 2002, a microbiology laboratory in the North West of England replaced their conventional wet preparation microscopy method with routine testing of all faecal specimens from patients with community-acquired diarrhoea using an EIA diagnostic method. Following the introduction of this new diagnostic method laboratory-based surveillance detected a temporal increase in the incidence of giardiasis in the population served by this laboratory. This report discusses the nature of these epidemiological changes and the possible implications of these findings on the surveillance and epidemiology of giardiasis in the wider setting.

Methods

In order to assess the impact of the introduction of the EIA test in 2002, surveillance data for the "intervention" area introducing the EIA test was compared to a neighbouring "control" area where the standard wet preparation/microscopy method based on selective "in-house" criteria for age and foreign travel had not changed. Statistical comparison of giardiasis trends for 1996-2006 was investigated using Poisson regression modelling.

The intervention area named "Central Lancashire" was served by a single laboratory and comprised a population of 337,600 people in the local government areas of Chorley, South Ribble and Preston. The control area named "North Lancashire and Cumbria" was served by two laboratories and comprised a population of 427,100 people in the local government areas of Blackpool, Wyre, Fylde and Carlisle.

Throughout 1996 - 2006 the microbiology laboratory serving the Central Lancashire screened for giardiasis all diarrhoeal samples submitted from the community by family doctors, hospital admission wards and paediatricians. Prior to November 2002 screening was done by light microscopy of a wet preparation. In November 2002 light microscopy was replaced by a monoclonal EIA antigen detection method (GIARDIA/CRYPTOSPORIDIUM CHEKTM, Techlab). Positive results indicating the presence of either *Giardia* or *Cryptosporidium* spp. were confirmed by light microscopy until April 2006, after which an immunochromatographic assay (RIDA®QUICK Giardia) was used.

All faecal samples were taken from clinical cases of diarrhoea. Laboratory-confirmed cases of giardiasis were identified through laboratory reports to the respective Health Authority (1996-2003) and to the Cumbria and Lancashire Health Protection Unit (2003-2006). Comparative national data was provided by the Health Protection Agency Environmental and Enteric Diseases Department surveillance database [1].

Statistical methods

Poisson regression modelling was performed to determine whether the observed increase in giardiasis following the introduction of the routine screening test was statistically significant and whether differences in age/sex specific incidence were significant. Giardia count was defined as the dependent variable, logarithm of the population at risk as the offset and age group (five-year age bands), sex, year, area (Central Lancashire versus North Lancashire and Cumbria) and test introduction phase (prior or post) as the independent variables. Baselines were arbitrarily chosen to be 0-4-year-olds, male, Central Lancashire and prior phase for the age, sex, area and phase variables respectively. As the introduction of the test occurred near the end of 2002, the statistical analysis took the years 1996 to 2002 inclusive to be the prior and 2003 to 2006 inclusive to be the post-test introduction phases respectively.

The modelling yielded relative risks either relative to a baseline or as a year-on-year increase in giardiasis.

The initial model consisted of all three-way interactions between the independent variables. Variables and interactions were considered significant if the associated p-value was less than 0.05. A backwards stepwise modelling procedure was adopted with the non-significant three-way interaction with the largest p-value being removed at each step until all three-way interactions were significant, at which point the non-significant two-way interaction with the largest p-value not involved in the remaining three-way interactions was removed at each step. The final model was reached when all interactions were significant. Independent variables were not removed from the model as they were all involved in one or more interactions. As the final model consisted of more than one interaction, a series of models were fitted, each with one interaction, thereby ignoring the other interactions. All statistical analysis was performed using STATA, version 9.2 [2].

