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# Ongoing outbreak of hepatitis A in Italy: preliminary report as of 31 May 2013

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**Since January 2013, an unusual increase in hepatitis A cases has been detected in northern Italy. A total number of 352 cases were reported to the integrated surveillance system between January and the end of May 2013 and this represents a 70% increase compared to the same period of the previous year. The outbreak is ongoing and the public health authorities are continuing their investigations to establish the transmission vehicle and to control the outbreak.**

From 1 January 2013 to 31 May 2013 a total of 352 cases of hepatitis A were reported to the Italian national surveillance system, corresponding to a 70%, 54%, and 34% increase in HAV notifications compared to the same period in 2012, 2011 and 2010, respectively. Here we describe the epidemiological features of the cases and the investigation of the outbreak.

## Surveillance of hepatitis A in Italy

Hepatitis A is a notifiable disease in Italy. According to the national legislation, laboratory-confirmed cases of hepatitis A virus (HAV) infection are reported by clinicians to the local health units (LHUs) which are responsible for the epidemiological investigation. From the LHUs, notifications are sent to the regional health authorities (RHAs) and from here to the Ministry of Health. However, the routine notification system does not collect information on risk groups and risk factors associated with hepatitis A and there is an important delay in the transmission of the data [1]. For this reason, in 1984, a specific sentinel surveillance system for acute viral hepatitis (SEIEVA -Sistema Epidemiologico Integrato Epatiti Virali Acute) was set up in parallel with the official notification system in Italy [2]. Data included in the SEIEVA system provide insight into the risk factors associated with the disease. Data collected by SEIEVA are provided by LHUs, which participate on a voluntary basis. A case is defined as a person with an acute illness including symptoms clinically compatible with hepatitis A, such as fever, fatigue, nausea, vomiting, abdominal pain, dark urine and jaundice, and positive for IgM anti-HAV. Cases are interviewed using a standardised online questionnaire collecting socio-demographic, clinical and laboratory information, and information on possible risk factors (shellfish consumption, contact with a jaundice case, travel to an endemic area, child attending daycare in the household, intravenous drug use in the last six months). After the alert issued by the northern European countries about a possible association between the hepatitis A cases and frozen berries [3], the consumption of mixed frozen berries was included as another possible risk factor in the SEIEVA questionnaire at the end of April 2013.

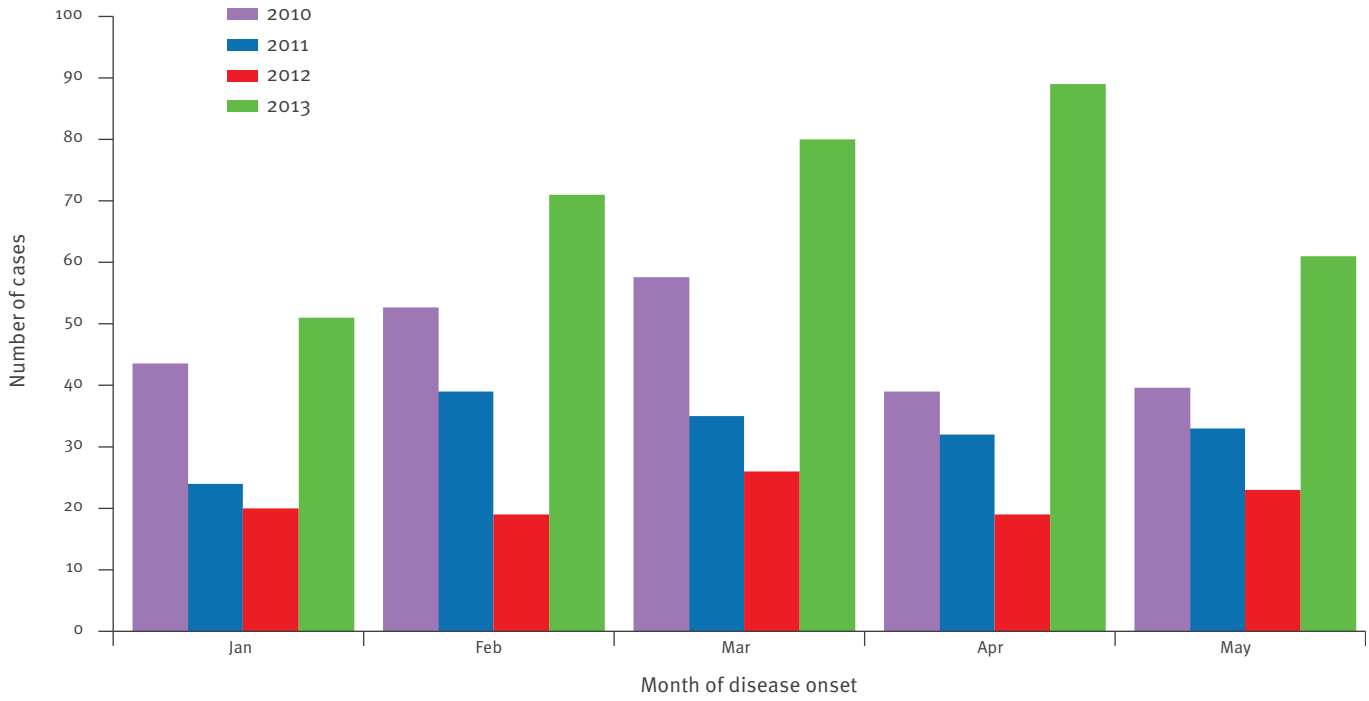
As of 31 May 2013, 76% of the Italian LHUs (139/181) participate in the SEIEVA. The participating LHUs are distributed all over the country and cover 70% of the population. Data were adjusted considering the total population of the LHUs' catchment areas.

## Epidemiological situation of hepatitis A in Italy

In recent decades, the epidemiological pattern of hepatitis A has changed. Italy is considered to be at low/intermediate endemicity for HAV [2,4]. The improved health and sanitary conditions have favored a progressive decrease of the infection rate in children, and a major shift of the population at risk, with the highest incidence reported in young adults. Outbreaks were described in 1996-1997 and 2004 mainly in southern Italian regions (Apulia and Campania) and were related to the consumption of contaminated raw shellfish [5,6]. From 1997, when the incidence was 19 per 100,000

**Figure 1**

Distribution of hepatitis A cases in Italy, January–May 2010 to January–May 2013




Source: Sentinel surveillance system for acute viral hepatitis (Sistema Epidemiologico Integrato Epatiti Virali Acute - SEIEVA)

population [2] to date, a decreasing trend in the incidence of HAV has been observed, to 1.1 cases, 0.7 and 0.8 per 100,000 population in 2010, 2011, and 2012, respectively [7].

## The 2013 hepatitis A outbreak in Italy

From 1 January 2013 to 31 May 2013 a total number of 352 cases of hepatitis A were reported to SEIEVA surveillance system, corresponding to a 70%, 54% and 34% increase in HAV notifications compared to the same period in 2012, 2011 and 2010, respectively (Figure 1).

The highest increase in the number of cases was observed in seven northern Italian regions (Trento and Bolzano, Emilia-Romagna, Lombardy, Friuli Venezia Giulia, Piedmont, and Veneto) that accounted for 193/352 (55%) of the total cases recorded in 2013. In these seven regions, the cumulative incidence was 2.66 per 100,000 population in the five-month reference period. Another region that showed an increase in the number of cases in 2013 is Apulia, in southern Italy, which recorded a 22% increase in the number of cases in 2013; 77 of the 352 cases were reported from this region.

The distribution of cases by age group and year is reported in Figure 2. The mean age of cases was 35 years (range: 2–63 years) and the median was 39 years; 23 cases (12%) were recorded in children under 14 years. The cases were equally distributed among men and women: 55% of the cases were men and 45% were women. A total of 159 persons were hospitalised, with the majority of hospitalised cases in the age group of 35-54 years. As of 31 May 2013, no acute liver failures and deaths occurred. Four cases had been vaccinated against hepatitis A, with one dose within the three weeks before the onset of symptoms, so these were not considered vaccine failures.

With regard to the risk factors, among those who answered the questionnaire (193 cases), 3% (7/193) reported to have travelled to Egypt, 17% (33/193) reported to have eaten raw seafood and 20% (37/193) mixed berries in the six weeks before the symptom onset. When considering risk factors distribution after the end of April (date of introduction of the question on the consumption of frozen mixed berries), the majority of cases (37 of 46) reported having consumed frozen mixed berries.

## Description of the 2013 hepatitis A outbreak in the provinces of Trento and Bolzano

In May 2013, Germany, the Netherlands and Poland reported trough the Epidemic Intelligence Information System for food- and waterborne diseases (EPIS-FWD) and the Early Warning and Response System (EWRS) 15 cases of HAV infection associated with a ski holiday in the autonomous provinces of Trento and Bolzano (northern Italy). The sequencing of the VP1-region of these five Italian isolates, from Trento province, showed 100% nucleotides homology with those isolated from two German and one Dutch case [8].

After the EPIS and EWRS notifications, a retrospective epidemiological investigation started in the provinces of Trento and Bolzano, contacting cases notified through the regional notification system. For the epidemiological investigation, a confirmed case was defined as a person resident in the provinces of Trento and Bolzano with an acute illness including symptoms clinically compatible with hepatitis A, such as fever, fatigue, nausea, vomiting, abdominal pain, dark urine and jaundice, and identified as positive for IgM anti-HAV after 1 January 2013.

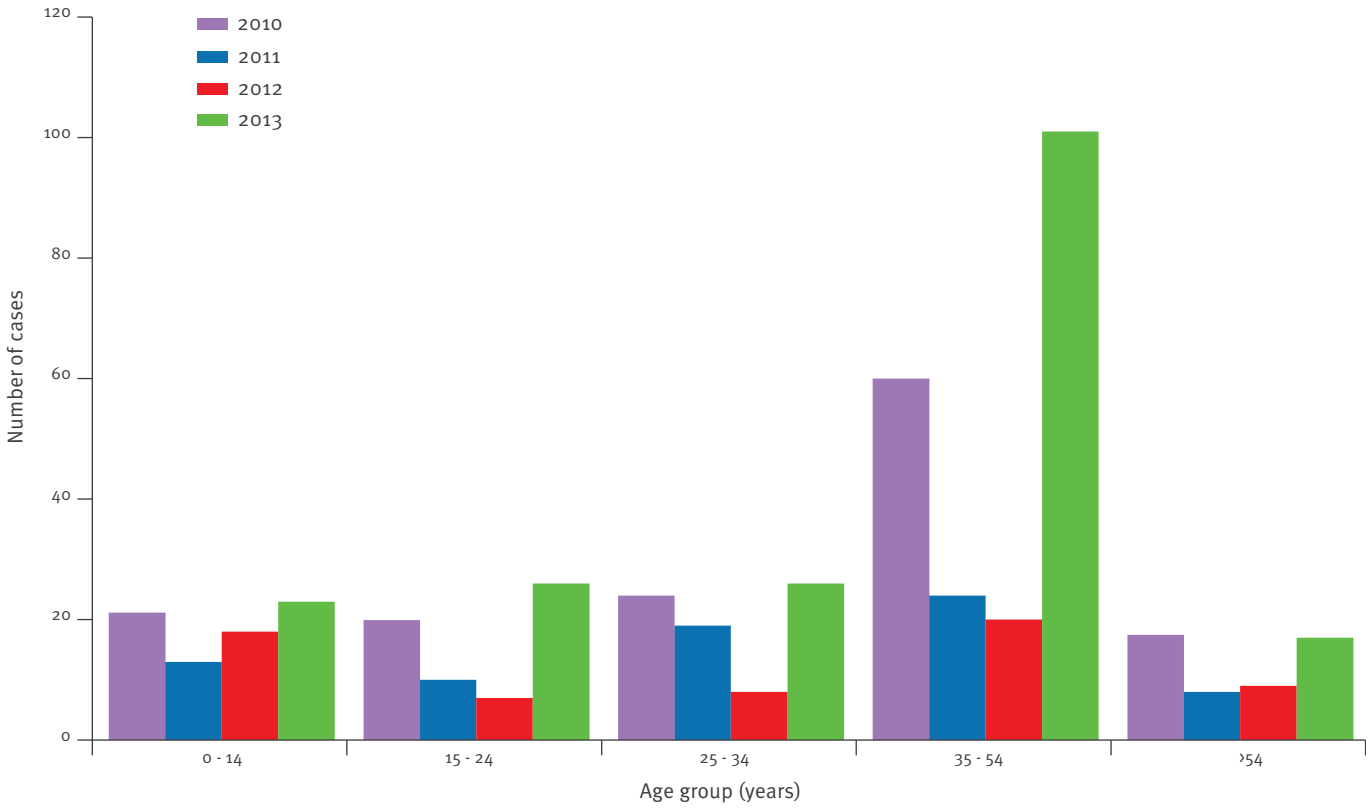
Between 1 January and 31 May 2013, 31 cases of HAV infection were notified in the province of Trento (a 13-fold, 19-fold and 6-fold increase approximately, compared to the same period in 2012, 2011, and, 2010 respectively). The first case reported the onset of symptoms on 2 February and the most recent case was identified on 31 May. Most of the cases had the onset of symptoms in May (15 cases).

In the province of Bolzano, seven cases were reported in the same period. The epidemic curve of the 38 confirmed HAV infection cases in these two provinces shows the evolution of the outbreak over time and suggests a common vehicle of transmission (Figure 3).

In these two provinces, the mean age of the cases was 36.3 years (range: 3–63 years) and the median was 38.5 years. Men were more represented than women (24 versus 14). A total of 31 persons were hospitalised and the majority of them were 35 to 54 years-old. There was only one case vaccinated and this case was reported

**Figure 2**

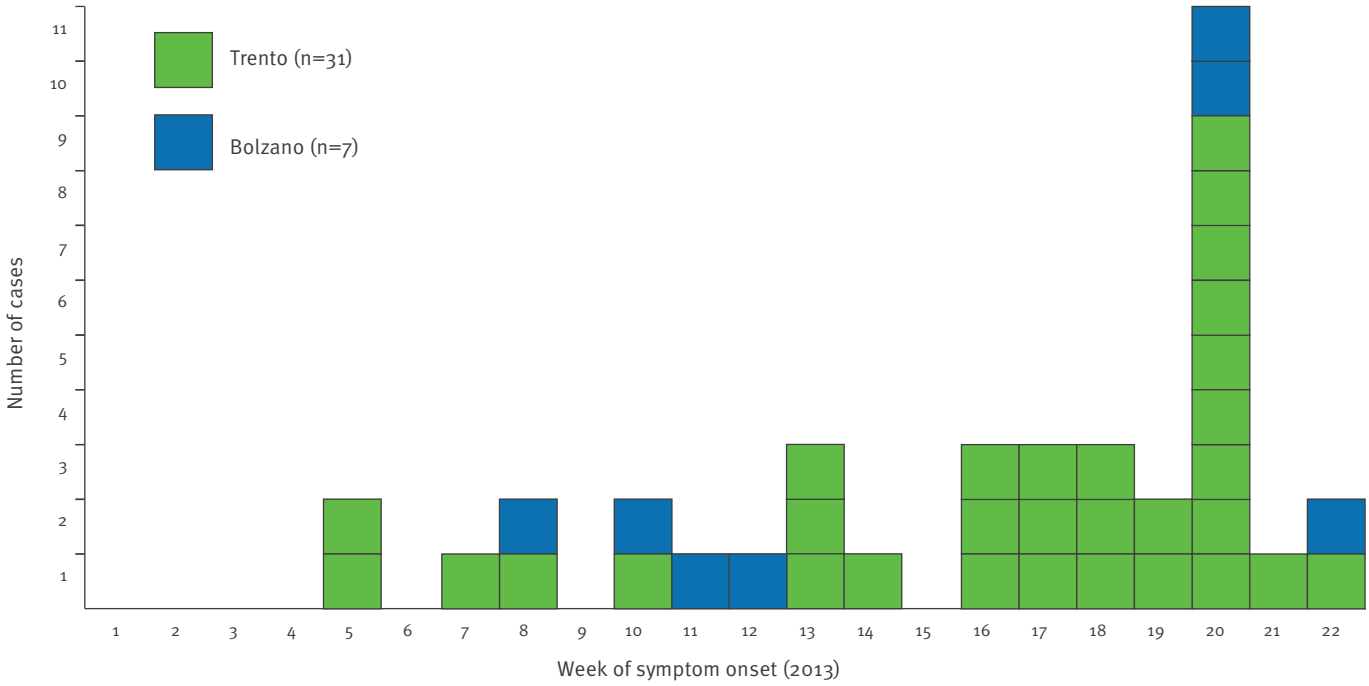
Distribution of hepatitis A cases by age group in seven Italian regions[a], January–May 2010 to January–May 2013




[a] Trento and Bolzano, Emilia-Romagna, Lombardy, Friuli Venezia Giulia, Piedmont, and Veneto.

