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Recent evidence of underestimated circulation of hepatitis C virus intergenotypic recombinant strain RF2k/1b in the Rhône-Alpes region, France, January to August 2014: implications for antiviral treatment

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Since the beginning of 2014, hepatitis C virus (HCV) recombinant forms RF2k/1b have been detected in the Rhône-Alpes French region in 10 patients originating from the Caucasus area. Circulation of this particular HCV strain is very likely to be underestimated. It is also prone to be misgenotyped when using genotyping methods based on the 5' region of the viral genome, which may lead to suboptimal treatment.

Here we report the detection of 10 patients infected with a particular recombinant form (RF) of hepatitis C virus (HCV), RF2k/1b, in two virology laboratories in the university hospitals in Lyon and Grenoble in the Rhône-Alpes region, France.

Case description

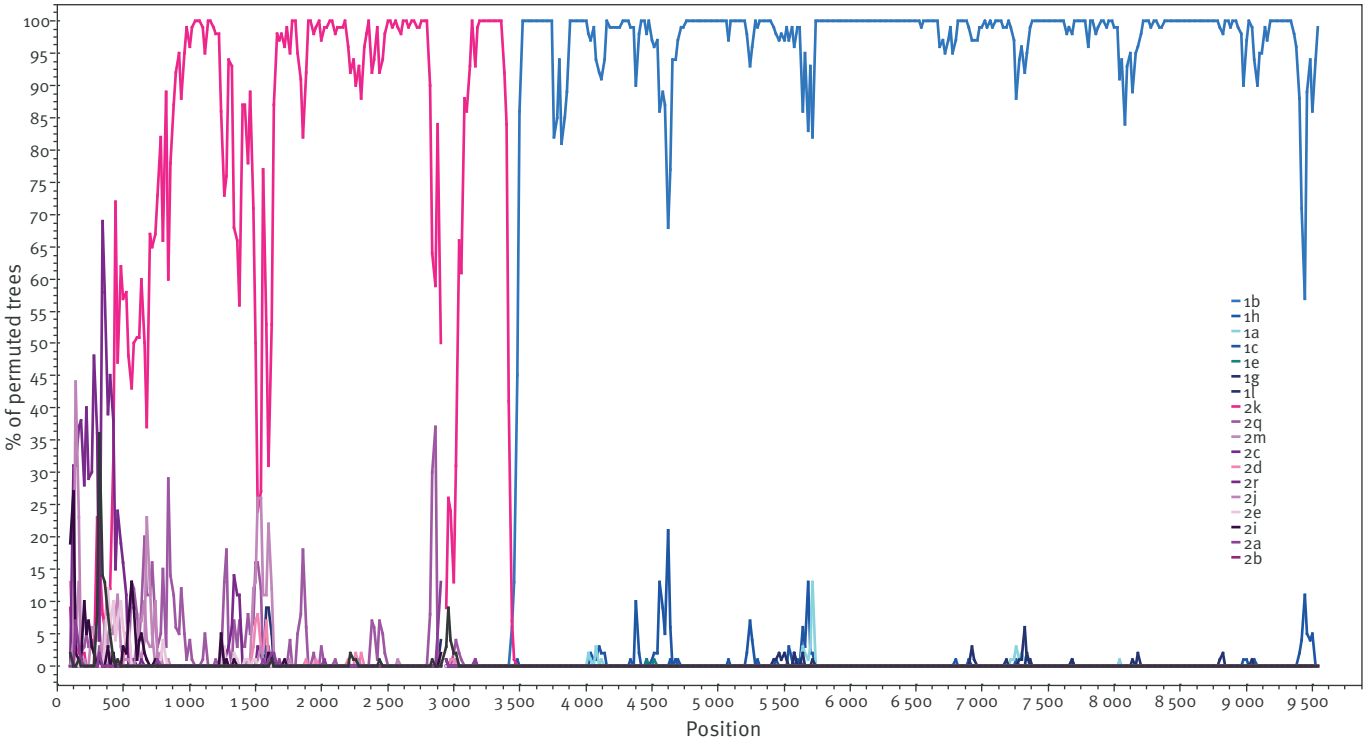
The first RF was identified in a patient in their 50s born in the Caucasus and followed up in a hepato-gastroenterology unit in Lyon for HCV-induced cirrhosis. This infecting virus was first classified as a genotype 2a/2c HCV strain, based on genotyping results using VERSANT HCV Genotype 2.0 Assay Line Probe Assay (LiPA, Siemens) which uses sequence information from both the 5' untranslated and the core regions of the viral genome. According to current European recommendations [1], a three-month course of sofosbuvir and ribavirin dual therapy was initiated in January 2014. Despite a rapid virological response, relapse occurred four weeks after treatment completion. Genotyping was repeated by sequencing of the NS3 region, which resulted in a clear classification as genotype 1b, highly suggestive of the presence of an HCV inter-genotypic recombinant form. To confirm this hypothesis, analysis was completed with near full-length sequencing of the HCV genome using next-generation sequencing (NGS) on the same sample. Results confirmed the presence of an RF2k/1b strain with a breakpoint between genomic regions of genotype 2k and 1b localised in the NS2 region between nucleotide position 3,189 and 3,200 on the H77 reference genome (Figure 1), as previously observed in the reference RF2k/1b genome sequence (GenBank accession number: AY587845) [2].