Figure 1

Reported number of cases of giardiasis in England and Wales, 1996-2006

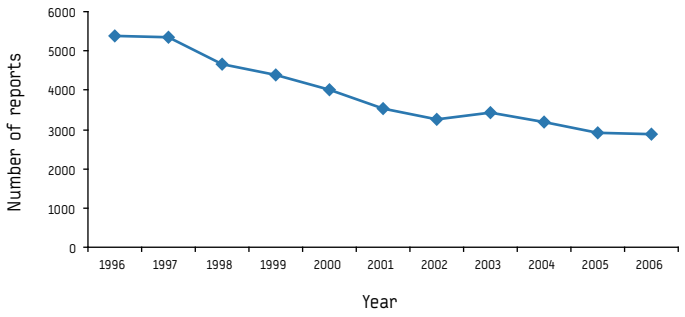


Figure 2

Reported number of cases of giardiasis in Central Lancashire versus North Lancashire and Cumbria, 1996-2006

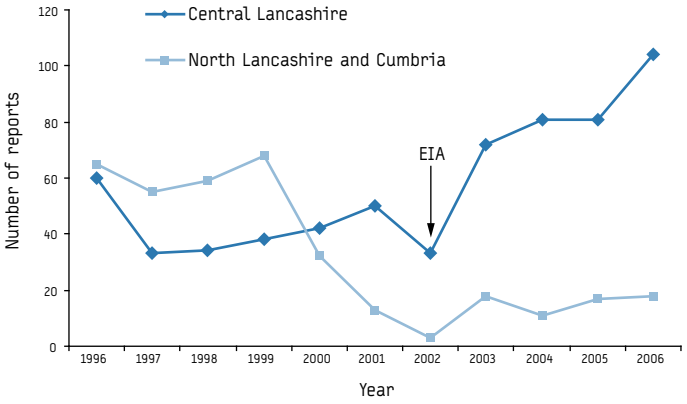
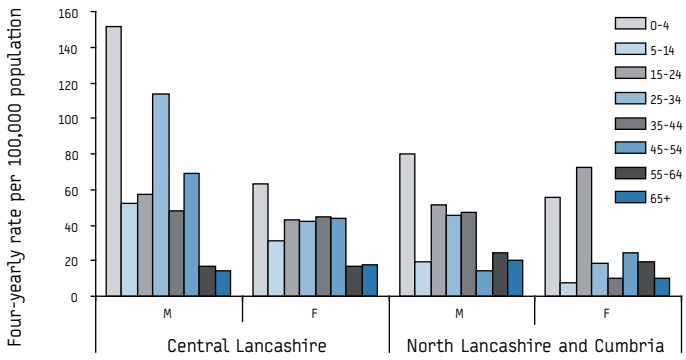


Figure 3

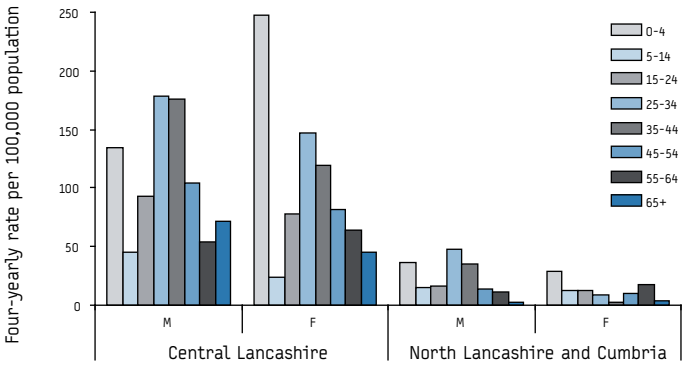
Age and sex-specific incidence of giardiasis, 1999-2002: Central Lancashire versus North Lancashire and Cumbria



Rates were calculated using 2000 population data available from: http://www.lancashireprofile.com

Figure 4

Age and sex-specific incidence of giardiasis, 2003-2006: Central Lancashire versus North Lancashire and Cumbria



Rates were calculated using 2004 population data available from: http://www.lancashireprofile.com

Results

Surveillance data for England and Wales demonstrate that the national number of reported cases of giardiasis has decreased steadily over the past decade falling from 5,379 cases in 1996 to 2,875 cases in 2006 [1] (Figure 1).