**Figure 3**

Hepatitis A cases by week of symptom onset, provinces of Trento and Bolzano, Italy, January–May 2013 (n=38)




Source: Sentinel surveillance system for acute viral hepatitis (Sistema Epidemiologico Integrato Epatiti Virali Acute - SEIEVA)

from the province of Trento; however, this case had been vaccinated with one dose within the three weeks before the onset of symptoms, so this was not considered a vaccine failure. Preliminary epidemiological investigation for the identification of risk factors and common exposures focused on consumption of contaminated food as no epidemiological link between the cases could be confirmed. The only common food consumed by all cases was mixed berries or food containing mixed berries (cakes).

Serum samples were collected during the acute phase of the disease from five of the 38 cases, all from the Trento province. The sequence of the VP1/2A region of the HAV 1A virus obtained from all of them (with GenBank accession number KF182323) showed a 100% nucleotides homology with sequences of the isolates from the German and Dutch cases.

## Investigation of food items implicated

The preliminary epidemiological investigation in the provinces of Trento and Bolzano showed that the only common food consumed by different cases was mixed berries or food containing mixed berries (cakes). Moreover, the hypothesis was strongly supported by the results of an epidemiological investigation conducted in a family cluster in Veneto region. Part of the mixed berries (redcurrant, blackberries, raspberries, blueberries) that the cases indicated to have eaten within the period of time compatible with the onset of clinical symptoms were still available and were sampled. The analysis for HAV detection in the sample of mixed berries provided positive results. As a consequence, on 17 May, the Italian Ministry of Health (which is the food safety authority at national level) communicated these findings through the European Rapid Alert System for Food and Feed (RASFF). Following these preliminary positive results, the surveillance of these food items was intensified. More samples of berries were collected throughout the country once they were identified as potential risk factors, and two sampled berries in Trento were found positive for HAV. On 30 May, two additional RASFF notifications were issued to inform about new HAV findings in frozen mixed berries from Italy. Environmental investigations have been done on the mixed frozen berries suppliers of raw material in six different countries. Results on samples collected are pending at the time of the present rapid communication.

## Control measures

On 23 May the Ministry of Health (the General Direction for Prevention together with the food safety authority) published a note for RHAs in order to enhance surveillance and awareness of HAV recommending to report within 24 hours any new HAV cases, to collect

additional epidemiological information on risk factors associated, and perform virus genotyping and sequencing from all new cases. In addition to the recommendation mentioned above, a case-control study in the regions that experienced the highest increase of cases was planned, in order to support the hypothesis of berries as source of infection, to find other potential risk factors and to identify appropriate control measures. The National Institute of Health (Istituto Superiore di Sanità, ISS) is responsible of the coordination of the virological and epidemiological investigations, and of the case-control study.

Moreover, after the positive results on the sampled frozen mixed berries from different regions, the Ministry of Health started the tracing back this food item. The investigation identified a dealer that received consignments of berries from different countries (mix made in Italy, with raw material from Bulgaria, Canada, Poland, and Serbia).

Following the RASFF notification from the Ministry of Health, regions recalled the lots that were identified positive for HAV and advised the population through the website of the Ministry of Health regarding the use of the leftover frozen mixed berries. Trace back investigations on food are ongoing for each new case notified.

The European Centre for Disease Prevention and Control (ECDC) performed a rapid risk assessment that was published on 16 April 2013 [8].

## Discussion

Preliminary analysis of the case interviews on possible risk factors associated with the ongoing outbreak identified consumption of frozen mixed berries (redcurrant, blackberries, raspberries, blueberries) as potential vehicle of infection. The hypothesis that they could be implicated is strongly supported by the detection of HAV virus in a sample of frozen mixed berries. The surveillance on these frozen mixed berries together with other food items potentially carrying the HAV (vegetables, seafood, and other food reported as potential risk factors by cases in the epidemiological investigation), has been intensified, to provide a clear picture of the distribution of the contaminated items and the risk of exposure through these.

The case-control study is currently ongoing; the results of this investigation will provide an opportunity to support the hypothesis of the likely source of infection and together with the molecular sequencing information will provide a picture of the genotypes in this outbreak to be compared to those circulating in the previous years in Italy and to those that are currently circulating in other countries [9-12].

Comparison of information from the tracing-back of positive frozen mixed berries with information obtained through the purchase history is ongoing.

Seven sequences of HAV genotype 1A isolated from cases in different countries (the Netherlands, Germany and Italy) and in different laboratories showed a 100% similarity. The genotype and the sequence of the virus isolated in the Italian outbreak is different from the currently ongoing outbreak with frozen berries as suspected vehicle described in northern European countries and in the United States [3,12,13].

Despite the great efforts made in the detection of positive food consignments and recall of those suspected or found positive, more cases are expected in the next weeks, due to the long incubation period of HAV (28–30 days; range: 15–50 days) [14], the notification delay, and the long shelf life that frozen berries have.

The Italian public health authorities are collaborating closely in order to confirm the source of the outbreak and to stop further transmission.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### Conflict of interest

None declared.

### Authors' contributions

CR contributed to the descriptive study and drafted the manuscript as the lead writer. MET contributed with data on Italian cases. VA and MCMR gathered data from the different regions implicated and to the descriptive study. DDM, LB, ME, SDP, GS, contributed to describe the investigation on food items implicated and commented on the manuscript. AC, RB, ME, ST contributed to the laboratory sequencing and analysis and commented on the manuscript. VC and SF contributed with data on Trento province cases. BN and MA contributed with data on Bolzano province cases.

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# Joint analysis by the Nordic countries of a hepatitis A outbreak, October 2012 to June 2013: frozen strawberries suspected

**Nordic outbreak investigation team**[1]
1. The members of the group are listed at the end of the article

**Citation style for this article:**
Nordic outbreak investigation team. Joint analysis by the Nordic countries of a hepatitis A outbreak, October 2012 to June 2013: frozen strawberries suspected. Euro Surveill. 2013;18(27):pii=20520. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20520

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**The Nordic countries faced a food-borne outbreak of hepatitis A that started in October 2012 and was ongoing with 103 reported cases as of 27 June 2013. A case–control study in Denmark, Finland, Norway and Sweden, combined with trace-back investigations, has identified frozen strawberries as the likely cause of the outbreak. The origin of the berries is still being investigated.**

Hepatitis A seroprevalence is under 10% in Nordic countries [1] where endemicity is very low [2]. In February 2013, Denmark noticed an increase in the number of notified hepatitis A virus (HAV) infections among individuals with no travel history. On 1 March 2013, following an urgent enquiry posted through the European Epidemic Intelligence Information System for food- and waterborne diseases (EPIS-FWD), Finland, Norway and Sweden reported a similar increase and identified cases that were infected with the same IB genotype and sequence (KC876797) as the Danish cases, as well as cases with closely related sequences [3]. In March 2013, a case–control study conducted in Denmark identified frozen berries, particularly frozen strawberries, as the likely vehicle of the outbreak, but could not exclude other frozen berries [3]. As a result of this finding, the four Nordic countries recommended boiling all frozen berries before consumption [3].

While public health institutes in the four country coordinated their data collection methods to pool the analysis of the country-specific case–control studies to identify the vehicle of the outbreak more precisely, investigators compared the outbreak strains with the HAV network (HAVNET) database [4] to gain information on the probable phylogenetic origin of the outbreak strains, and food agencies analysed product distribution and tested fruit specimens.

## Methods

### Case definitions

A *probable* case was defined as a person living in Denmark, Finland, Norway or Sweden who developed clinical illness compatible with HAV infection on or after* 1 October 2012 (1 December 2012 for Sweden) and was positive for IgM antibodies against HAV. We excluded cases who (i) reported travel outside of Western European countries two to six weeks before onset of symptoms, (ii) were living in the same household as a patient with HAV infection typed with a genotype or sequence not belonging to the outbreak and (iii) reported other risk factors for hepatitis A exposure including injection drug use, homelessness or male-to-male sexual contact (the exposures under (iii) were not ascertained in Swedish and Finnish cases).

A *confirmed* case was defined as a probable case infected with HAV genotype 1B with the sequence identified by GenBank number KC876797 (hereafter called sequence 1) or a sequence that differed by no more than 2% from sequence KC876797 [3], and was isolated in at least two of the four affected countries.

A *secondary* case was defined as a probable or confirmed case with symptom onset two to six weeks after close contact with a primary probable or confirmed case.

### Descriptive epidemiology

We described the distribution of cases by age, sex, country of residence, disease status (confirmed, probable, secondary) and HAV sequence.

### Case–control studies

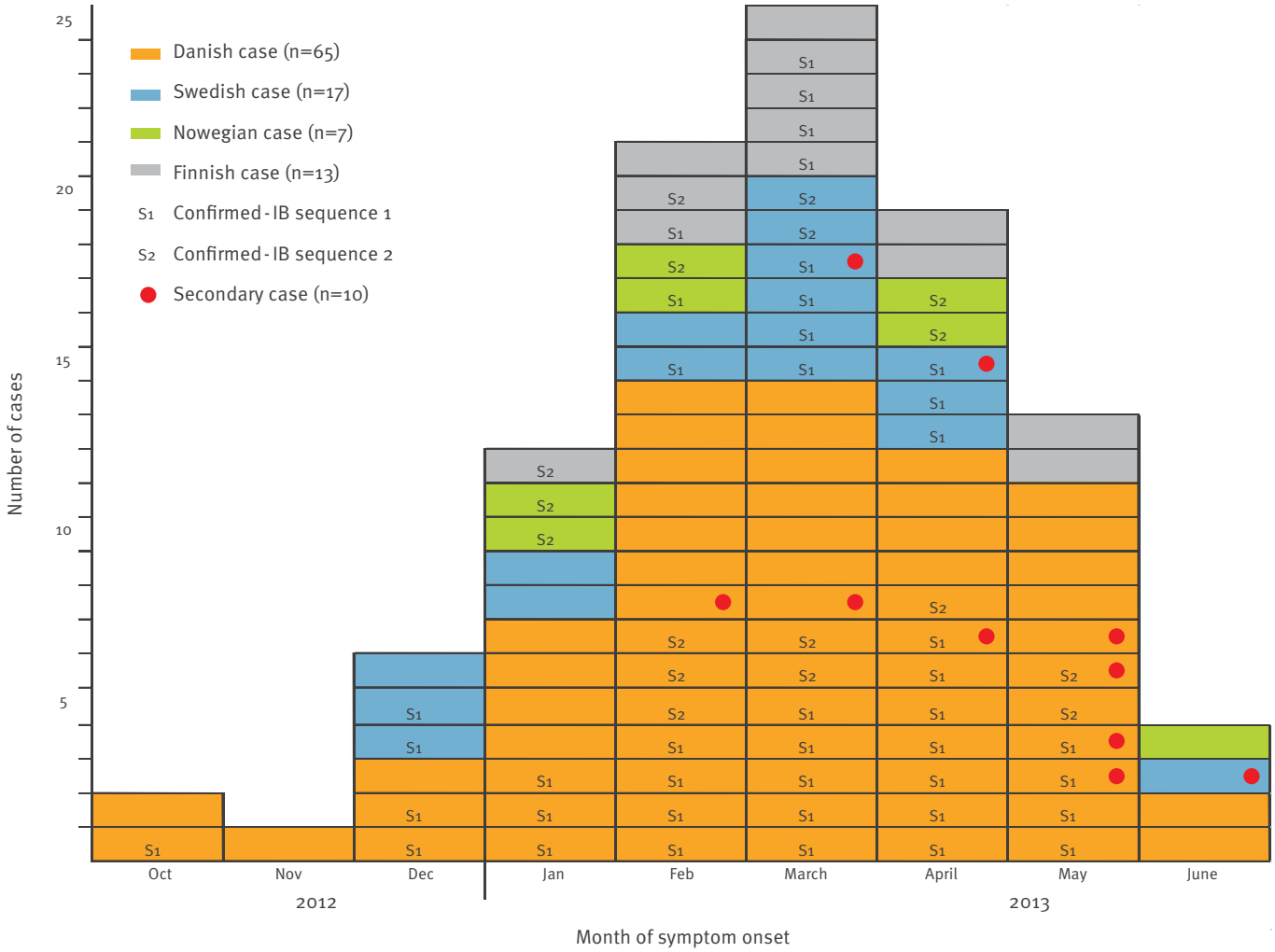
Each country conducted a matched case–control study based on the Danish case–control study protocol, modified according to findings from their own trawling questionnaires. Early and regular communication by email and teleconferences, as well as sharing of study plans and questionnaires, ensured that data could be used for joint analysis.

#### Control selection and invitation

Each country randomly selected controls using national population registries, matched on age, sex and place of residence (municipality in Denmark, Norway and

**Figure 1**

Distribution of hepatitis A cases over time, Denmark, Finland, Norway and Sweden, October 2012–June 2013 (n=103)




Finland; county in Sweden). Controls were excluded if they were vaccinated against HAV, reported a previous HAV infection, or if they had been travelling for more than two weeks in western Europe or any length of time outside of western Europe in the six weeks prior to recruitment.

## Data collection

In Denmark, investigators telephoned potential controls until two controls per case had been recruited. In Norway, the same procedure was followed for two to three controls per case. Sweden invited six controls per case by post and Finland invited 30 controls per case, by phone. The latter two countries included all controls who accepted the invitation.

Cases and controls were asked about the consumption of a range of food items, including berries, during the six weeks before onset of illness (cases) or recruitment (controls). Norway asked controls about exposures during the period corresponding to the exposure period of the cases (January to February 2013).

## Data analysis

The pooled analysis regrouped primary confirmed cases included in the national studies with at least one matched control (Denmark, Norway, Sweden) or before 24 May (Finland). The strength of the association between HAV infection and consumption of food items present in at least three country questionnaires was estimated using matched odds ratio (mOR) and 95% confidence intervals (CI) using conditional logistic regression. Statistically significant exposures at the alpha=0.05 level were fitted in a multivariable conditional logistic regression model to adjust for confounding. We stratified the analysis by HAV sequence isolated in cases [3] and compared cases with HAV sequence 1 and cases with HAV sequence 2 in terms of consumption of berries using Fisher's exact test.