Genotyping

Following this identification, the virology laboratory in Lyon, as well as the collaborating laboratory in Grenoble, decided to perform two genotyping methods, namely NS3 sequencing and either LiPA 2.0 or core sequencing, for each new genotyping request when one of the following criteria were met: patients with previous treatment failure infected with HCV genotype 2 (based on previous LiPA 2.0 results), NS3 or NS5B sequence clustering with available RF sequences in GenBank or patients born in Russia or in the countries of the former Soviet Union, as these regions represent the major source of circulating HCV recombinants described to date. This strategy led to the detection of the RF2k/1b strain in nine additional patients (two in Grenoble and seven in Lyon). Of these 10 patients, six were born in Georgia, three in Armenia and one in Azerbaijan. Five patients were people who inject drugs (PWID) or had been in the past. Two patients had a history of surgical interventions performed in their home country. For the remaining three patients, putative ways of transmission were unclear. Based on anamnestic data, it was very likely that they had acquired

Figure 1

Bootscan plot of percentage permuted trees over nucleotide positions for the recombinant hepatitis C virus RF2k/1b strain NA_Armenia, isolated in France, May 2014



Trees were constructed with Kimura 2-parameter distances and the neighbour-joining algorithm. Subtypes with their corresponding colour code are indicated on the right.

HCV before their migration to France, but it was not possible to be absolutely certain on this point for two patients. At the time of the study, treatment had not yet been initiated in any of the nine additional patients, and a survey to test potential contacts, particularly in PWID, was still underway.

When HCV genotyping using LiPA 2.0 was performed, four or five positive bands were detected (numbers 5, 9, 10, 11, +/-12) leading to classification of the strains as genotype 2 or 2a/2c. Phylogenetic analysis of core sequences was performed for seven patients, and of NS3 sequences for all 10 patients (Figure 2). Core sequencing confirmed that the 5' fragment of these genomes belonged to genotype 2k (Figure 2a), whereas NS3 sequencing led to the classification of the 3' fragment of the viral genome region as 1b (Figure 2b). Overall, core and NS3 sequences from all strains clustered with previously described RF2k/1b and were distinct from reference sequences of non-recombinant genotype 2k and genotype 1b, respectively. GenBank accession numbers for all sequences obtained in this study are indicated in the Table.

Since the beginning of 2014, HCV from 21 patients originating from the Caucasus (i.e. born in Armenia, Azerbaijan or Georgia) have been genotyped in the virology laboratory in Lyon: eight were RF2k/1b, eight were of genotype 1b, four of genotype 3a and one of genotype 4a. Patients in Grenoble are not described here in detail as some of their demographic data were incomplete.