By contrast, in Central Lancashire there was a small increase in the number of reported cases of giardiasis between 1997 and 2001 and a marked increase from 2002 onwards. The start of the rise in 2002 corresponds in time with the introduction of the EIA diagnostic method. In North Lancashire and Cumbria, the reported cases of giardiasis decreased between 1999 and 2002, and have since remained at a low baseline (Figure 2)

In 2005 the incidence of giardiasis in England and Wales was 5.5 cases per 100,000 per year [1]. Similarly, the incidence of giardiasis in North Lancashire and Cumbria in 2005 was 4.0 cases per 100,000 per year. In Central Lancashire, however, the annual incidence of giardiasis increased from 10.1 cases per 100,000 in 2002 to 33.6 cases per 100,000 in 2006 – i.e. to more than six times the national rate. The increase in giardiasis in Central Lancashire was seen in all age groups except for males aged 0-14 years and was most marked in males aged 25-44 years and females aged 0-4 years (Figures 3 and 4).

The final Poisson regression model fitted was: AGE + SEX + INTRO + YEAR + AREA + AGE.SEX + AGE.AREA.YEAR + AREA. INTRO.YEAR, where INTRO referred to prior/post introduction of the screening test; and age, sex, area and intro were fitted as categorical covariates and year as a continuous covariate. The AGE.SEX, AGE.AREA.YEAR and AGE.INTRO.YEAR interactions had p-values of 0.007, <0.001 and <0.001, respectively. These interactions indicated that the incidence rates among the age groups were statistically significantly different between the sexes, the annual trends were statistically significantly different between each age group and area combination and age group and introduction phase, respectively.

The model clearly represented a complicated picture of the occurrence of Giardia. To try to understand the situation better, the following three models were fitted:

AGE + SEX + INTRO + YEAR + AREA + AGE.SEX,
AGE + SEX + INTRO + YEAR + AREA + AGE.YEAR.AREA,
AGE + SEX + INTRO + YEAR + AREA + INTRO.YEAR.AREA.

It appears that the rates are higher in males than in females with rates for males reaching their peak in the range 15-44 years of age, whereas for females the corresponding peak is in the range 15-34 years of age. The trend generally increases with age for Central Lancashire while for North Lancashire and Cumbria there is a decreasing trend in the rates which are consistent across all ages. There is an increasing trend following the introduction of the screening test in Central Lancashire, whereas for North Lancashire and Cumbria there is a decreasing trend prior to test introduction, but no statistically significant change in the post introduction phase (Table).

Table

Summary of the model of giardiasis incidence in Central Lancashire versus North Lancashire and Cumbria, 1996-2006

Model*	Variable	Age (years)	Relative risk	95% CI	
Averaging over AGE, YEAR and AREA, and INTRO, YEAR and AREA interactions	Males	0-4	1.00		
		5-14	0.31	0.21	0.45
		15-24	0.59	0.42	0.81
		25-34	0.79	0.58	1.06
		35-44	0.64	0.47	0.87
		45-54	0.43	0.30	0.60
		55-64	0.25	0.16	0.38
		65+	0.28	0.19	0.40
	Females	0-4	1.00	0.71	1.42
		5-14	0.21	0.14	0.32
		15-24	0.58	0.42	0.80
		25-34	0.51	0.36	0.71
		35-44	0.32	0.22	0.46
		45-54	0.32	0.22	0.46
		55-64	0.27	0.18	0.40
		65+	0.12	0.08	0.19
Averaging over AGE,SEX and INTRO,YEAR and AREA interactions	Trend (per year) by age group in **Central Lancashire**	0-4	0.99	0.92	1.07
		5-14	0.96	0.87	1.06
		15-24	0.94	0.87	1.01
		25-34	1.05	0.98	1.12
		35-44	1.10	1.02	1.18
		45-54	1.01	0.94	1.09
		55-64	1.06	0.95	1.18
		65+	1.04	0.94	1.14
	Trend (per year) by age group in **North Lancashire and Cumbria**	0-4	0.75	0.67	0.83
		5-14	0.70	0.70	0.89
		15-24	0.72	0.66	0.78
		25-34	0.80	0.73	0.87
		35-44	0.82	0.75	0.91
		45-54	0.76	0.67	0.85
		55-64	0.82	0.73	0.92
		65+	0.79	0.69	0.92
Averaging over AGE,YEAR and AGE,YEAR and AREA interactions	Trend (per year) by test introduction in **Central Lancashire**	Prior	0.97	0.91	1.03
		Post	1.11	1.01	1.23
	Trend (per year) by test introduction in **North Lancashire and Cumbria**	Prior	0.77	0.72	0.82
		Post	1.03	0.83	1.29