**Table**

Frequency of selected exposures among confirmed hepatitis A cases (n=26) and controls (n=56) in a matched analysis by the Nordic countries[a] and an unmatched analysis in Sweden, October 2012–June 2013

| Exposure | Multicountry investigation | | | | | | | | | | National investigation | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cases | | | Controls | | | Crude mOR | 95% CI | Adjusted mOR | 95% CI | Sweden[b] | |
| | Total | Exposed | % | Total | Exposed | % | | | | | Odds Ratio | 95% CI |
| Frozen strawberries | 26 | 22 | 84.6 | 53 | 19 | 35.9 | 8.8 | 2.5- 30.5 | 11.4 | 1.9-69.9 | 24.5 | 1.9-1179.7 |
| Frozen raspberries | 25 | 16 | 64.0 | 54 | 16 | 29.6 | 7.3 | 2.1-26.0 | 5.1 | 0.9-26.4 | 2.1 | 0.3-18.4 |
| Berries in smoothie | 21 | 15 | 71.4 | 44 | 13 | 29.5 | 8.3 | 1.8-37.8 | - | - | 6.7 | 0.7-69.7 |
| Frozen mixed berries | 25 | 8 | 32.0 | 51 | 5 | 9.8 | 10.6 | 1.3-86.4 | 11.4 | 0.9-132.7 | 0.8 | 0.0-13.2 |
| Berries in other forms | 19 | 9 | 47.4 | 45 | 8 | 17.8 | 2.4 | 0.5-7.2 | - | - | 2.7 | 0.3-23.7 |
| Other frozen berries | 21 | 6 | 28.6 | 41 | 5 | 12.2 | 3.6 | 0.9-15.1 | - | - | 1.5 | 0.0-34.5 |
| Frozen blueberries | 24 | 11 | 45.8 | 53 | 15 | 28.3 | 2.6 | 0.9-7.6 | - | - | 0.7 | 0.0-9.9 |
| Berries in desert | 22 | 11 | 50.0 | 45 | 16 | 35.6 | 3.7 | 0.9-14.5 | - | - | 1 | 0.1-7.3 |
| Nuts | 13 | 10 | 76.9 | 34 | 25 | 73.5 | 1.6 | 0.3-9.4 | - | - | 0.85 | 0.1-7.7 |
| Grapes | 13 | 11 | 84.6 | 33 | 27 | 81.8 | 1.8 | 0.3-10.1 | - | - | 3.5 | 0.3-188.8 |

CI: confidence interval; mOR: matched odds ratio.

[a] Denmark, Finland, Norway, Sweden.

[b] Unmatched study, eight cases (confirmed), 18 controls.

The Swedish case–control study (including confirmed cases only) was also analysed individually. As controls could not be recruited for every case, the match was broken (after checking that matched and unmatched ORs were of the same magnitude) and odds ratios (OR) with 95% CI were calculated using logistic regression, adjusting for age.

## Identification of strain origin

Laboratory confirmation of the cases has been described previously [3]. To look for indications of the geographical origin of the outbreak strains, we analysed the phylogeny using the HAVNET database that contains sequence data of viruses from patients from non-endemic countries, many of whom contracted the infection in a foreign country (67% of sequences in HAVNET are from isolates from Dutch patients). A total of 442 nucleotides in the VP1-P2A region and 466 nucleotides in the VP1 region of the genome were analysed separately. The probable origin of the cluster of sequences including the outbreak strains was ascertained as previously described [5].

## Product distribution analysis

In Norway and Denmark sales receipts obtained from the cases were used to ascertain the types and brands of berries purchased before symptom onset. Supermarkets chains assisted in tracing suppliers and countries of origin of the berries.

Danish, Finnish, Norwegian and Swedish, national food authorities collected soft fruit specimens (berries and mango) from confirmed cases' freezers and from shops selling suspected batches. Denmark, Norway and Sweden analysed the specimens applying the same standardised HAV detection method [6] based on reverse transcriptase polymerase chain reaction (RT-PCR). In addition, Denmark and Norway followed a protocol specifically developed for soft fruits [7]. Specimens from Finland were tested in Denmark.

## Results

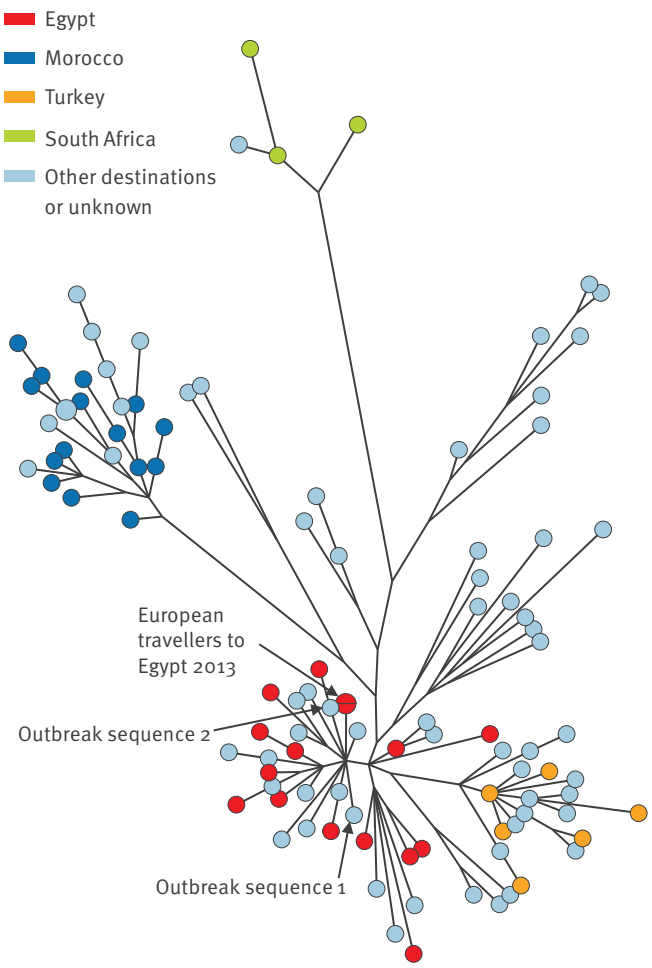
As of 27 June 2013, 103 cases (59 confirmed, 34 probable and 10 secondary) were reported, 66 in Denmark, 17 in Sweden, 13 in Finland and seven in Norway (Figure 1). The age range was 4–76 years (median: 24 years) and 61% were female. No cases with the outbreak sequences were excluded because of other hepatitis A risk factors. The most recent case (as of June 27) was reported in Norway (illness onset 14 June 2013). Sequence 1) was isolated from 42 of 59 confirmed cases, whereas a second sequence with 1.7% difference to sequence 1 over 847 bp (GenBank accession number KC876799, hereafter called sequence 2) was isolated from 17 of 59 cases.

## Case–control studies

After excluding cases that did not have a matched control, the multicountry analysis included 26 confirmed cases (Denmark: 12, Sweden: 6, Norway: 4, Finland:

**Figure 2**

Comparison of hepatitis A virus sequences included in the HAVNET database with the two sequences involved in the outbreak in the Nordic countries[a], October 2012–June 2013




[a] Denmark, Finland, Norway, Sweden.

Maximum parsimony tree of 442 nucleotides of the VP1/2A junction region of hepatitis A virus type IB, using 253 sequences deposited in the Dutch HAVNET database. This database contains 67% Dutch travellers. The two sequences involved in the outbreak in the Nordic countries are indicated by arrows

4) and 56 controls, with one to five controls per case. In the univariable analysis, eating frozen strawberries (mOR 8.8; 95% CI 2.5–30), frozen raspberries (mOR 7.3; 95% CI 2.1–26), berry-containing smoothies (mOR 8.3; 95% CI 1.8–38) and frozen mixed berries (mOR 11; 95% CI 1.3–86, Table) was associated with being a case. Of the 26 confirmed cases, 22 reported eating frozen strawberries, 16 reported eating frozen raspberries, and eight reported eating frozen mixed berries. When including strawberries, raspberries and mixed berries in a multivariable model, only strawberries remained significantly associated with being a confirmed case (mOR 11.4; 95% CI 1.9–70, Table). When restricting the analysis to cases with sequence 1, eating strawberries and raspberries were both associated with HAV infection (crude mOR 6.1; 95% CI 1.7–22 and mOR 5.6; 95% CI 1.1–27, respectively). However, only eating strawberries was associated with being a confirmed case in multivariable analysis (mOR 5.8; 95% CI 1.2–27 for strawberries, mOR 5.3; 95% CI 0.77–36 for raspberries). When restricting the analysis to cases with sequence 2, eating frozen raspberries was also associated with being a confirmed case (crude mOR 11; 95% CI 1.3–92). Since all sequence 2 cases consumed frozen strawberries, an mOR for frozen strawberries could not be calculated, but the association was statistically significant. Cases with sequence 1 were as likely to have eaten strawberries and raspberries as cases with sequence 2 (Fisher's exact test: p=0.54 and p=0.35, respectively).

The Swedish case–control study included eight confirmed cases and 18 controls. Frozen strawberries were the only exposure significantly associated with HAV infection (crude OR: 24; 95% CI 1.9–1,200) (Table), which remained significant after adjusting for age (OR: 82; 95% CI 1.7–3,929).

### Identification of strain origin

The most frequently represented countries of infection included in HAVNET were Egypt (n=30), Morocco (n=21) and Turkey (n=15). All samples were from travellers. A comparison of the two investigated genomic regions indicated that the outbreak strains were associated with strains commonly isolated in travellers infected in Egypt (p<0.001, Figure 2). Sequence 1 and 2 differed by 1.22% over 1,233 bp and by 1.26 % over 397 bp, respectively, from the strain causing a concurrent outbreak in travellers returning from Egypt [4].

### Product investigation analysis

As of 27 June, 54 soft fruit specimens (17 from Denmark, 14 from Finland, 11 from Sweden, 12 from Norway) were tested, 23 of which were strawberries. HAV was not detected in any of the specimens.

Trace-back analysis was ongoing as of 27 June 2013, pointing at strawberries from several countries.

## Public health actions

On 22 May 2013 Public Health and Food agencies in Denmark, Finland and Norway issued statements identifying strawberries as the likely vehicle of the outbreak, but maintained previous recommendations to boil all frozen berries before consumption due to the potential implication of other berries. Sweden restricted the boiling notice to strawberries only. On 30 May 2013, one supermarket chain in Denmark, Norway and Sweden voluntarily recalled frozen strawberries from Egypt and Morocco packed in Belgium [8,9].

## Discussion

After pooling data from the four affected countries, consumption of frozen strawberries, frozen raspberries and mixed frozen berries (which can contain both strawberries and raspberries) were significantly associated with being a confirmed case. Although the pooled analysis was restricted to earlier confirmed cases, this is unlikely to have introduced bias since there was no reason to believe later cases were different from earlier ones. Hepatitis A outbreaks have been previously linked to both strawberries [10,11] and raspberries [12,13], and frozen mixed berries are suspected in two hepatitis A outbreaks that were ongoing as of 27 June 2013 in Italy and the United States (US) [14,15]. Frozen strawberries were most strongly associated with being a confirmed case and were the product most commonly eaten by cases. Additional elements pointed to the strawberries as the vehicle of the outbreak: Firstly, strawberries were most strongly associated with being a case in the Swedish national case–control study as well as national case–control studies in Denmark and Norway [3]. Secondly, strawberries were the only exposure significantly associated with being a confirmed case in the multicountry multivariable analysis. Thirdly, the preliminary food trace-back investigations pointed towards strawberries. Finally, when restricting the analysis to individuals who did not consume raspberries, the association between being a confirmed case and frozen strawberries remained significant.

The strength of association between raspberries and being a confirmed case in the multicountry analysis was weaker than for strawberries. In addition, raspberries were not associated with illness in the Swedish study. Restricting the analysis to people who had not eaten strawberries was difficult because only four cases did not eat strawberries. Trace-back analysis found no evidence to implicate raspberries, but based on the epidemiological analyses, we cannot completely exclude that raspberries or frozen mixed berries may have played a role in the outbreak.

The outbreak included two distinct (less than 2% different) HAV sequences that were found in the four countries during the outbreak period. There was no evidence that one outbreak strain was more strongly associated with one vehicle than the other. The HAV mutation rate is low [16], suggesting the two sequences identified in the outbreak represented distinct strains rather than a sporadic mutation. Such multi-strain food-borne hepatitis A outbreaks have been reported previously [17,18]. At this stage we do not know where in the production chain or in which country the contamination occurred. While the comparison of strains in the HAVNET database indicated an association with strains from travellers infected in Egypt, we cannot exclude that these strains also circulate in other countries. As HAV is not genotyped routinely, the known genetic diversity is biased towards more densely sampled regions. Finally, the trace-back investigation has not yet pointed to a single country. The origin of the contaminated strawberries or the point of contamination can therefore not be identified at this moment.

This outbreak occurred in the context of several hepatitis A outbreaks affecting Europe and EU residents [4,14,19] as well as another genotype 1B outbreak in the US related to frozen mixed berries [15]. The US and Nordic HAV strains are both genotype 1B and originate from the same geographic region but there is no evidence so far they are related [15].

HAV has as yet not been detected in the tested berries. Possible reasons could be that the HAV concentration in the samples collected may have been below the detection limit, or that other berries from the same batch contained HAV.

In conclusion, during this outbreak, combined evidence from case–control studies and the food trace-back contributed to implicate frozen strawberries as the source of the outbreak, leading one supermarket chain voluntary recall this product. Investigations of the source will continue in order to identify the producer and batch and to test berries in the laboratory. In view of the long incubation period of hepatitis A [20] and of notification delay, more cases can be expected to occur for at least another few months, and possibly even later, despite interventions, as frozen berries can be stored in freezers for up to two years.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## Conflict of interest

None declared.

## Authors' contributions

ME contributed to the study design, collection and analysis of data for the joint case–control study, led the Swedish case–control study and drafted the manuscript as the lead writer. SGL contributed to the study design and was in charge of the joint descriptive epidemiology.BS contributed to the study design and led the Danish case–control study.SEM and HTV contributed to the laboratory sequencing and analysis in Denmark. TKF contributed to interpretation of the virological data in Denmark.TJ was in charge of the trace-back in Denmark and drafted the trace-back section of the manuscript.KM and SE contributed to the design of the Danish case–control study and to the epidemiological investigations. AS contributed to the study design. AS and LV were in charge of the epidemiological investigations including the case–control study in Norway. LJ was in charge of the trace-back investigation of in Norway, with assistance from ML and TS. KSJ was in charge of the laboratory typing and analysis in Norway. RRF was in charge of the epidemiological investigations in Finland.MK was in charge of the laboratory typing and analysis in Finland. HL was in charge of the trace-back in Finland. ML were in charge of the epidemiological investigations in Sweden. LS was in charge of the laboratory typing and analysis in Sweden.MS was in charge of the trace back in Sweden. LV, HV and MK were in charge of the phylogenic analysis. SGL, KM, BS, TKF, TJ, AS, LV, LJ, RRF, HL, ST, ML, LS, AW, MS, LV, MK reviewed the first draft of the paper. All authors approved the final version.

## *Authors' correction:

On request of the authors, the probable case definition was corrected from "after 1 October 2012" to "on or after 1 October 2012". This change was made on 4 July, 20:15.