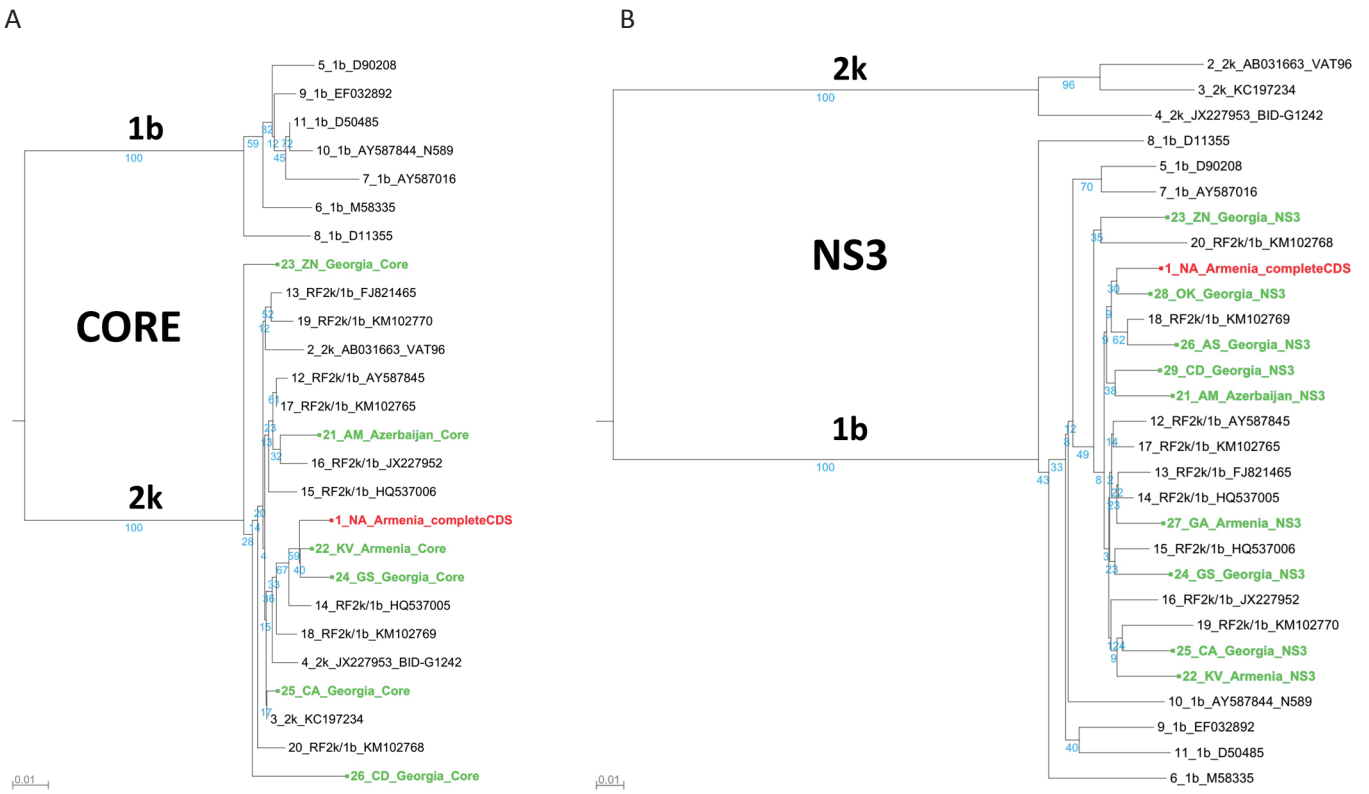
Discussion

The first natural HCV recombinant form, RF2k/1b, was described in Saint Petersburg in 2002 [3]. Since then, 17 recombinant forms of HCV have been identified worldwide [2,4], but the RF2k/1b strain is the only circulating recombinant form for which several isolates with a supposed common origin have been described. Recombinant forms probably emerged in patients exposed to multiple HCV strains [5]. Concerning RF2k/1b, the time of its emergence has been estimated to be between 1923 and 1956, which coincides with the development of blood donation centres in the former Soviet Union [6]. However, despite a high rate of mixed infections among certain risk groups, in particular PWID and haemophiliacs, recombinant strains appear to constitute the minority among HCV circulating strains, probably due to the constraints of viral replication [6].