*AREA=Central Lancashire or North Lancashire and Cumbria, INTRO=pre or post EIA introduction
Note: The modelling yields relative risks for trend either relative to a baseline or as a year-on-year increase in giardiasis. Baselines are 0-4-year-olds, male, Central Lancashire and prior phase for the age, sex, area and test introduction variables respectively.

Discussion

This report describes a localised increase in the incidence of giardiasis after introduction of a sensitive diagnostic test. The results presented need to be treated cautiously for two reasons: firstly relative risks have been obtained for one interaction at a time, ignoring the others, and secondly there are various caveats with regard to the data, not least the low number of cases for North Lancashire in 2002 which was about the time when a new surveillance system came into operation. However it appears that the epidemiological change is in part due to increased detection following the introduction of the EIA diagnostic method. This was suspected from an "in-house" comparison of the sensitivity of microscopy versus EIA prior to EIA introduction. Some 601 faecal samples were tested and positive stools by either method were further tested by giardia PCR. The 18 samples that tested positive by EIA were all corroborated by PCR while microscopy missed three of these. Thus the additional yield of EIA in this survey was 17%. It is likely this would be greater in routine practice as the EIA is less demanding in terms of technical expertise.

Although statistical analysis is not conclusive it supports increased detection as the most likely explanation for the increased incidence as indicated by the relative risk of 1.11 (1.01, 1.23) post-EIA introduction in Central Lancashire. This explanation is further

supported by the association in time (Figure 2), the absence of other satisfactory explanations (i.e. no identified outbreaks, no systematic changes in overseas travel, water supply or stool sampling policy between the two surveillance areas) and the scientific plausibility of this explanation. For example, EIA diagnostic methods have been shown to be both highly sensitive (95% [3] and 88.6-100% [4]) and specific (100% [3] and 99.3–100% [4]). The sensitivity of conventional microscopy of single stool samples is operator-dependent and has been shown to be around only 70% [3,5]. PCR detection of *Giardia* and *Cryptosporidium* spp. is 22 times higher than that of conventional microscopy methods [6] suggesting that the currently used diagnostic systems are likely to considerably underestimate the incidence of these parasites.

The findings of this report have been based on arbitrary choices of baselines. Since the relative risks have been well estimated with these choices, different conclusions would not have been reached by choosing a different set of baselines. Indeed, some other choices may have led to relative risks being less well estimated.

From a practical perspective the EIA test was simple to perform and was readily incorporated into laboratory practice. The additional reagent costs were more than offset by the increased efficiency of skilled laboratory staff who no longer needed to undertake relatively time-consuming microscopy. The EIA also had the advantage of simplifying the diagnosis of cryptosporidiosis as it was a combined test. However the extra cost of the test is probably the main obstacle preventing laboratories from introducing the EIA test.

We have been unable to find similar reports in the literature of an increase in the incidence of giardiasis following the introduction of an EIA or similar method. For example, following implementation of a similar enzyme immunoassay screening test for *Giardia* and *Cryptosporidium* in a Canadian laboratory, although the timeliness and efficiency of diagnosis of these parasites improved, the total percentage of cases with enteric parasite infection remained stable [7]. However changes in laboratory methods have been associated with changes in epidemiology of infections caused by other organisms, e.g. *Bordetella pertussis* [8].