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# Pet rat harbouring Seoul hantavirus in Sweden, June 2013

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**We report the first detection of Seoul hantavirus (SEOV) in a pet rat in Sweden. SEOV-specific antibodies were detected in the pet rat blood by focus reduction neutralising test (FRNT), and SEOV RNA in lung tissue was confirmed by reverse transcription-nested polymerase chain reaction (RT-PCR) followed by sequencing. The discovery follows the recent reports of SEOV infected pet rats, as well as associated human cases of severe haemorrhagic fever with renal syndrome (HFRS), in England and Wales.**

In June 2013, Seoul hantavirus (SEOV) was detected for the first time in a pet rat in Sweden. The rat had been imported to Sweden from England in 2011. During the winter 2012/13, the presence of SEOV, both in wild- and in pet rats, as well as associated severe human cases of haemorrhagic fever with renal syndrome (HFRS), were reported in England and Wales [1,2]. In Sweden, this raised concerns among owners of pet rats imported from the United Kingdom (UK) that such animals may be infected. During late spring, three rat owners initially came forward to have their pet rats tested by the National Veterinary Institute (SVA), and among the three respective rats tested, one was found to be SEOV infected.

## Background

Hantaviruses (family *Bunyaviridae*) are three-segmented, negative-stranded RNA viruses transmitted by rodents, insectivores and bats. Over 40 hantavirus species, or potential species, are currently known, and most of them are restrained to a single reservoir host species. Several hantaviruses are human pathogens (notably all those associated with rodents), causing up to 50,000 disease cases annually worldwide [3 and references therein].

The bank vole *Myodes glareolus* is the reservoir of Puumala hantavirus (PUUV), which causes nephropathia epidemica (NE), a milder form of HFRS that accounts for the majority of hantavirus-related disease incidence in Europe. Other European hantavirus pathogens causing HFRS, are Dobrava (DOBV) and Saaremaa (SAAV) viruses, carried respectively by mice of the species *Apodemus flavicollis* and *A. agrarius* [3-6]. These are the species listed by the International Committee on Taxonomy of Viruses, but the nomenclature of the European *Apodemus*-derived hantaviruses has been and still is, under debate and revision: in the literature DOBVvariants in *A. flavicollis* are also referred to as DOBV-Af, and variants in *A. ponticus* as DOBV-Ap. Some strains recovered from *A. agrarius* are described as a genotype DOBV-Aa [6].

As hantaviruses are strictly associated with their rodent, bat or insectivore hosts, the distribution of hantaviruses is limited to that of their respective host species. SEOV is an exceptional hantavirus in that it has a global distribution due to the worldwide dispersal of its carrier host, the rat of the species *Rattus norvegicus*, mainly through global trade. Although rats are unaffected by the virus, SEOV causes a more severe form of HFRS in humans (mortality approximately 2%), and this has been observed mainly in Asia [6]. Outside of Asia, SEOV has been found, by molecular methods, in rats in the Americas [7] and in Europe (Belgium, France and the UK) [8-11]. During the winter 2012/13, human cases of severe HFRS caused by SEOV were reported in the UK and France [1,2,11]. In the UK, some of the SEOV cases were associated with pet rats [2]. This report describes the first finding of SEOV in a pet rat in Sweden.

## Virus investigation

The sampling of the pet rats' blood and organs (e.g. lungs, kidneys, liver) was conducted at the SVA. Approximately 500 mg of the rat lungs were cut to smaller pieces and vortexed with 1 ml of PBS. The lung tissue samples were centrifuged for 1 min and the supernatant diluted 1:5 and mixed 1:1 with virus dilution resulting in a final concentration of 1:10 (corresponding, based on earlier comparisons of serum

antibodies and antibodies extracted from rodent lung tissue in our laboratory, to a serum concentration of approximately 1:200) for the initial screening.

Focus reaction neutralisation test (FRNT), the gold standard for typing of hantavirus antibody responses, was performed as described earlier [12]. An 80% reduction of the number of foci, as compared to the virus control, was used as the criterion for virus neutralisation titres.

The initial screening clearly showed the presence of SEOV-specific neutralising antibodies in a lung tissue sample (number 1466) from one of the three rats investigated. When titrating the supernatant, the result was repeated with a complete neutralisation at the 1:10 dilution. The corresponding serum dilution of the sample from the lung is hard to define, but we estimate it to correspond to a serum dilution of at least 1:200.

220 mg of lung tissue from the FRNT-positive rat was homogenised with 2,5 ml of Trizol (Life technologies) and RNA was extracted according to manufacturer´s instructions.

Reverse transcription (RT)-nested polymerase chain reaction (PCR) was performed on the purified RNA of the pet rat as described before [10]. Partial sequence of the hantavirus L segment (nt 2,968 to 3,300) was targeted by the PCR. The obtained sequence was analysed by sequence alignment to other hantavirus sequences available from GenBank and by phylogenetic analysis. Multiple sequence alignment was conducted with the SeqApp 1.9a169 programme. Phylogeny was inferred using the Phylogeny Inference Package (PHYLIP) programme [13]. Five-hundred bootstrap datasets were generated using the 'Seqboot' programme. Genetic distances were calculated using the 'Dnadist' programme under the maximum likelihood model for nucleotide substitutions and the resulted distant matrices were analysed with the neighbour joining (NJ) tree-fitting algorithm ('Neighbour' programme). The bootstrap support values were calculated with the 'Consense' programme.

The analyses showed that the sequence was indeed derived from a SEOV species and the newly detected strain was designated SEOV/Sweden/Rn1466/2013, or Sweden1466 for short. The Swedish strain was found most closely related to SEOV strains from Indonesia (1 substitution, sequence identity of 99.7%) and Belgium (9 substitutions, sequence identity of 97.3%). All nucleotide substitutions were silent, i.e. the deduced partial amino acid sequences of Swedish, Indonesian and Belgian strains were 100% identical. On the phylogenetic tree (Figure) these three strains formed SEOV genetic lineage number 7 which was clearly distinct from lineages formed by strains from the UK (Humber), South Korea (80-39) and China (L99, Z37, ZT10, ZT71). The strain from Sweden differed from the UK strain Humber by 23 point mutations, all silent.

The pet rat diagnosed with SEOV was a male which had been imported to Sweden for breeding purposes in 2011 and had a proven pedigree. It had come from England together with 19 other pet rats. All 20 pet rats had been grouped together for transport reasons, but had different backgrounds in the UK, and were separated when they were delivered to different families in Sweden. Because they had been in close contact with the infected rat, the 19 co-imported pet rats from England to Sweden were delivered to the SVA and are in the process of being tested, as they are suspect for SEOV infection.

## Control measures

Transmission of hantaviruses to humans most frequently occurs through breathing of aerosols of virus-contaminated rodent excreta [6]. Large quantities of infectious virus are excreted in the urine, saliva and faeces of the infected rodents. Recent results on PUUV have shown; (i) that the secreted virus is surprisingly stable, and thereby infectious over long time-periods outside the rodent host [14], and (ii) the virus is secreted for several months, or even years, after the infection [15; data not shown].

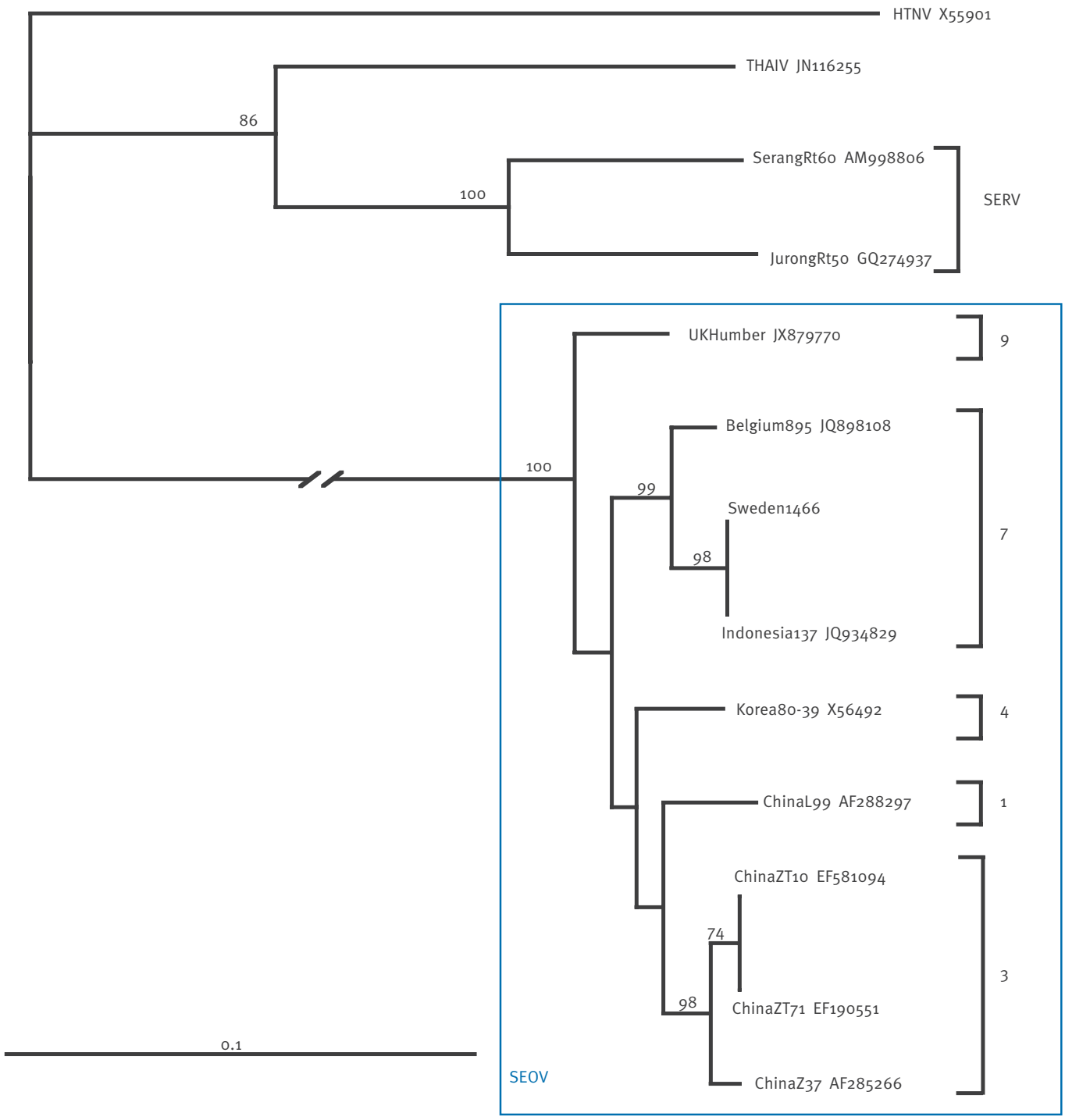
The unique finding in this study of a strain of SEOV in a Swedish pet rat, rather than in wild rats, poses a challenge for infection control and will involve a multi-disciplinary panel including medical/science experts from e.g. the Swedish Institute for Communicable Diseases (SMI), the SVA, the National Board of Health and Welfare (SoS) and the Swedish Board of Agriculture. At this time, the owner of the SEOV-infected rat is being offered clinical follow-up and all the concerned pet handlers both in Sweden and the UK have been informed of the finding and offered advice. Human samples as well as more rats are in the process of being tested. Interim guidance on minimising the infection to the pet rat community has earlier been published in the UK (http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/Hantaviruses/) and in Sweden (www.sva.se), and will continue to be updated as the investigations progress. Recommendations for managements of any future finding of infection in a pet rat would be made on a case-by-case basis. Further studies are planned to collect evidence on the prevalence of this virus in the pet rat community, as well as in wild rats in selected geographical areas of Sweden (e.g. international harbours where rats originating from different geographical areas may be found), which will inform future risk assessment and the provision of appropriate public health guidance.

## Discussion and conclusions

In December 2012, our colleagues in the UK reported the first discovery of SEOV in wild *Rattus norvegicus* that were associated to a severe case of HFRS in the UK [1]. In February 2013, pet rats as a source of SEOV, and also associated severe human disease, were reported from England and Wales [2].

## Figure

Phylogenetic analysis of a sequence derived from a pet-rat suspected of hantavirus infection, Sweden, June 2013




HTNV: Hantaan virus, strain 76-118; SEOV: Seoul virus; THAIV: Thailand virus, strain ThaiR5370; SERV: Serang virus.

The phylogenetic tree is based on partial L segment sequences (nt 2,968 to 3,300) of hantaviruses. GenBank accession numbers of the sequences figure on the tree. The HTNV sequence was used as an outgroup. Bootstrap support values greater than 70% are shown at the nodes. SerangRt60 and JurongRt50 sequences were used as representative strains of SERV. Belgium895, Indonesia137, UKHumber, Korea80-39, ChinaL99, ChinaZ37, ChinaZT71, and ChinaZT10 sequences were strains of SEOV. For sequences or clusters of sequences belonging to the SEOV, the corresponding lineage number is indicated to the side of the phylogenetic tree.

We now report the first SEOV variant ever found in Sweden, similar, but genetically distinct from the available SEOV strains reported to date. At this moment, we cannot tell if the rat had been infected in the UK, or later in Sweden. Further investigations will reveal the relationship of various SEOV strains in Europe, and how they are associated to pet rat and wild rat populations. So far, representatives of two genetic lineages of SEOV have been found in Europe: lineage number 9 (including strain Humber from the UK) and lineage number 7 (including the strain from Sweden reported here as well as strains from Belgium and most likely also strains from France, since Belgian and French strains are very close in their S and M segment sequences [10]).

Currently, the prevalence of SEOV in the Swedish pet rat community is unknown, but further investigations have been planned. Should overall findings indicate that further health protection advice is necessary, SMI and SVA will work with the relevant partners to provide this. At this time there is no evidence of human (HFRS type) disease associated with pet rats in Sweden.

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## Conflict of interest

None declared.

## Authors' contributions

Åke Lundkvist – Planning and responsible for the study, writing of the manuscript. Jenny Verner-Carlsson – Performed the serological analyses. Angelina Plyusnina – Performed the molecular analyses. Linda Forslund – Performed the animal sampling. Ricardo Feinstein – performed the animal sampling. Alexander Plyusnin – Responsible for the molecular analyses and interpretation. All co-authors reviewed the manuscript.

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# Distribution of *Salmonella enterica* isolates from human cases in Italy, 1980 to 2011

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We describe trends of *Salmonella enterica* serovars isolated from humans in Italy from January 1980 to December 2011. A total of 229,279 *Salmonella* isolates were reported during this period. Serovars Enteritidis, Typhimurium, Infantis, Derby, 4,[5],12,:i:-, and Napoli accounted for 135,783 (59%) of these isolates. Temporal trends from 2000 to 2011 varied by serovar: Enteritidis and Infantis decreased significantly (with a mean of -3.0% and -2.8% isolates per year, respectively, p<0.001); Typhimurium remained stable; while 4,[5],12:i:-, Derby and Napoli increased significantly (+66.4%, p<0.001; +8.1%, p<0.001; and +28.2%, p<0.05, respectively). Since 2000, Enteritidis fell consistently below Typhimurium, which is the most reported serovar in Italy in contrast to the international situation where Enteritidis still ranks at the top despite its significant decrease. Most serovars showed a marked seasonality, increasing over the summer months and peaking in August/September. Typhimurium, 4,[5],12:i:-, and Napoli were most likely to be isolated from children, whereas Enteritidis, Derby, and Infantis from adults. We conclude that the applied control measures are not equally efficient against the considered *Salmonella* serovars and that sources of infection other than those of Enteritidis (laying hens and eggs) have become increasingly important. Further investigations on the emerging serovars and on the causes related to their emergence are needed to define and implement newly tailored control measures.