Among our patients, the RF2k/1b strain was, along with 1b, the most frequent genotype detected in patients

Figure 2

Phylogenetic analysis of hepatitis C virus RF2k/1b strains identified in this study and reference strains from GenBank, France, January–August 2014 (n=16)



Two dendrograms are presented based on 423 nt of the core region (A) and 465 nt of the NS3 region (B). Both analyses were performed using MAFFT software (version 7).

originating from the Caucasus area. Thus the recombinant RF2k/1b strain seems to have spread widely and appears to be one of the major strains infecting patients from these countries. Moreover, this RF was identified in 10 new patients during a single eight-month period, whereas only 37 isolates of this particular strain have been reported in GenBank to date since the first description in 2002 [2,4]. Based on these observations, it can be hypothesised that the number of patients infected with the RF2k/1b HCV strain (and maybe other recombinant HCV forms) is underestimated. During the study period, HCV genotyping has been verified by NS3 sequencing in 16 patients not born in the Caucasus region who were infected with HCV previously classified as genotype 2 by either LiPA or sequencing of the 5' non-coding region. No RF was detected in any of these patients; however, it cannot be excluded that this strain has spread outside of the Caucasus population. Indeed, the spread of these RF in Western European countries is poorly described and only a few previous reported cases of HCV RF2k/1b are available in studies from Cyprus, France, Ireland and the Netherlands [6-9]. In all these cases, the patients had their origin in Russia or Georgia. Supplementary studies will be necessary to determine the frequency of this strain in France and other European regions, in particular among PWID.

HCV genotyping remains an essential criterion when considering the choice of antiviral treatment protocols, and the circulation of recombinant HCV strains must be taken in account. Even when using new highly efficient anti-HCV direct acting agents (DAA) such as sofosbuvir, misgenotyping may lead to a suboptimal treatment choice and eradication failure, as illustrated by the case reported here. Moreover, it has been shown in a recent study by Hedskog et al. that the response to the sofosbuvir and ribavirin combination, a regimen for HCV genotype 2, was similar in patients infected with different HCV RF2/1 recombinant forms and in patients infected with genotype 1 [4]. In particular, three of the four patients infected with HCV RF2k/1b in the study by Hedskog et al. relapsed following treatment with this regimen. HCV genotyping methods based on sequencing of portions of the NS3–3'UTR region of the genome should therefore be recommended and standardised, and particular attention should be paid to patients from the Caucasus region.

Table

GenBank accession numbers for hepatitis C virus sequences used in the phylogenetic analysis of this study, France, January–August 2014 (n=16)

Patient	GenBank accession number	
	Core	NS3
AM_Azerbaijan	KM591900	KM591886
KV_Armenia	KM591899	KM591887
ZN_Georgia	KM591898	KM591888
GS_Georgia	KM591897	KM591889
CA_Georgia	KM591896	KM591890
AS_Georgia	ND	KM591892
GA_Armenia	ND	KM591893
OK_Georgia	ND	KM591894
CD_Georgia	KM591895	KM591891
Near full-length genome		
NA_Armenia	KM495736	

ND: not done.

Conflict of interest

None declared.

Authors' contributions

Christophe Ramière, Pauline Tremeaux, Mary-Anne Trabaud, Patrice André, Sylvie Larrat performed laboratory diagnostics and viral characterisation. Christophe Ramière, Pauline Tremeaux, Alban Caporossi, Marie-Ange Thélu, Patrice Morand, Patrice André, Sylvie Larrat performed sequencing data analysis. Fanny Lebossé, François Bailly, Jean Nana, Vincent Leroy provided clinical care. Christophe Ramière, Pauline Trémeaux, Patrice Morand, Patrice André, Sylvie Larrat wrote the paper. All authors reviewed the manuscript before submission.

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Exceptionally low rotavirus incidence in the Netherlands in 2013/14 in the absence of rotavirus vaccination

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An unexpected drop in rotavirus (RV) detections was observed in the Netherlands in 2014, without RV vaccination. The estimated decrease in RV detections and gastroenteritis consultations in under five year-olds, in January–April 2014, compared to the same months in previous years, was 72% and 36%, respectively. The low birth rate, mild winter, high RV incidence in the previous year and the introduction of RV vaccination in neighbouring countries may have contributed to this decrease.

We describe an unexpected and large decrease in rotavirus (RV) detections in the Netherlands in the winter of 2013/14 compared to previous years. We corroborated this finding with our analysis of syndromic disease data on acute gastroenteritis (GE) in children under five years old, in which we also found a reduction and no sign of the usual peak in March. We believe that our findings are of great importance to the European public health community to alert other countries on the unusual RV epidemiology of the 2013/14 winter.