The introduction of the EIA method in 2002 does not fully explain the continuing increase in the reported incidence of giardiasis in 2006. The most likely explanation for this is the replacement of light microscopy confirmation by a more sensitive immunochromatographic assay in April 2006. Giardiasis is known to have a bimodal age distribution with a large peak in children under five and a smaller peak in adults aged 25-39 [9,10]. The high incidence in males aged 25-44 years in our series is particularly interesting as this is not a group that frequently seeks medical attention [11] and therefore has fewer stool samples collected. Given they are not a traditional high risk group for giardiasis this raises the question as to whether as yet undetermined risk factors may be contributing to the increased incidence and to the change in age- and sex-related epidemiology.

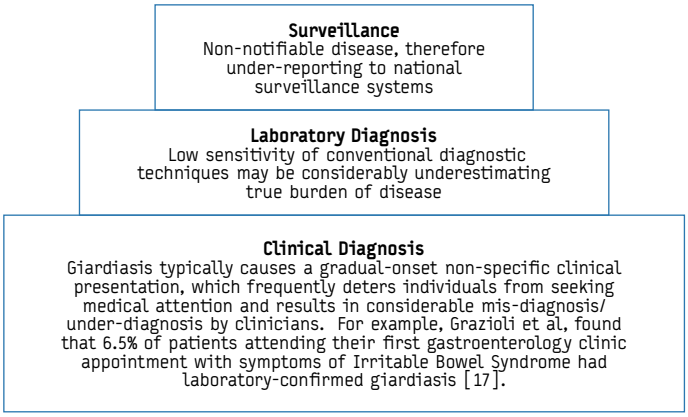
The majority of non-travel associated cases of giardiasis in the UK tend to be acquired sporadically rather than being associated with outbreaks. However, most information on risk factors for giardiasis has come from investigation of outbreaks abroad. A case-control study of sporadic giardiasis in Southwestern England identified swallowing water while swimming, recreational fresh water contact, drinking treated tap water and eating lettuce as independent risk factors for giardiasis [12]. Nevertheless, the relative importance of the various sources and transmission routes of giardiasis are poorly understood and a clear quantitative understanding is required [13].

This report highlights several general issues regarding the epidemiology and surveillance of giardiasis. Firstly, the true burden of clinical disease attributable to giardiasis may currently be considerably underestimated as a result of substantial under-diagnosis at all stages of reporting. Although this underestimation of community-acquired gastrointestinal diseases by national surveillance is a well recognised issue [14], this is likely to be particularly true for giardiasis [15,16] (Figure 5).

In one study *G. lamblia* was present in 9 out of 137 (6.5%) of patients with Irritable Bowel Syndrome, a finding which if replicated in further studies, would add to the public health importance of giardiasis [17]. Secondly, the non-standardisation of laboratory diagnostic methods makes interpretation of routine surveillance data and comparisons at regional, national and even international level difficult. Finally, the increasing incidence of giardiasis and the changes in age and sex-related epidemiology noted in this report emphasise the lack of knowledge regarding the relative importance of the various transmission routes for the acquisition of giardiasis in European countries such as England and Wales.

FIGURE 5

Underestimation of burden of disease due to giardiasis



Conclusion

The increase in giardiasis following introduction of the sensitive enzyme immunoassay test was at least in part due to increased detection. Additional research is required to evaluate the enzyme immunoassay test more widely. The test may assist in standardisation of diagnostic methods for giardiasis and enable more accurate estimation of disease burden and transmission routes, particularly in non-traditional high-risk groups.

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This article was published on 11 September 2008.

Citation style for this article: Ellam H, Verlander NQ, Lamden K, Cheesbrough JS, Durband CA, James S. Surveillance of giardiasis in Northwest England 1996-2006: impact of an enzyme immunoassay test. Euro Surveill. 2008;13(37):pii=18977. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18977

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.