## Introduction

In the European Union (EU), *Salmonella* infection is the primary cause of confirmed foodborne outbreaks and the second most reported zoonosis, behind *Campylobacter* infection [1]. Recently it has been estimated that approximately 6.2 million cases of human salmonellosis occur in the EU general population each year, 298,000 of which in Italy (approximately 60 million population) [2].

More than 2,500 serovars of *Salmonella enterica* have been described [3]. Although virtually all these serovars are capable of infecting humans, most human infections are caused by a limited number of serovars. *S.* Enteritidis and *S.* Typhimurium are among the serovars most frequently associated with human illness in the EU, accounting for up to 68% of confirmed human cases identified at serovar level [1]. Poultry, and particularly laying hens for table egg production, have long been identified as the primary source of human *S.* Enteritidis infection, whereas it is widely accepted that human *S.* Typhimurium infection primarily originates from pigs [4].

*Salmonella* serotyping is an important tool for surveillance purposes that allows for trends to be monitored over space and time. Serotyping is also a useful classification scheme to support the investigation of foodborne outbreaks and the attribution of human cases to different sources of infection and routes of transmission [4].

In Italy, the laboratory-based surveillance system for human *Salmonella* infections has changed substantially over time to follow the evolution of the surveillance activities for infectious diseases undertaken at the national and international level [5]. The former system was created in 1967 and was based on the Reference Centres for Enterobacteriaceae (RCE) [5,6], which became part of the European *Salmonella* Network (SALM-NET) project in 1992 [5]. In 1997, SALM-NET was further changed into the Enteric Pathogen Network (ENTER-NET) [7]. Italy's ENTER-NET (IT-ENTER-NET) is a passive, laboratory-based surveillance system for enteropathogens based on a network of more than 140 clinical microbiology diagnostic laboratories covering about 65% of the Italian territory and is complementary to the Italian National Surveillance System for Infectious Diseases (SIMI) [8,9]. Since October 2007, the European ENTER-NET has been coordinated by the European Centre for Disease Prevention and Control (ECDC), European Food- and Waterborne Disease and Zoonoses Surveillance Network (FWD-Net) [10].

In Italy, IT-ENTER-NET collects basic microbiological information (at least the serovar) on *Salmonella* isolates from human cases each year. These isolates correspond to approximately 50% of the total number of human salmonellosis cases notified to the SIMI [11]. Since 2002, the IT-ENTER-NET laboratories are also invited to submit *S.* Enteritidis and *S.* Typhimurium isolates to the Istituto Superiore di Sanità (Italian National Institute of Health) for phage and molecular typing and antimicrobial susceptibility testing.

The aim of this study was to describe the distribution of *Salmonella* serovars isolated from humans in Italy from January 1980 to December 2011, with a focus on the six most frequently reported serovars.

## Method

Data of *Salmonella* isolates from human cases were obtained from different laboratory-based surveillance systems depending on the considered time period. Data from 1980 to 1992 were obtained from published statistics of the RCE [6]. Data from 1993 to 1997 were obtained from the SALM-NET records and those from 1998 to 2011 from IT-ENTER-NET (http://www.iss.it/salm/?lang=1&id=1&tipo=4). In all of these three systems, the common case definition was 'an isolate of *Salmonella enterica* with identified serovar from a human specimen'.

For the purposes of this study, a minimum set of comparable information about each serotyped isolate was collected, including the patient sex, age and residence location, the laboratory that reached the microbiological diagnosis and the date of isolation thereof. This set of information was not systematically collected and made available before 2000, when only the serovar and the year of isolation were available.

A data set including *Salmonella* isolates of the whole study period (1980–2011) was created by merging the data obtained from the three systems (RCE, SALM-NET, and IT-ENTER-NET). This data set contained 254,418 records (i.e. isolates) with information on the serovar and date of isolation.

Another data set that included the isolates collected by IT-ENTER-NET from 2000 to 2011 (56,546 records) was created. This data set contained a number of duplicate entries, i.e. different isolates from a same case (because of the follow-up of patients with *Salmonella* infection after the first isolation) that were not always indicated. Therefore, 24,492 duplicate entries for an isolate that matched on serovar, laboratory reaching the microbiological diagnosis, and date of birth of the patient within the same or the consecutive month of isolation were discarded. Moreover, during the study period there were 2,122 cases related to 1,475 outbreaks (subjects tested within the framework of 'epidemiological investigation' in the IT-ENTER-NET data set) so we also discarded 647 outbreak-related entries, choosing only one isolate from each outbreak. The resulting data set included a total 31,407 records. Data management procedures were performed using ACCESS, version 2002 (Microsoft, Redmond, USA).

Data analysis focussed on the six most frequently reported serovars in the whole study period. The distribution of isolates by year was examined from 1980 to 2011, whereas the distribution by sex, age group (‹1, 1–5, 6–14, 15–64, and ›65 years) and month of isolation (January–December) was examined using the 2000–2011 data set. Mean annual isolation rates per 100,000 population were calculated by serovar, sex, age group, and province of residence standardised to the 2008 Italian reference population provided by the Italian National Institute of Statistics (ISTAT) (http://demo.istat.it/).

The inter-annual trend in the number of isolates from 2000 to 2011 was tested for statistical significance using the Cuzick's test for trend [12] (alpha: 0.05). Data analysis was performed using EpiInfo2000, version 3.3.1 (Centers for Disease Control (CDC), Atlanta, USA), and STATA, version 11.2 (StataCorp, College Station, USA).

Shapefile of Italy with provincial administrative boundaries was obtained from the ISTAT (ED-1950-UTM coordinate system, zone 32 N). Mean annual isolation rates per 100,000 population were presented using a choropleth map (with 4 classes determined according to Jenks' natural breaks method) in ArcGis, version 9.0 (ESRI, Redlands, USA).

## Results

### Inter-annual trends

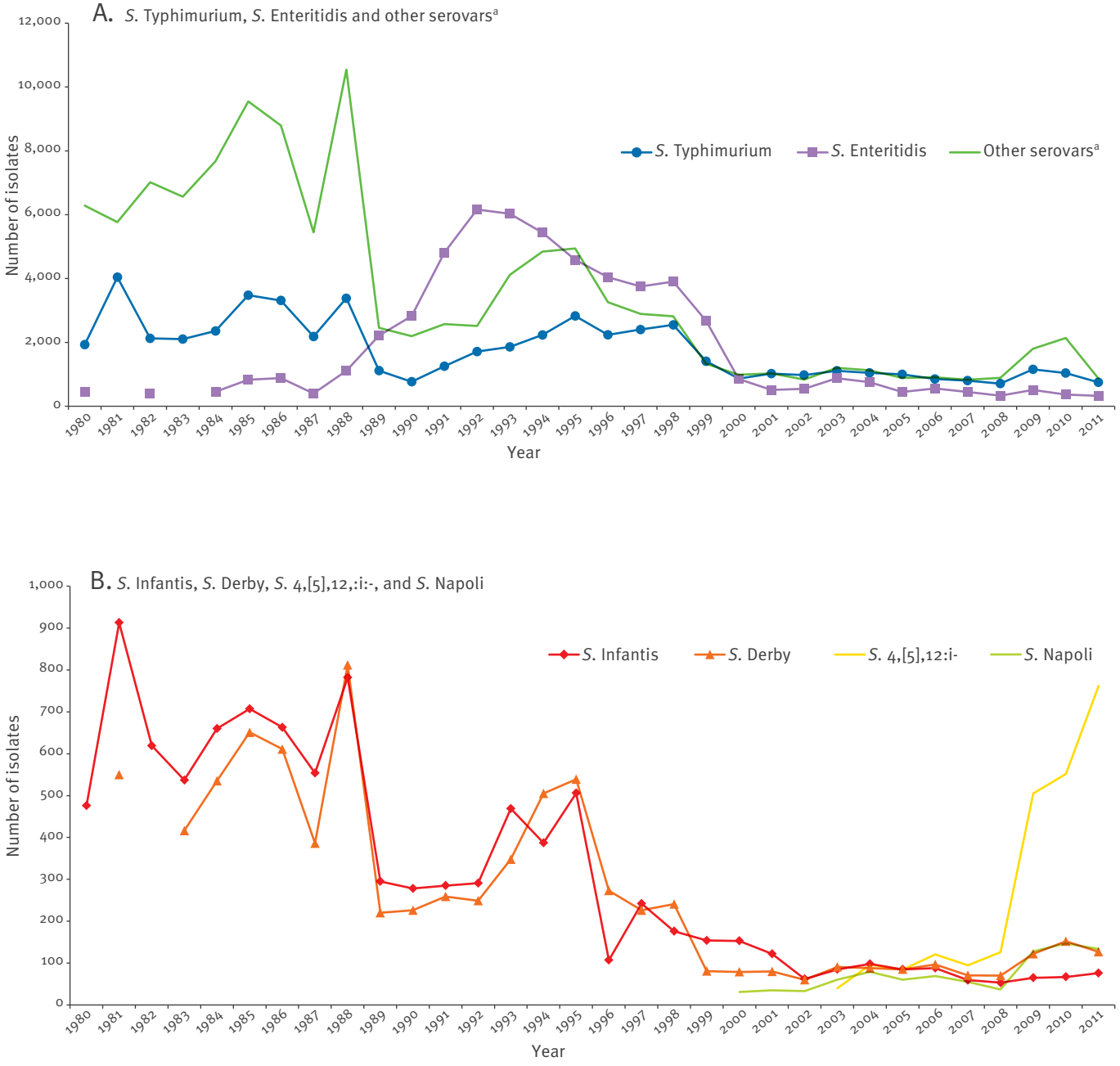
After exclusion of duplicate and outbreak-related entries (if more than one per outbreak), a total of 229,279 *Salmonella* isolates were reported from 1980 to 2011. The annual number of isolates decreased from an annual mean of 10,286 isolates in the period from 1980 to 1995 to an annual mean of 4,043 isolates in the time between1996 and 2011, with a more marked reduction from the year 2000 onwards (2,618 isolates on mean per year).

During the whole study period, the top six reported serovars were *S.* Enteritidis (57,499 isolates; 25.1% of the total number of *Salmonella* isolates; mean isolation rate: 2.99 isolates per 100,000 population/year), *S.* Typhimurium (56,671; 24.7%; 2.95 per 100,000 population/year), *S.* Infantis (10,114; 4.4%; 0.53 per 100,000 population/year), *S.* Derby (8,250; 3.6%; 0.43 per 100,000 population/year), *S.* 4,[5],12,:i:- (2,381; 1.0%; 0.12 per 100,000 population/year) and *S.* Napoli (868; 0.4%; 0.04 per 100,000 population/year). The other serovars accounted cumulatively for 93,496 isolates (40.8%; 4.87 per 100,000 population/year) (Figure 1).

*S.* Typhimurium was the predominant serovar from 1980 to 1988, but in 1989 *S.* Enteritidis overtook *S.*

**Figure 1**

Temporal trend of the top six reported *Salmonella enterica* serovars, Italy, 1980–2011 (n=229,279)

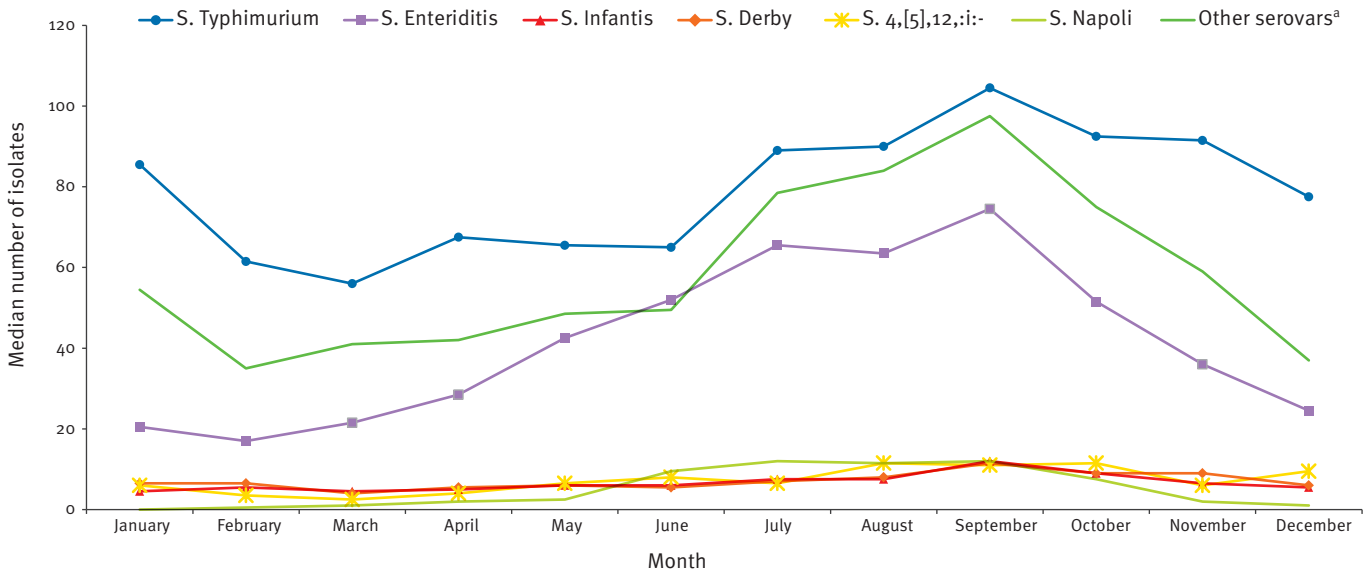



Some points in the charts representing the annual number of isolates for the *S. enterica* serovars Enteridis (panel A) and Derby (panel B) stand alone and are not included in the curve relating the annual number of isolates throughout the study period. This is because these two serovars were not reported for all years during the period up to the mid-1980s, resulting in missing data in the time series.

[a] Other serovars include all serovars other than *S.* Enteritidis and *S.* Typhimurium.

**Figure 2**

Median number of isolates of the top six reported *Salmonella enterica* serovars by month of isolation, Italy, 2000–2011 (n=31,407)




[a] Other serovars include all serovars other than *S.* 4,[5],12,:i:-, *S.* Derby, *S.* Enteritidis, *S.* Infantis, *S.* Napoli and *S.* Typhimurium.

Typhimurium and dramatically increased in the following years, reaching a peak in 1992. Since then, *S.* Enteritidis started decreasing, and from 2000 onwards *S.* Typhimurium returned to being the predominant serovar (Figure 1).

*S.* Infantis and *S.* Derby alternated as having the position of the third most frequently reported serovar during the whole study period (Figure 1). A decrease in the annual number of isolates for both serovars occurred from the mid-1990s, and from 2002 to 2008 the respective annual number of isolates remained below 100 isolates per year. Starting from 2009, however, the number of isolates of *S.* Derby per year increased and approximately doubled the number of *S.* Infantis isolates.

In 2000 and 2003, *S.* Napoli and *S.* 4,[5],12,:i:- emerged, respectively. *S.* Napoli increased from 31 isolates in 2000 to 134 isolates in 2011. *S.* 4,[5],12,:i:- was isolated for the first time in Italy in 2003 with 40 isolates (1.3% of the total number of isolates of that year). Since then, it increased steadily, reaching 762 isolates (39.1%) in 2011.

From 2000 to 2011, a significantly increasing temporal trend in the number of isolates was observed for *S.* Derby (mean of +8.1% isolates per year, p<0.001; mean isolation rate: 0.16 isolates per 100,000 population/year), *S.* Napoli (+28.2%, p = 0.032; 0.22 per 100,000 population/year) and *S.* 4,[5],12:i:- (+66.4%, p<0.001; 0.33 per 100,000 population/year), whereas a significantly decreasing temporal trend was observed for *S.* Infantis (-2.8%, p<0.001; 0.14 per 100,000 population/year) and *S.* Enteritidis (-3.0%, p<0.001; 0.91 per 100,000 population/year) isolates. *S.* Typhimurium isolates did not show any significant trend from 2000 to 2011 (p = 0.11; 1.58 per 100,000 population/year).