Background

RV is a leading cause of GE in infants and young children. A number of European countries have recently implemented universal RV vaccination, including neighbours of the Netherlands (Belgium in 2006, UK (UK) and Germany in 2013) [1]. In the Netherlands, however, RV vaccination is not part of the national vaccination programme and a national recommendation for its use has not been issued so far. On average, only 41 doses were distributed per year between 2011 and 2014 (personal communication, Truus Maessen, IP International Pharmacy GmbH, September 2014).

RV incidence in the Netherlands usually follows the typical seasonal pattern observed in temperate climates with an annual epidemic during winter months, peaking in February–March [2]. Up to 2014, RV was responsible for 3,300–4,800 hospitalisations annually in children under five years old, of which 85% occurred between January and April with the highest incidence in those aged between six months and two years [3,4].

Virological surveillance data showed an unexpected low number of RV detections in the Netherlands in the winter of 2013/14. We studied this observation to assess whether it reflects a decreased circulation of RV and to discuss implications for the control of RV in the Netherlands and beyond.

Epidemiological surveillance for rotavirus infection, data collection and analysis

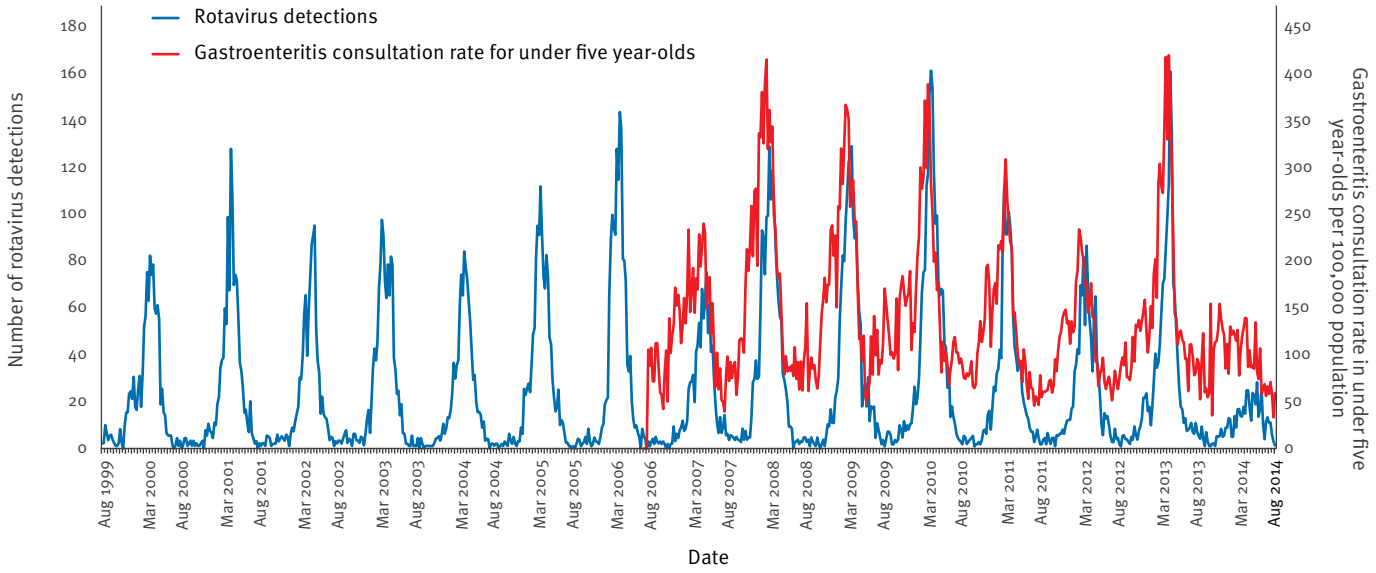
We studied RV laboratory detections from virological laboratory surveillance and all-cause GE consultations in under five year-olds from a Dutch sentinel General Practice (GP) network.

The virological surveillance collects weekly numbers of virus detections from between 17 and 21 virological laboratories registered with the Dutch Working Group for Clinical Virology (NWKV), serving primary care, hospitals and long-term care facilities. RV diagnostic testing is not routinely recommended for suspected infectious GE, but stool tests are performed in approximately two-thirds of children admitted for GE and in 10% of children with GE visiting primary care [3]. We analysed data from 1999 to 2014 by RV epidemiological year, defined as running from August to July of the following year.