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)

Figure 1

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

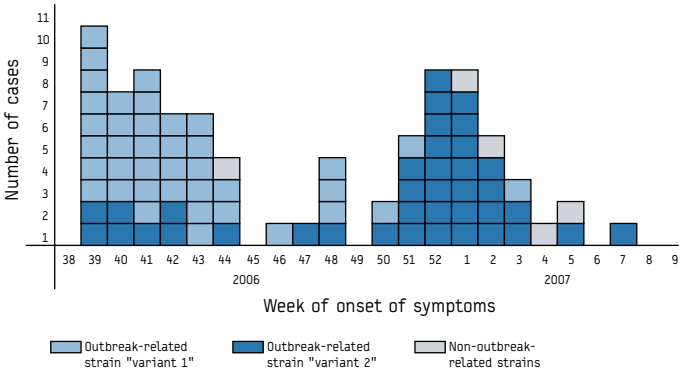


Table 2

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		

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 ≥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 Sliced chicken	18/34 (53) 7/34 (21)	44/77 (57) 2/57(4)	0.8 7.1	0.4-1.9 1.4-36.7	0.68 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) Raclette	21/35 (60) 4/31 (13)	72/80 (90) 1/67 (2)	0.2 9.8	0.1-0.5 1.0-91.5	<0.001 0.03
Soft cheese (any) Soft cheese "brand X"	20/35 (57) 12/34 (35)	43/79 (54) 5/73 (7)	1.1 7.4	0.5-2.5 2.4-23.4	0.79 0.0002

Microbiological analysis

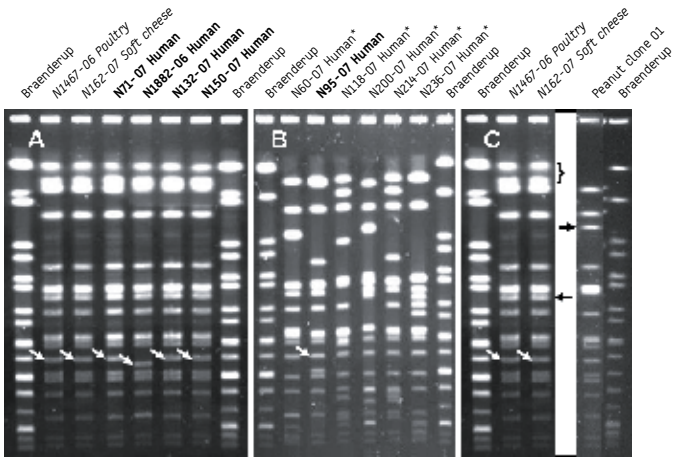
Within the outbreak period, NENT identified 91 isolates of *S.* Stanley from human samples, one from chicken imported from Hungary and two from soft cheese "brand X". Two variants of an outbreak related clone were identified by molecular analysis. Comparing the PFGE patterns, these variants differed 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).

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).

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.

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.

Acknowledgements

We thank Pierre-Alain Raeber, head of the Division of Communicable Diseases, and Simone Graf, Peter Helbling, Linda Nartey, Sandra Oesterreicher in the FOPH for their support in the data collection. We thank the ARQAH microbiological laboratory in Moudon, for their essential contribution to the microbiological investigations. We are also grateful to Marta Valenciano (European Programme of Intervention Epidemiology Training - EPIET) for scientific advice. We acknowledge John Threlfall's providing the PFGE pattern of the 2001 peanut outbreak clone of *S.* Stanley. We thank Grethe Sägesser for the excellent experimental microbiological work and technical support.

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This article was published on 11 September 2008.

Citation style for this article: Pastore R, Schmid H, Altpeter E, Baumgartner A, Hächler H, Imhof R, Sudre P, Boubaker K. Outbreak of Salmonella serovar Stanley infections in Switzerland linked to locally produced soft cheese, September 2006 – February 2007. Euro Surveill. 2008;13(37):pii=18979. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18979

Q fever in the Netherlands – what matters is seriousness of disease rather than quantity

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To the editor: We have read with interest the article by Schimmer B *et al.* [1] about Q fever in the Netherlands, an outbreak that captured the attention of the mass media and was labelled as "unique in the world". We wish to contribute our experience regarding the clinical manifestations and potential seriousness of this disease and make some comments about the difficulties of diagnosis encountered in endemic areas.