## Seasonal distribution

In the period from 2000 to 2011, the largest proportion of *Salmonella* isolates was observed in September (4,025/31,407 cases, 13%) and the smallest in February (1,698/31,407 cases, 5%). The median number of isolates in these two months was 335 and 139 respectively (Figure 2). Although this seasonal pattern was consistent for most serovars, *S.* Napoli and *S.* Derby showed slight variations. *S.* Napoli increased steeply in June (median = 10 isolates) and peaked in July (median = 12 isolates), remained at high levels until September (median = 12 isolates) and then decreased rapidly in October (median = 8 isolates). *S.* Derby peaked in September (median = 12 isolates) but remained at a high level until November (median = 9 isolates), when thereafter a stepwise decrease occurred until March, the month for which the median number of isolates was at the lowest (median = 4 isolates) (Figure 2).

## Age and sex distributions

During the period between 2000 and 2011, the highest isolation rate was for children aged one to five years,

**Table**

Distribution of the annual isolation rates of the top six reported *Salmonella enterica* serovars in Italy, by age and sex, Italy, 2000–2011 (n=31,407)

| Serovar | Annual isolation rates[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0–11 months | 1–5 years | 6–14 years | 15–64 years | ≥65 years | Female | Male |
| *S.* Typhimurium | 3.95 | 14.40 | 3.24 | 0.43 | 0.77 | 1.42 | 1.71 |
| *S.* Enteritidis | 2.47 | 6.03 | 1.99 | 0.43 | 0.45 | 0.91 | 0.96 |
| *S.* 4,[5],12:i:- | 0.69 | 2.36 | 0.58 | 0.06 | 0.16 | 0.23 | 0.27 |
| *S.* Derby | 0.44 | 0.84 | 0.15 | 0.06 | 0.18 | 0.14 | 0.15 |
| *S.* Infantis | 0.36 | 0.70 | 0.18 | 0.06 | 0.13 | 0.13 | 0.15 |
| *S.* Napoli | 0.64 | 1.03 | 0.20 | 0.02 | 0.08 | 0.10 | 0.12 |
| Other serovars | 4.98 | 7.19 | 1.66 | 0.52 | 1.02 | 1.12 | 1.25 |
| **Total** | **13.54** | **32.54** | **8.01** | **1.60** | **2.80** | **4.06** | **4.62** |

[a] The annual isolation rates are the annual number of isolates of each serovar/100,000 population of the age group or sex under consideration.

at 32.54 isolates per 100,000 population/year, followed by infants aged <1 year (13.54 per 100,000 population/year) and children aged six to 14 years (8.01 per 100,000 population/year). In the other age groups, the mean isolation rate was <3 isolates per 100,000 population/year. There were no evident differences in isolation rates between males and females (4.62 and 4.06 isolates per 100,000 population/year, respectively) (Table).

Of the total 31,407 isolates reported from 2000 to 2011, 1,005 (3.2%) were from cases of *Salmonella enterica* infection aged less than one year, 12,217 (38.9%) from cases aged one to five years, 5,339 (17.0%) from cases aged six to 14 years, 8,449 (26.9%) from cases aged 15 to 64 years, and 4,397 (14.0 %) from cases aged ≥65 years.

Considering the top six reported serovars, *S.* Typhimurium showed the highest isolation rate in all age groups except for cases aged 15 to 64 years, where *S.* Typhimurium and *S.* Enteritidis accounted for the same proportion of isolates (2,264/8,449 and 2,261/8,449; 26.8%). *S.* Typhimurium accounted for 3,469/12,217 (28.4%) and 2,322/5,339 (43.5%) of isolates from children aged one to five and six to 14 years, respectively. *S.* 4,[5],12:i:- had a visibly higher isolation rate than *S.* Derby and *S.* Infantis in cases aged one to five years but not in cases aged 15 to 64 years, where *S.* 4,[5],12:i:-, *S.* Derby, and *S.* Infantis had almost the same isolation rate. Moreover, while *S.* Napoli was the fourth most isolated serovar in cases aged ≤14 years, it was the least represented in those aged >14 years.

## Spatial distribution

Figure 3 presents the distribution at the province level of the mean annual isolation rate per 100,000 population of the top six reported serovars (2000 to 2011). The highest isolation rates were observed in the northern provinces of the country, particularly in the provinces of Sondrio, Trento, and Varese, whereas the southern provinces showed considerably lower isolation rates. Such spatial distribution was also observed in the isolation rate of the different serovars.

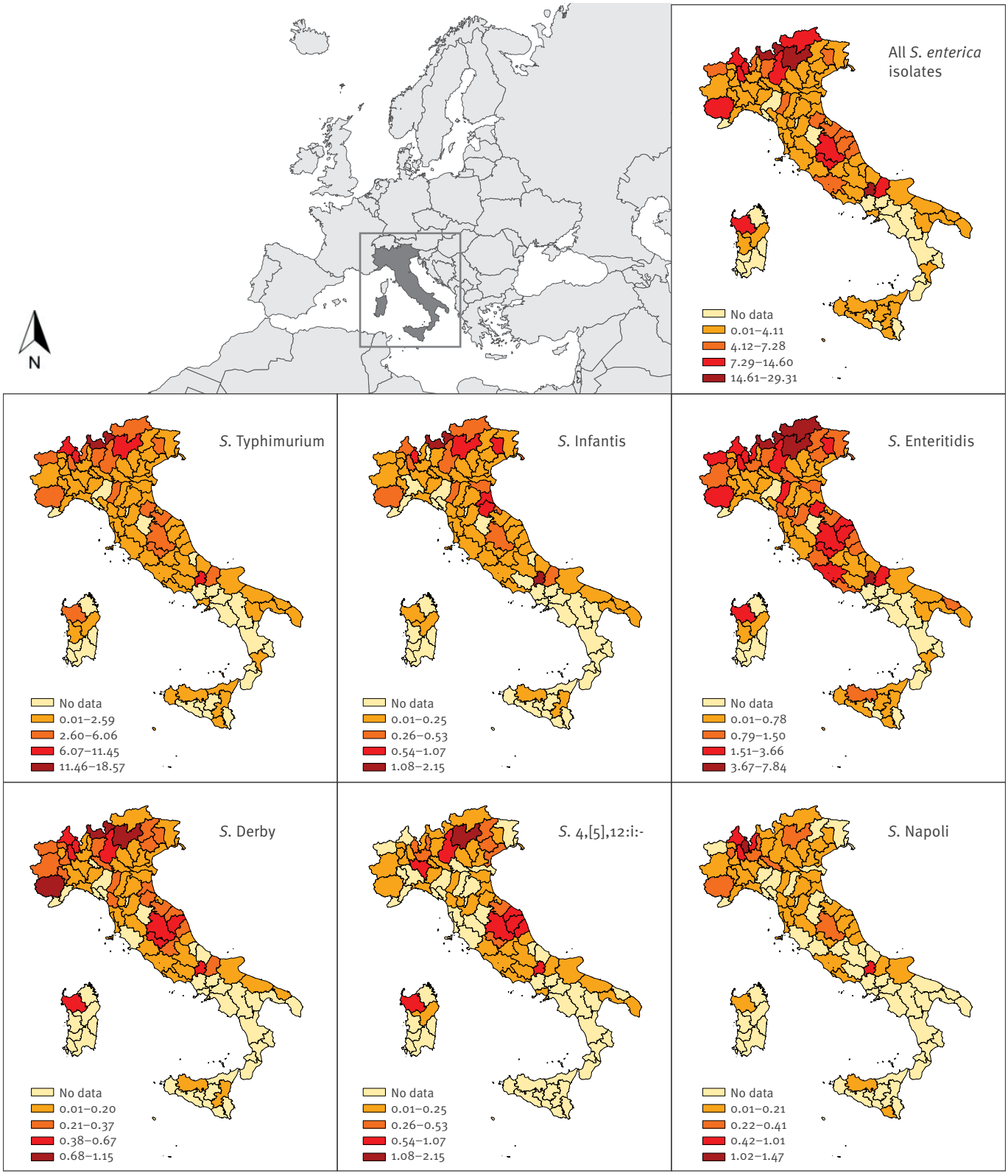
## Discussion

Evidence that human salmonellosis in Italy has decreased since the late 1990s has previously been provided through the analysis of cases notified to the SIMI [9]. This study shows that, since 2000, the decrease has concerned only specific serovars, namely *S.* Enteritidis and *S.* Infantis, whereas other serovars have emerged (*S.* 4,[5],12:i:-, *S.* Derby, and *S.* Napoli) or remained fairly stable (*S.* Typhimurium).

After the global emergence of *S.* Enteritidis in the late 1980s that apparently filled the ecological niche vacated by the eradication of *S.* Gallinarum from poultry [13], a sustained decrease in the number of human *S.* Enteritidis infections was observed globally starting from the late 1990s [4,14-17]. Several factors, including the implementation of new on-farm control measures against *Salmonella* in poultry (e.g. the introduction of live vaccines), improved hygiene and education of consumers and food-workers, have probably contributed to this decrease, at least in the EU [1,4] and in the United States [15,18]. In 1992, the European Parliament issued a directive (Council Directive 92/117/EEC) [19] establishing measures for protection against specified zoonotic agents in animals and foods of animal origin. This Directive proposed that the EU Member States establish monitoring systems and control measures in poultry breeding flocks. In 2003, to enforce these measures, the European Parliament and the EU Council introduced the Regulation No. 2160/2003 [20] to ensure that proper and effective measures were undertaken to control *Salmonella* at all relevant stages of production, processing, and distribution of poultry

## Figure 3

Province-level maps of the mean annual isolation rates per 100,000 population of *Salmonella enterica*, Italy, 2000–2011 (n=31,407)



products. The observed decrease of human cases of *S.* Enteritidis suggests that these measures have succeeded in reducing the burden of human *S.* Enteritidis infection.

We observed a peculiar profile of serovars in Italy, as *S.* Enteritidis fell consistently below *S.* Typhimurium since 2000, whereas in most other EU countries, despite the significant decrease of *S.* Enteritidis, *S.* Typhimurium has never became the most reported serovar, at least until the second half of the first decade of the 2000s [17]. This is particularly evident in the EU, where in this period few countries in addition to Italy have experienced this shift in the dominant serovar, i.e. Belgium, Denmark and France [4]. In 2011, *S.* Typhimurium had been predicted to become the most common serovar in England and Wales by 2012 as a result of the decrease of *S.* Enteritidis [21].

Given the distribution of serovars from humans and animal sources in the period from 2007 to 2009, it has been estimated that pig and pork products are the most important source of human salmonellosis in Italy, accounting for 73% of human infections [4]. This is in line with our results, as pigs constitute in fact the most important reservoir for *S.* Typhimurium [4].

As laying hens are the most likely source of human *S.* Enteritidis infection in Europe [4], the drastic decrease of human cases of *S.* Enteritidis in Italy may be explained, to some extent, by the structure of the Italian poultry industry (which is largely developed through the vertical integration system) and by the fact that poultry meat and table egg production in Italy is self-sufficient to meet the internal market demand. Vertical integration means that all major stages of poultry production (e.g. feed mills, breeder farms, hatcheries, grower farms and processors) are parts of a streamlined poultry production system, usually united through a common owner. This enables companies to harmonise biosecurity measures, housing technologies, feeding regimens, vaccination schemes and testing protocols among farms, so as to control the (microbiological) quality of both input and output products. Moreover, since 2003, the level of biosecurity and hygiene practices in the Italian poultry industry have greatly been enhanced to address the legal requirements provided for the control of avian influenza epidemics [22]. These improvements may have had a particularly significant impact on the effectiveness of the applied control measures against *S.* Enteritidis in the Italian poultry industry, as both the production and consumption of poultry products are rather closed to external influences.

The monophasic variant of *S.* Typhimurium, *S.* 4,[5],12:i:-, characterised by the antimicrobial resistance to Ampicillin, Streptomycin, Sulphonamide, and Tetracycline (pattern ASSuT) is emerging and extensively circulating in Denmark, Italy, the United Kingdom and also recently in Greece [11,23, 24]. In Italy, *S.* 4,[5],12:i:-, showed a dramatic increase since 2003, both in humans and in animals farmed for food production, particularly pigs and bovines [25]. Also *S.* Napoli is an emerging serovar although it is not emerging homogenously over the whole EU, with most of the cases (87%) reported between 2000 and 2006 having occurred in Italy, France, and Switzerland. It has been suggested that the environment can act as the main reservoir for *S.* Napoli, and from there this serovar can spill over to animals and humans [10].

Most serovars showed a marked seasonality, increasing over the summer months and peaking in August/ September, and then decreasing gradually. Although the reasons of this pattern are not entirely known, it may be related to the parallel *Salmonella* shedding trend in animal hosts, and/or insufficient refrigeration and mishandling of foods during the warm months [26,27].

As expected, isolation rates were highest in children. This may be due to the greater proportion of symptomatic infections among the young but also to the higher propensity to take samples by paediatricians (i.e. detection bias) [27]. However, consistent with other studies [10,11,27], we observed that cases with *S.* Typhimurium, *S.* 4,[5],12:i:-, or *S.* Napoli infection were most likely to be children aged ≤14 years, whereas cases with *S.* Enteritidis, *S.* Derby, or *S.* Infantis infections were more likely to be adults aged ≥15 years. This may be due to the different serovar-specific risk factors to which individuals are exposed at varying age groups [28].

This study is based on reported data from laboratories that are not homogenously distributed in the Italian territory; thus, there may be differences in representativeness of the data from different regions. It has been showed that the surveillance systems of northern regions of Italy are generally more sensitive in detecting cases of infectious gastroenteritis, leading to significantly higher notification rates of salmonellosis compared to the national average [9]. Moreover, diagnostic capacity for enteropathogens differs from laboratory to laboratory in Italy [29]. These may be the reasons as to why we observed that the isolation rates were considerably lower in the southern part of the country.

Regarding the selection of isolates included in our analyses, we deleted duplicates and most of the outbreak-related cases in order to represent as much as possible the role of the different serovars without any 'artificial' replication of isolates due to outbreaks. Documented major outbreaks of human salmonellosis that occurred in Italy during the study period have concerned mainly *S.* Typhimurium [e.g. 30] and *S.* Enteritidis [e.g. 31].

In conclusion, *Salmonella* serotyping is useful for informing and addressing public health actions, providing data about the emerging serovars (which may

reveal the presence of a previously unrecognised source of infection) and the efficacy of intervention measures.

We found that *S.* Enteritidis has decreased dramatically in Italy and that *S.* Typhimurium has become once more the most reported serovar as of 2000. It is noteworthy that between 2000 and 2011, while *S.* Enteritidis and *S.* Infantis decreased, *S.* Typhimurium remained stable and *S.* 4,[5],12:i:-, *S.* Derby, and *S.* Napoli increased. This suggests that the applied control measures are not equally efficient against these serovars and that other sources of infection have probably become increasingly important (e.g. unconventional, wild and free-range animals, fruit and vegetables). Therefore, further investigation into the potential causes of spread of the emerging serovars, against which newly tailored control measures should be implemented, is warranted.