We analysed GP consultations for all- cause GE in under five year-olds using routine electronic health record data from general practices participating in the NIVEL

Figure

Weekly rotavirus detections[a] (August 1999–August 2014) and general practice gastroenteritis consultation rate for children under five years old (August 2006–August 2014), the Netherlands



[a] Adjusted for the weekly number of reporting laboratories by multiplying the number of rotavirus detections by the average number of reporting laboratories / the number of laboratories reporting that week.

Primary Care Database (NIVEL-PCD). Here, data were available from August 2006 to July 2014. Consultations are coded according to the International Classification of Primary Care (ICPC) [5]. Data are provided by 240 general practices covering a population of 1 million people, ca 5.5% of the Dutch population. Every Dutch citizen is obliged to be registered with a general practice. The GP acts as a gatekeeper for specialised, secondary healthcare. The electronic medical records kept by the GP, therefore, provide the most complete picture of the population's health.

Descriptive statistics were used to summarise data by RV epidemiological year and by seasonal month. Time-series analysis was performed to assess trends in weekly counts of RV detections and in GE consultations in under five year-olds, and to compare the 2013/14 RV season (January–April) to the same months in previous years. Negative binomial regression models were fitted to each data source separately. We adjusted the models for seasonal patterns (adding an indicator variable for month), for potential long-term linear trends and for variations in the number of reporting laboratories or person-time under observation, respectively. Both models were corrected for residual autocorrelation by adding lagged residuals.

Results

The weekly number of RV detections and the all-cause GE consultation rate in children under five year-old correlate well and were both much lower between August 2013 and August 2014 than in previous years (Figure).

Between August 1999 and July 2013, the average annual number of RV detections in virological surveillance, adjusted for the number of reporting virological laboratories, was 1,362 (range: 1,001–2,000). The adjusted number of RV detections between August 2013 and July 2014 was 570 (a 58% drop). This RV epidemiological year registered by far the lowest number of RV detections in the entire time series. Reductions were most pronounced during February and March 2014 when the number of adjusted RV detections were down by 75% and 88%, respectively. In contrast, an elevated number of RV detections was observed in July 2014 compared to previous years (Table). The peak in weekly RV detections in 2014 was shifted to May, whereas the peak was usually observed in March in previous years.

The mean weekly GE consultation rate in under five year-olds in the GP sentinel surveillance for the RV epidemiological years 2006–2013 was 152 per 100,000 person-weeks in under five year-olds (range: 111–201). The mean consultation for the 2013/14 season was 97 per 100,000 (a 36% decrease). In accordance with RV laboratory detections, the decrease in GE consultations was most pronounced during February (55%) and March (61%) (Table). Furthermore, there was no sign of the usual peak in February–March in GE consultations.

In time-series analyses, both the model for RV detections and for the GE consultation rates confirmed significantly lower activity in the 2013/14 season compared to previous years ($p < 0.0001$). The mean estimated decrease in RV detections for the 2014 winter season (January–April) compared to the same period in

Table

Weekly rotavirus detections and general practice gastroenteritis consultation rate by month, the Netherlands, 2013/14 (August–July) compared to previous years

Month	Weekly mean number of RV detections[a]				Weekly mean rate of GE consultations[b] in children under five years old per 100,000 population			
	Aug 1999–Jul 2013	Aug 2013–Jul 2014	Change in 2013/14 (%)	P-value[c]	Aug 2006–Jul 2013	Aug 2013–Jul 2014	Change in 2013/14 (%)	P-value[c]
Aug	3.0	5.3	+80	0.0910	90.6	69.4	-23	0.0218
Sep	3.3	5.4	+63	0.3299	86.2	79.7	-8	0.6515
Oct	3.2	1.8	-44	0.0626	89.7	83.3	-7	0.8065
Nov	5.0	3.7	-26	0.2408	139.3	99.4	-29	0.0703
Dec	11.9	6.9	-42	0.0046	171.4	135.3	-21	0.0059
Jan	25.7	8.8	-66	<0.0001	180.7	105.7	-42	0.0001
Feb	58.2	14.1	-76	<0.0000	269.8	122.3	-55	<0.0001
Mar	85.2	15.0	-82	<0.0001	277.2	107.4	-61	<0.0001
Apr	70.4	19.8	-72	<0.0001	205.4	117.5	-43	0.0006
May	31.3	21.4	-32	0.0103	112.3	95.3	-15	0.1996
Jun	12.5	14.3	+15	0.7158	95.3	75.5	-21	0.1675
Jul	5.1	11.7	+131	<0.0001	88.5	62.3	-30	0.0001

GE: gastroenteritis; RV: rotavirus.