Between 1984 and 2004, as a result of routine clinical diagnoses and without active surveillance, 1,261 cases of Q fever were detected in our region (Gipuzkoa, Basque country, north of Spain, 690,000 inhabitants) [2]. The annual number of cases ranged from 19 to 153, with two major outbreaks, at the beginning and at the end of the 1990s. The Gipuzkoa experience showed several similarities with that of the south of the Netherlands, both regions having a temperate oceanic climate and generally abundant rainfall spread out over the year. The most common clinical manifestation of acute Q fever in our report was atypical pneumonia (79%), with hepatitis being very infrequently found. Our study also revealed that most cases occurred in men (75%), in adults (70% between 25 and 54 years) and between March and June (64.3%), a period that coincides with the greatest parity of sheep and goats and the season when people frequently take walks in the countryside. There were no deaths, fewer than 2% of the patients required admission to the intensive care unit, and only two cases of chronic Q fever (two patients with prosthetic valve endocarditis) were detected. In regions with the same climate, the seriousness of the disease and clinical manifestations are similar while differing from those in other climatological regions, suggesting that different *Coxiella burnetii* types circulate in regions with different climates.

To date, diagnosis of Q fever relies on serology, immunofluorescence (IF) being the reference technique [3]. The diagnostic criteria used by us are more restrictive than those usually employed. For acute Q fever, using the IF, *C. burnetii* phase II, Bio-Mèrieux, these criteria were as follows: seroconversion of IgG and/or IgM (from negative to ≥1/128), a four-fold or greater increase in IgG titre in two paired sera, or IgM titre ≥1/256 in a single sample after removing rheumatic factor. The Tissot-Dupont cut-off for acute Q fever (*anti–phase II* IgG titre ≥1/200 and anti–phase II IgM titre ≥1/50) [3] would not be useful in our area, since many patients without acute Q fever would meet these criteria.

We reviewed the presence of these lower titres among the serological results obtained in two years: one year with an outbreak (1992: 153 cases) and another without outbreaks (2002: 43 cases). If an IgM titre of 1:50 with IgG titre ≥1/200 had been used as the diagnostic criteria, the number of acute cases would have increased by 22.2% (187 cases instead of 153) and 39.5% (60 instead of 43) in 1992 and 2002, respectively. These patients were excluded after we ascertained that they showed no clinical manifestations of infection. Moreover, we detected IgM titres ≥1/64 with IgG ≥1/256 in a further 46 patients without chronic infection, who had already been diagnosed with acute Q fever five or more months previously. In 24 of these patients, high antibody titres persisted for over one year.

As the number of detected cases may be closely related to the intensity of the search and the methods and criteria used in the diagnosis, we believe that exhaustive surveillance under pressure from the media could lead to over-diagnosis and unnecessary treatments which, like that of pregnant women, are not free of risk.

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This article was published on 11 September 2008.
Citation style for this article: Cilla G, Montes M, Pérez-Trallero E. Q fever in the Netherlands – what matters is seriousness of disease rather than quantity . Euro Surveill. 2008;13(37):pii=18975. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18975

Authors reply: Q fever in the Netherlands – what matters is seriousness of disease rather than quantity

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We thank G. Cilla, M. Montes and E. Pérez-Trallero for their comments on our article *'Large ongoing Q fever outbreak in the south of the Netherlands, 2008'* [1].

Since the publication the number of Q fever notifications received at the National Institute for Public Health and the Environment has increased to 880 (data from 1 January to 3 September 2008). As we have already indicated in our article [1], increased awareness for Q fever has certainly led to more diagnoses and laboratory-confirmed cases. Clinicians were regularly informed about the epidemic and its medical consequences. Numerous reports published in the newspapers will have increased the awareness about the disease among medical doctors and patients. We think this has led to accurate diagnosis of acute Q fever cases that would otherwise have gone undetected. As we have pointed out the notification criteria in the Netherlands require clinical signs and symptoms that are consistent with Q fever infection. A positive laboratory result without clinical disease is not sufficient for notification. We are currently retrospectively collecting data from physicians to get a more precise idea of the severity and duration of clinical disease.