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# Pilot study to introduce a notification card for partner notification of sexually transmitted infections in Catalonia, Spain, June 2010 to June 2011

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We conducted a cross-sectional study in 10 primary care centres in Catalonia, to determine applicability, acceptability and effectiveness of partner notification cards used by patients diagnosed of a sexually transmitted infection (STI) and to characterise these and their sexual partners. Statutorily notifiable STIs included *Chlamydia* infection, gonorrhoea, syphilis, human immunodeficiency virus (HIV) infection or other STIs as deemed necessary by the treating physician. Between June 2010 and June 2011, 219 index cases were enrolled, of whom 130 were men (59.4%), 71 of them men who have sex with men (54.6%). *Chlamydia* infection (41.1%), gonorrhoea (17.8%) and syphilis (16.0%) were the STIs most frequently diagnosed. HIV infection accounted for 4% of cases. A total of 687 sexual partners were reported, and 300 of these were traceable through the notification card (45.7%). Those who did not report traceable contacts were older (mean age: 34 years versus 31 years, p=0.03). The main reason for not distributing the card was anonymous sexual intercourse (38%). Patient referral notification cards can reach a high percentage of sexual partners at risk. However, only few notified sexual partners attended participating health centres. Internet-based partner notification may be considered in order to reach those partners not otherwise traceable.

## Introduction

Partner notification is the process through which sexual partners of a patient diagnosed with a sexually transmitted infection (STI) are informed that they have been exposed to infection, so they can be assessed, diagnosed and treated [1]. Partner notification is based on the assumption that the transmission chain of STI can be interrupted when both symptomatic and asymptomatic exposed individuals are assessed, diagnosed and treated appropriately [2]. Partner notification for STIs is specifically indicated in cases of *Chlamydia* infection, gonorrhoea, syphilis, or human immunodeficiency virus (HIV) infection [3,4]. Although there is no formal indication or enough evidence to recommend partner notification for other STIs, it may be reasonable in certain circumstances and at the discretion of the treating physician. Clinicians are asked to perform partner notification, but other health professionals such as nurses or social agents can also play a role. Patient referral partner notification seems to be the most cost-effective method compared with other partner notification strategies such as provider referral, conditional referral (where the provider informs the sexual partner(s) in case the patient fails to do so within an agreed period of time) or patient-delivered partner therapy. In a patient referral methodology, only the index case is responsible for notifying their sexual partners of possible infection. The effectiveness of this

process can be increased with the use of a notification card [5,6].

In recent years, Catalonia has experienced a re-emergence of syphilis and HIV infections, the emergence of *Neisseria gonorrhoeae* strains resistant to ceftriaxone [7,8], and outbreaks of Lymphogranuloma venereum [9]. Catalonia has an adult population (15 to 64 years) of almost 5 million [10], and more than 600 new HIV cases were reported in the year 2011 (rate: 8.5/100,000 inhabitants) [11]. Moreover, increased mobility of people, the use of the Internet to find sexual partners (casual and anonymous), and the decreased use of barrier methods are all contributing to the spread of all STI [12-15]. In Spain, no formal guidelines for partner notification have been published yet, nor have there been studies evaluating partner notification. In contrast, 11 of the 24 European Union and European Economic Area (EU/EEA) countries that responded to the survey have regulated partner notification by law [16,17], although only three (Finland, Norway and Sweden) currently observe compulsory partner notification by the health provider and the patient. In Catalonia there has been an increasing interest in implementing partner notification within primary care services since 2007, when gonorrhoea, syphilis, Lymphogranuloma venereum and HIV infection were included as statutorily notifiable infections. The latest version of the STI guidelines published by the Catalan Department of Health strongly recommends partner notification [18], but no standard guidelines or specific support for partner notification have been developed, although there are health centres that have designed their own notification card. For these reasons, a notification card was designed specifically for this study, in order to increase the coverage and efficiency of partner notification as well as to unify and standardise the available tools.

The goal of this study was to facilitate the introduction and standardisation of partner notification for STIs in primary care centres in Catalonia, including the specialist STI unit. We evaluated the applicability (ability of this tool or procedure to be used under real conditions in primary health centres and STI units), and acceptance of this method (willingness and satisfaction of the staff with the use of the tool or procedure to be used under real conditions in primary health centres and STI units), as well as its effectiveness in notifying as many sexual partners of the index case as possible with the support of a notification card. Secondly, we aimed to describe the profile of patients with STIs and their sexual partners.

## Methods

### Study population

Patients diagnosed with an STI (either clinically or by laboratory test) and attending, during the study period from June 2010 to June 2011, primary care centres (primary health physicians, gynaecologists and midwives) or the Sexually Transmitted Infection specialist unit (STI unit) were eligible to be enrolled in the study.

### Study design

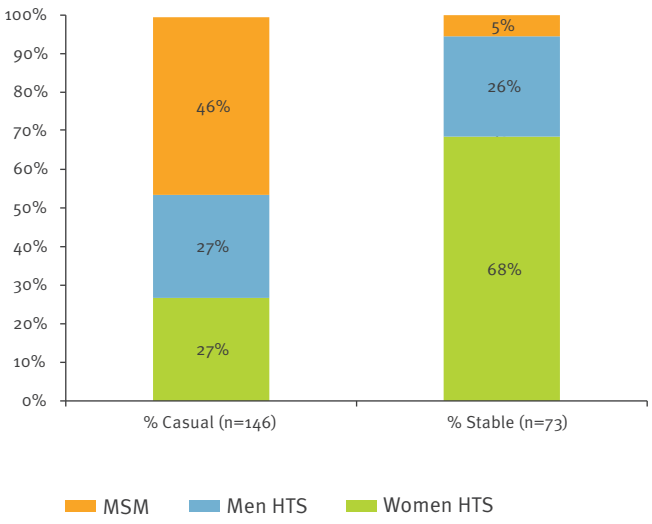
A cross-sectional study was carried out. The study was set up in 10 primary care centres in Catalonia. These centres represented different models of primary STI care (public STI reference units, public and private primary care) and were located in either rural or urban areas. Patients were continuously enrolled during the study period (June 2010 to June 2011). All health professionals participating in the study shared the same standard approaches to STI, defined in the current guidelines for the control and management of STIs in Catalonia [14]. Biological samples were collected from symptomatic patients from the anatomical site of suspicious symptoms, and followed the same laboratory procedures. No further standard criteria were established for additional collection of samples or the screening of asymptomatic partners, e.g. rectal or pharyngeal swabs, these being taken entirely at the discretion of the treating clinician.

Once a laboratory-confirmed STI or suspected (syndromic) case was diagnosed, the index case filled in a specific epidemiological questionnaire. Data collected included: date of index case presenting to the health centre, age, sex, sexual orientation, number of sexual partners during the theoretical infectious period according to clinical guidelines [18], type of relationship (casual or stable), number of partners eligible for being contacted independently of the tool (paper card, SMS, telephone, internet, etc) and of these, the number of partners suitable for notification using the notification card, and reasons why the notification card was not used. Eligible partners were all those that, for each specific infection, had a sexual relationship with the index case within the period of infectiousness, defined by days or months backwards from the date of onset of symptoms in the index case. Eligible partners were classified as: eligible for partner notification using the paper card and those eligible for partner notification using other methods than the card. Partners testing positive were also enrolled as new index cases.

A number of cards equal to the number of partners eligible for being contacted by notification card were distributed to each index case. The health professional received personally the notification card from the notified partner, holding information of date of diagnosis of infection, type of infection, syndromic or laboratory-confirmed, and treatment given to the index case. Syndromic diagnoses were specifically written in a blank space in the notification card: (e.g. urethral syndrome). By counting the notification cards received from notified partners at health centres and recording the date of the partner presenting at the health centre we evaluated the effectiveness of using this notification card.

**Figure 1**

Type of relationship of index cases by sex and sexual orientation, partner notification study, Catalonia, June 2010 to June 2011 (n=219)




HIV: human immunodeficiency virus; HTS: heterosexual; MSM: men who have sex with men.

Index cases stating that it would not be possible for them to trace any of their eligible partners for notification were qualified as non-notifier index cases.

Laboratory results from the index case were included once they were available (including negative results without any alternative diagnosis). For STIs such as HIV infection and syphilis, partner notification did not start until laboratory results were available. A presumptive clinical diagnosis of Lymphogranuloma venereum in men who have sex with men (MSM) triggered a request to subtype *Chlamydia trachomatis*. For other STIs such as *Trichomonas* or herpes simplex infections, causing urethral syndrome or genital herpes, syndromic diagnosis was considered sufficient to start partner notification procedures.

The notification card and the epidemiological questionnaire were piloted by two health professionals with some of their patients for a week prior to their use during the study. This allowed us to adapt both the notification card and the questionnaire, so as to include more understandable words and sentences. Data from patients interviewed during the pilot study were not included in the study.

## Data management and analysis

Data collected from the index case's epidemiological questionnaire, from the notification cards and from the questionnaire on acceptability completed by health professionals, were validated and entered into a database designed specifically for the study. Data analysis was done using STATA 10.0 (Statacorp, Texas, United States). Mean, range, 95% confidence interval (CI) and standard deviation (SD) were calculated for quantitative variables. Proportions and 95% CI for binomial distributions were calculated for qualitative variables. Chi-square and Fisher's exact test were used for bivariate analysis of qualitative variables and Student's T-test for quantitative variables. Men were stratified into two groups: i) MSM, including bisexuals, and ii) heterosexual men. All women were included in a single category. Casual sexual intercourse was defined as an occasional relationship with a partner not considered stable. Relationships lasting more than three months were considered stable. Index cases with negative laboratory results were not excluded from the analysis, having established that there were no socio-demographic differences between index cases with negative and those with positive results. Primary, early latent and secondary syphilis were included in the same category.

## Ethical issues

Partner notification is indicated once there is a diagnosis of a statutorily notifiable STI [17]. Informed consent form was therefore not obtained by the health professional. This study was approved by the Ethics Committee of the Hospital Universitari Germans Trias i Pujol. Data in the coordinating centre (Centre for Epidemiological Studies on Sexually Transmitted Infections and HIV/AIDS of Catalonia; CEEISCAT) were treated strictly confidentially following standard procedures. Health professionals participating in the study used their daily practice to contribute to this study.

# Results

## General description of index cases and distribution of sexually transmitted infections

During the study period, 219 index cases were included (mean age: 32.2 years, SD: 9.3 years, range: 15–57 years), 97 (44%) of whom were recruited in the STI Unit. They were 130 (59%) men and 89 (41%) women. Among men, 71 (55%) were MSM. Most of the MSM were seen at the STI unit (n=63, 89%). MSM were older than heterosexual cases (men and women) (34.8 versus 30.9 years, p=0.001).

Casual relationships were more frequently mentioned by index cases (67%) than stable relationships (33%) (Table 1). Fifty (68%) of the 73 index cases reporting stable relationships were heterosexual women. In contrast, the most frequent sexual orientation among the 146 index cases reporting casual relationships were MSM (46%) (Figure 1). We registered 213 laboratory-confirmed STIs from 239 STI diagnoses (syndromic and laboratory-confirmed), including 20 cases with multiple infections (19 subjects with two infections and one with three infections). A detailed description of the index cases is shown in Table 1. The total number of statutorily notifiable STIs (*Chlamydia* infection, gonorrhoea and syphilis, excluding HIV infection) included in

## Table 1

Characteristics of the index cases, partner notification study, Catalonia, June 2010 to June 2011 (n=219)

| Variable | Number of cases | Percentage of index cases | 95% CI |
|---|---|---|---|
| Age (n=219) | | | |
| 15–25 | 53 | 24 | 18.7–30.1 |
| 26–35 | 98 | 45 | 38.0–51.6 |
| 36–45 | 44 | 20 | 15.0–26.0 |
| >45 | 24 | 11 | 7.1–15.9 |
| Sex (n=219) | | | |
| Men | 130 | 59 | 52.5–65.9 |
| Women | 89 | 41 | 34.0–47.5 |
| Sexual orientation (n=219) | | | |
| Heterosexual women | 89 | 41 | 34.0–47.5 |
| Heterosexual men | 59 | 27 | 21.2–33.3 |
| MSM | 71 | 32 | 26.3–39.1 |
| Relationship (n=219) | | | |
| Casual | 146 | 67 | 60.0–72.9 |
| Stable | 73 | 33 | 27.1–40.0 |
| Laboratory and syndromic diagnosis (n=239 diagnoses)[a] | | | |
| *Chlamydia* infection | 90 | 42[b] | 31.4–44.1 |
| Lymphogranuloma venereum | 2 | 2[c] | 0.3–7.8 |
| Gonorrhoea | 39 | 18[b] | 11.9–21.6 |
| HIV infection | 9 | 4[b] | 1.7–7.0 |
| Syphilis | 35 | 16[b] | 10.4–19.8 |
| Primary syphilis | 14 | 40[c] | 23.9–57.9 |
| Secondary syphilis | 10 | 29[c] | 14.6–46.3 |
| Early latent syphilis | 4 | 11[c] | 3.2–26.7 |
| Latent syphilis | 7 | 20[c] | 8.4–36.9 |
| Other laboratory-positive and syndromic STI[d] | 40 | 19[b] | 12.2–22.1 |
| Laboratory-positive and syndromic STI | 213 | 89[e] | 84.4-92.7 |
| Laboratory-negative | 26 | 11[e] | 7.2–15.5 |
| Number of sexual partners mentioned by index case (n=687 partners) | | | |
| 0–1 | 103 | 47 | 40.3–53.9 |
| 2–3 | 65 | 30 | 23.7–36.2 |
| >3 | 51 | 23 | 17.9–29.5 |
| Notification card distributed by index case (n=300 cards) | | | |
| 0 | 48 | 22 | 16.6–28.0 |
| 1–3 | 161 | 73 | 67.1–79.2 |
| >3 | 10 | 5 | 2.2–8.2 |
| Reason for not using the notification card (n=99 index cases)[f] | | | |
| Anonymous sexual partner | 43 | 38 | 27.5–45.4 |
| Sexual partner lives far | 34 | 30 | 21.8–39.4 |
| It is not necessary to notify | 8 | 7 | 3.1–13.5 |
| Other | 28 | 25 | 17.1–33.8 |

CI: confiidence interval; HIV: human immunodeficiency virus; MSM: men having sex with men; STI: sexually transmitted infection.

[a] Includes 19 patients with two infections (*Neisseria gonorrheae+Chlamydia trachomatis*, n=7; *C. trachomatis+Trichomonas* vaginalis, n=2; *C. trachomatis+Ureaplasma urealyticum*, n=1; *C. trachomatis+Treponema pallidum*, n=2; *N. gonorrhoeae+T. pallidum*, n=1; HIV+*C. trachomatis*, n=1; HIV+*T. pallidum*, n=2; HIV+*T. vaginalis*, n=2; *U. urealyticum+Mycoplasma genitalium*, n=1) and one patient with three infections (*C.* trachomatis+N. gonorrhoeae+U. urealyticum).

[b] Percentage of the type of infection over all laboratory-positive and syndromic STI (n=213).

[c] Percentage of cases within each category.

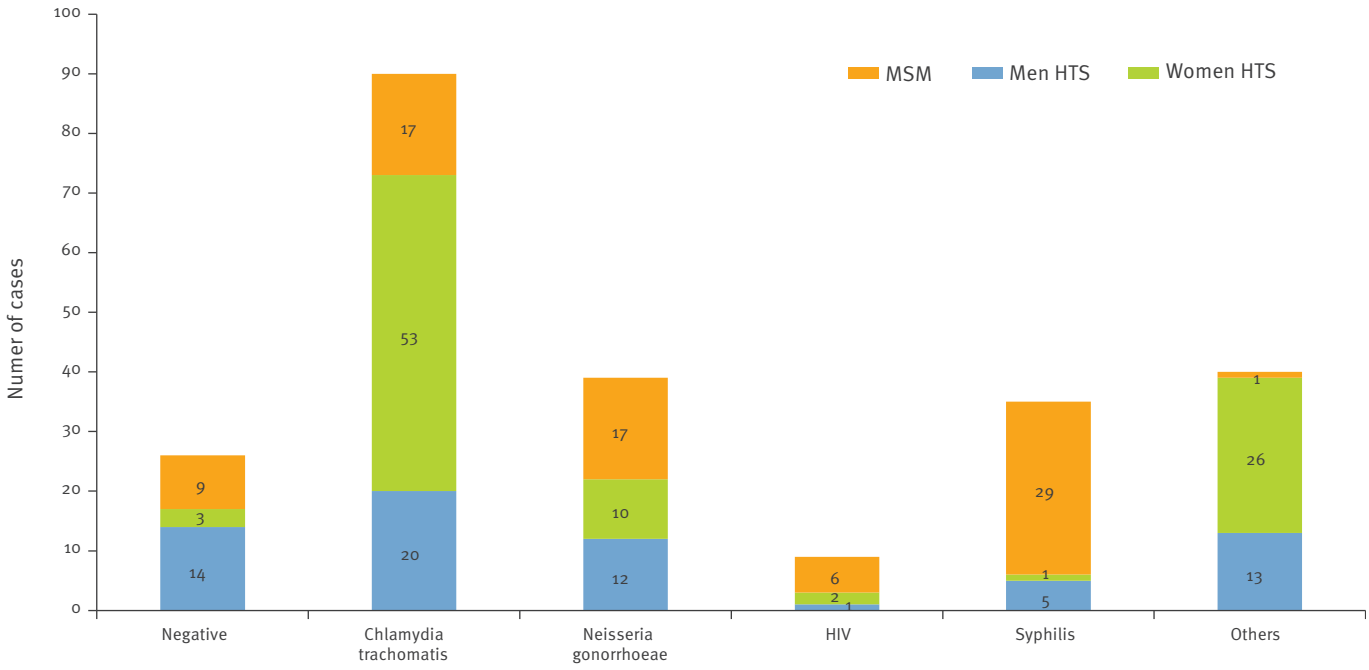
[d] *T. vaginalis* (n=20), Human papillomavirus (n=6), Hepatitis B virus (n=4), *U. urealyticum* (n=4), *M. genitalium* (n=3), Herpes simplex (n=3).

[e] Percentage of results over all laboratory and syndromic diagnoses (n=239).

[f] This question accepted more than one answer.

**Figure 2**

Distribution of sexually transmitted infections by sex and sexual orientation, partner notification study, Catalonia, June 2010 to June 2011 (n=219)[a]




HIV: human immunodeficiency virus; HTS: heterosexual; MSM: men who have sex with men.

[a] The 219 cases had a total of 239 diagnoses and included 19 patients with two infections (*Neisseria gonorrheae+Chlamydia trachomatis*, n=7; *C. trachomatis+Trichomonas vaginalis*, n=2; *C. trachomatis+Ureaplasma urealyticum*, n=1; C. *trachomatis+Treponema pallidum*, n=2; *N. gonorrhoeae+Treponema pallidum*, n=1; HIV+*C. trachomatis*, n=1; HIV+*Treponema pallidum*, n=2; HIV+*T. vaginalis*, n=2; *U. urealyticum+Mycoplasma genitalium*, n=1) and one patient with three infections (*C. trachomatis+N. gonorrhoeae+U. urealyticum*).

the study was 164 representing 15% of all STIs declared in Catalonia during the study period (n=1,158).

The most frequent STIs were: *Chlamydia* infection, including two cases of Lymphogranuloma venereum, (41%, n=90), gonorrhoea (18%, n=39) and syphilis (16%, n=35). HIV infection accounted for 4% (n=9) and all were newly diagnosed HIV infections. Other STIs represented 18% (n=40) of the sample. Twenty-six patients had negative laboratory results and their diagnosis was therefore exclusively clinical and syndromic (12%).

Figure 2 shows the number of STIs by diagnosis, sex and sexual orientation. The proportion of women among *Chlamydia*-infected patients was higher than that of heterosexual men (53 of 90 versus 20 of 90, p=0.002). Syphilis was proportionally more frequent among MSM than heterosexual men (29 of 35 versus 5 of 35 p<0.001). Most HIV infections were in MSM compared with the heterosexual population (6 of 9 versus 3 of 9, p=0.03). Both cases of Lymphogranuloma venereum were diagnosed in MSM.

## Number of sexual partners and cards distributed and recovered

Overall, the index cases reported having had 687 sexual partners during the infectious period (range: 1–30, mean: 3 partners per index case). Female index cases reported a mean of 1.7 sexual partners, heterosexual men of 1.7, and MSM of 6.2 (p<0.001).

A total of 300 notification cards were reported to be distributed by the index cases to their sexual partners (corresponding to 300 sexual contacts eligible to be contacted using the notification card). The remaining 387 partners were those eligible to be notified by other means than partner notification paper card, including those that may be impossible to trace by the index case. There were 59 notification cards distributed to heterosexual men, who reported a total of 100 sexual partners (ratio of cards distributed/contacts reported: 0.59, 95%CI: 0.48–0.68). There were 108 notification cards distributed to women who reported a total of 144 sexual partners (ratio: 0.75, 95% CI: 0.62–0.79) and 133 to MSM who reported 443 sexual partners (ratio: 0.30, 95% CI: 0.26–0.35).

**Table 2**

Characteristics of non-notifier[a] group of index cases, partner notification study, Catalonia, June 2010 to June 2011 (n=48)

| Variable | Number | Percentage[b] | p value[c] |
|---|---|---|---|
| Sexual orientation (n=48) | | | |
| Heterosexual women | 10 | 11 | 0.003[d] |
| Heterosexual men | 20 | 34 | |
| MSM | 18 | 25 | |
| Relationship (n=48) | | | |
| Casual | 37 | 25 | 0.08[d] |
| Stable | 11 | 15 | |
| Age (n=48) | | | |
| 15–25 | 7 | 13 | 0.17[d] |
| 26–35 | 21 | 21 | |
| 36–45 | 12 | 27 | |
| >45 | 8 | 33 | |
| Number of sexual partners referred (n=48) | | | |
| 0–1 | 29 | 28 | 0.08[d] |
| 2–3 | 9 | 14 | |
| >3 | 10 | 20 | |
| Laboratory diagnosis (n=55)[e] | | | |
| *Chlamydia* infection | 16 | 18 | 0.2 |
| Gonorrhoea | 9 | 23 | 0.8 |
| HIV | 3 | 33 | 0.4 |
| Syphilis | 9 | 26 | 0.6 |
| Other STI | 8 | 20 | 0.8 |
| Negative | 10 | 38 | 0.03 |

HIV: human immunodeficiency virus; MSM: men having sex with men; STI: sexually transmitted infection.

[a] Non-notifier index cases are those stating that it would not be possible for them to trace any of their eligible partners for notification either using a notification paper card or by other means.

[b] The denominator was the total of individuals included in the respective groups as presented in Table 1 (e.g. 10 non-notifier HTS women among 89 HTS persons included in the study).

[c] The baselines are considered as the group of notifiers.

[d] P test for trend.

[e] The number of laboratory results (n=55) exceeds the number of non-notifier index cases because some of them had more than one STI.

The ratio of cards distributed/contacts reported was lowest among MSM, followed by heterosexual men and heterosexual women (p test for trend=0.003). Overall, thirty-one cards were returned to participating health centres (10%) and the card holders were assessed by health professionals and treated as necessary following diagnosis. This percentage was higher in the STI Unit (20%, p=0.003). The main reason for not using a notification card was that sexual contact was anonymous 38%. Those partners that were notified by index cases but did not deliver a notification card or mention it on arrival at the health centre were not registered as contacts and were only enrolled as index cases.

## Non-notifier index cases

Of the 219 index cases enrolled in the study, 99 (45%) stated that it would be impossible to use the notification card at least for one of their partners. Among them were 48 (48%) for whom it was impossible to notify any of their partners (non-notifiers). These non-notifier index cases were older than other index cases (34 versus 31 years, p=0.03), independently of sexual orientation. In Table 2 we show a detailed description of non-notifier index cases.

## Discussion

This is the first study evaluating partner notification for STIs in Catalonia or Spain. Most of our findings are in line with recently published studies in the United States (US) and Switzerland [19,20], which reported higher-risk behaviour by MSM (greater number of sexual partners, including casual and anonymous sexual intercourse), and a higher proportion of syphilis among MSM and *Chlamydia* infection among women.

It is of note that female index cases indicated that they were able to give the card to their sexual partners more often than heterosexual men (ratio 0.75 versus 0.59). This can be explained by socio-cultural perceptions, or more probably, by the type of relation maintained with sexual partners (more frequently stable). We suspect that women were more frequently infected by their stable partner than other groups.

One finding of our study is the relatively low yield of partner notification cards distributed and recovered (patients returned). However, we consider this number as an underestimation. This study was not designed for collecting returned cards, although a certain number were collected by the participating centres. Given that there are hundreds of primary care and private centres in Catalonia that can see individuals with suspected STIs, the study was unable to include all these centres, and could thus only focus on a limited and representative number of centres. Moreover, a certain number of contacts may have visited a health centre without presenting the card (uncontrolled).

We also suspect that a lack of awareness and lack of concern about asymptomatic sexually transmitted infections may be one of the explanations for the low proportion of sexual partners presenting to health centres after being notified by the index case.

It is important to mention that a larger proportion of cards were retrieved in the STI unit compared with the rest of the participating centres (not specialised, 20%). This relatively high percentage may be attributable to the specialised attention given to patients in the STI

unit and the higher probability of effectively assessing and treating sexual partners of index case seen in this unit.

The main finding of our study with respect to partner traceability is that close to half of all sexual partners were traceable through a notification card distributed by the index cases (n=300 of 687, 44%) but it is also important to note the high number of partners that could not be contacted due to anonymous and casual sexual intercourse.

Consequently, additional notification strategies should be implemented to reach a higher proportion of exposed contacts and to overcome communication barriers. One of the strategies is email or website notification using pseudonyms. The use of the internet is becoming highly popular for sexual partner research, especially among high-risk groups. The use of email and specially designed websites under the control of health authorities can guarantee confidentiality and quality of the information given to sexual partners exposed. In fact, this may be the only way to contact a majority of sexual partners [21-24]. Some clinics in the US and Australia are already using this technology with promising results [25,26]. However, most of the literature about partner notification for STI was carried out in other countries with different socio-cultural contexts such as Australia, Canada, the US, Guatemala and Kenya. We cannot ascertain the real impact of this strategy (number of sexual partners finally screened in health services), and we relied on the previous studies that evaluated these indicators [2,6,27,28]

Since this study was done under real conditions in each health centre, a standard protocol to test sexual partners was not used in our study. Therefore, the testing of sexual partners may have been addressed differently by the participating centres. Comprehensive routine or sexual practice-based screening of different anatomical sites (rectal and pharyngeal swabs in the case of anal or oral intercourse) was not standard procedure. Although sexual partners attending health centres with a notification card were assessed according to their sexual orientation, sexual practice and symptoms, we cannot ensure that comprehensive testing was done by all participating centres in all sexual partners, independently of the presence of symptoms. In Catalonia, despite current guidelines recommending partner notification, there is still no clear partner notification strategy, a gap which needs to be closed. Considering the high proportion of asymptomatic STIs, comprehensive screening of sexual partners, irrespective of the presence of symptoms, should be offered by all health professionals treating STIs, in order to optimise partner notification practices and improve their effectiveness.

In conclusion, partner notification through the use of a notification card is a feasible (applicable and acceptable) strategy in Catalonia given its high acceptability among healthcare workers and index cases. Nevertheless, there are some variations in the use of the notification paper card, MSM being the ones with more difficulties to use it due to the highest number of anonymous sexual contacts. Moreover, the capacity of the card to bring contacts to the healthcare system is very sensitive to the awareness of health professionals and the site where they are working (STI unit, Care Programme for Sexual and Reproductive Health or primary healthcare physician), and also depends on whether the population knows about the services provided by each centre. Therefore, there is room for improvement in the healthcare system derivation procedures.

In addition, further strategies should be developed and implemented to maximise the impact of partner notification strategies, such as web-based notification for anonymous contacts using pseudonymous name. Finally, there is a need for a clear partner notification strategy including guidelines on testing procedures according to sexual orientation and sexual practice, and independently of the presence of symptoms.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# ECDC launches the second version of the EPIS-FWD platform

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On 8 July 2013, the European Centre for Disease Prevention and Control (ECDC) will launch a new version of the Epidemic Intelligence Information System for food- and waterborne diseases (EPIS-FWD) with new features that will contribute to multidisciplinary collaboration during FWD outbreak investigations.

As part of its mandate, ECDC identifies, assesses and communicates threats to human health from FWD [1]. ECDC launched in 2010 the first EPIS-FWD, a restricted web-based communication platform bringing together multidisciplinary experts to ensure the early detection and coordination of the response to multi-state outbreaks through the timely sharing of cross-sectorial information [2, 3]. Based on Microsoft SharePoint technology, this system gathers more than 350 epidemiologists, microbiologists but also policymakers and risk managers. The majority of them are from the 27 European Union (EU) Member States and the three European Economic Area (EEA) countries (Iceland, Norway and Lichtenstein); however, experts from Australia, Canada, Japan, New Zealand, South Africa, Switzerland, Turkey and the United States also contribute actively to the information exchange (as a follow-up of Enter-net [3]). For the past three years, EPIS-FWD has proved to be successful in strengthening the collaboration between stakeholders and also in ensuring the timely detection and smooth coordination of the response to food-borne outbreaks [4, 5].

The second version of the EPIS-FWD platform will include, among other things, two new features. The first is the Molecular Typing Cluster Investigations (MTCI), an area dedicated to the assessment of microbiological clusters of *Salmonella*, Shiga toxin-producing *Escherichia coli* (STEC) and *Listeria monocytogenes* infections detected through The European Surveillance System (TESSy). This area is targeted at microbiologists from the EU/EEA countries while the information will be available to epidemiologists from all the affected countries. The second new feature consists of the Urgent Inquiries and Urgent Inquiries associated forums, which are the outbreak alert and investigation tools. The Urgent Inquiries are by default open to the entire EPIS-FWD network (all 38 present members of the network). The Urgent Inquiries associated forums are dedicated areas linked to the Urgent Inquiries to share information about the outbreak investigation among a restricted number of experts. For each forum, experts from the network are invited to contribute. In addition, nominated experts outside the EPIS-FWD network, such as food-safety experts, veterinarians, environmental experts, from the network countries or any expert or organisation outside the network can also be invited to join in a timely manner. These forums may include discussions, questionnaires, working documents for co-editing and line listings. These restricted forums should facilitate the exchange of information between the countries and sectors. In addition, the new version of the EPIS-FWD platform encompasses a geographic information system allowing the display of cases up to the Nomenclature of Units for Territorial Statistics (NUTS) level 3 [6].

EPIS-FWD is part of the EU-wide systems to combat food-borne diseases. Effort should be made to integrate EPIS-FWD with systems such as TESSy, the Rapid Alert System for Food and Feed (RASFF) and the Early Warning and Response System (EWRS), with the aim of strengthening multidisciplinary collaboration and consequently preventing the occurrence of human infections.

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