Cilla *et al.* rightfully point to the lack of standardisation in interpretation of serology results. Interpretation of cut-off values for immunofluorescence (IF) antibody titres to diagnose acute Q fever depends on the antigens used. "Moreover, IF can be used for surveillance purposes for which higher cut-off values may be more appropriate. For diagnosis of acute Q fever, serology must be matched with clinical signs and symptoms. In case of doubt, follow-up samples should be taken to confirm diagnosis. In our cases serology was only performed in a clinical setting with a differential diagnosis that included Q fever. All cases were examined clinically after notification, and all initially dubious serologic results had to be confirmed by follow-up serum samples to obtain significant rise in titers and thus confirm the diagnosis. A case control study was carried out in 2007 in a small cluster area [2] in the southeast of the Netherlands. For this study indeed higher cut-off values were used than in the present outbreak.

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This article was published on 11 September 2008.

Citation style for this article: Schimmer B, Morroy G, Dijkstra F, Schneeberger PM, Weers-Pothoff G, Timen A, Wijkmans C, van der Hoek W. Authors reply: Q fever in the Netherlands – what matters is seriousness of disease rather than quantity. Euro Surveill. 2008;13(37):pii=18976. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18976

EpiWords: a new online English-Russian glossary of medical, statistical, microbiological and epidemiological terms

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EpiNorth project had launched a new feature – EpiWords. EpiWords is a bilingual glossary (English – Russian) of medical, statistical, microbiological and epidemiological terms. It will help to diminish the terminology difficulties in professional communication among health care specialists using English and Russian language.

Currently, the EpiWords section contains more than 800 terms and word combinations in Russian and English and is freely available. The terms are arranged in alphabetical order. EpiWords is a complementary source and is not intended to replace medical information provided by other published or online dictionary/glossary sources.

EpiNorth project staff plans to find and publish new terms in a timely manner and invites interested parties to send suggestions, comments or questions to EpiNorth project at epinorth@fhi.no

EpiWords can be accessed at the following link:
http://www.epinorth.org

This article was published on 11 September 2008.

Citation style for this article: Editorial team. EpiWords: a new online English-Russian glossary of medical, statistical, microbiological and epidemiological terms. Euro Surveill. 2008;13(37):pii=18980. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18980

WHO Regional Office for Europe and UNAIDS report on progress since the Dublin Declaration

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The Joint United Nations Programme on HIV/AIDS (UNAIDS), the WHO Regional Office for Europe and its partners recently published a report to take stock of the progress made since 2004 in implementing the Dublin Declaration on Partnership to Fight HIV/AIDS in Europe and Central Asia [1]. This declaration, which sets out targets for fighting HIV, was formally adopted by 55 countries at a conference that took place in Dublin on 23 and 24 February 2004 [2].

The report is broken down into 15 thematic chapters and addresses issues ranging from political leadership through injecting drug use to HIV in prisons, followed by a number of country profiles. It is aimed at supporting the member states of the WHO European Region to monitor and implement the provisions of the declaration.

It describes the indicators explaining the efforts to fight HIV and gives a guide to interpreting the latest data collected on these indicators and to reinforcing the battle against HIV. Apart from the comprehensive progress report itself, a 35-page summary is available as a policy brief outlining the relevance of each topic addressed and giving key findings and key recommendations for each thematic area. This is important since the European Region now has the fastest rate of growth of HIV prevalence in any region of the world [1].

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This article was published on 11 September 2008.

Citation style for this article: Editorial team. WHO Regional Office for Europe and UNAIDS report on progress since the Dublin Declaration. Euro Surveill. 2008;13(37):pii=18981. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18981