The usual RV season (January–April) is highlighted.

[a] Adjusted for the weekly number of reporting laboratories by multiplying the number of rotavirus detections by the average number of reporting laboratories / the number of laboratories reporting that week.

[b] International Classification of Primary Care (ICPC) code D73.

[c] Derived by performing t-tests.

2000–2013 was 72% (95% CI: 59–81%). The estimated decrease in GE consultation rate for the 2013/14 winter season, which was adjusted for the presence of a significant linear time trend, was 36% (95% CI: 17–50%).

Discussion

We observed an exceptionally low number of RV detections and a low primary care GE consultation rate in children under five years of age, with nearly complete absence of winter excess, during the period of the typical RV season in 2014. This is a striking finding in the absence of RV vaccination in the Netherlands. The observation that the decrease was found in both datasets indicates that the low number of RV detections is not a surveillance artefact, and likely reflects reduced RV circulation. Our RV data did not include the number of RV tests performed. However, there have been no changes in diagnostic guidelines or reimbursement policy that could have impacted RV testing practices in 2014 compared to previous years.

Potential contributing mechanisms for the reduced RV circulation in 2013/14 include the mild winter, the relatively high RV epidemic season in the previous year, a low birth rate and, possibly, RV vaccination programmes in the neighbouring countries [6–8].

RV transmission can be affected by weather-related differences in human behaviour or virus survival. For the Netherlands, it has been estimated that for every degree Celsius rise in temperature above a threshold of 4 degrees the number of symptomatic RV infections in the Dutch population decreases by 9%. Winter 2013/14 was unusually mild in the Netherlands: the average daily temperature was 2.6 degrees above the average of 3.4ºC, while four out of five preceding winters were considerably colder than the average [9]. This may have contributed to reduced RV transmission in 2013/14.

The recent history of the intensity of RV-seasons is a likely determinant of the future incidence, through depletion of susceptibles. In our RV time-series, this effect is not clearly discernible in the pattern of high and low epidemic peaks. Nevertheless, the relatively high incidence in 2012/13 is likely to have contributed in this manner to the low incidence in 2013/14. The 2013 introduction of RV vaccination in the UK and Germany may also have contributed to a reduction of RV circulation in the Netherlands by reducing the number of introductions of RV into the Netherlands. In Germany, the RV vaccine has been used in some eastern federal states since 2006, with evidence of local impact [10]. Notifications dropped by 36% in 2014 compared to 2013 [11]. This suggests that coverage increased only moderately since the introduction of a national RV vaccine recommendation. Furthermore, we are unaware of reports of such effects in other countries bordering regions with universal infant RV vaccination and no decline in RV activity in the Netherlands was observed after Belgium introduced RV vaccination in 2006 with immediate high uptake. In France, a country without routine RV vaccination where between

5 and 10% of infants are vaccinated each year in the private sector, the threshold for epidemic GE was, for the first time since 1992, not reached in the winter of 2013/14. This suggests that RV transmission was low in France also (personal communication, Daniel Lévy-Bruhl, September 2014).

Mathematical modelling studies have suggested that recruitment of susceptible infants, as determined by birth rate and RV vaccination coverage, is a main determinant in timing of RV season and in generating annual or biennial epidemics [6]. In the Netherlands, the birth rate has dropped to an all-time low of 10.2 per 1,000 population in 2013 [12]. This may now have reached a level at which timing of epidemics is shifted towards April–May and occur biennially, similar to the pattern observed in the United States, where recruitment of susceptibles is nowadays diminished due to the widespread use of RV vaccination.

Circulation of an unusual RV strain causing relatively mild disease could also result in lower numbers of RV related hospitalisations and GP visits. However, RV genotype surveillance for the 2013/14 season did not demonstrate an abnormal pattern of RV strains in the Netherlands. G1P[8] was the dominant strain until 2012. Since 2012 a varying mixture of G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8] has been observed (unpublished data).

Conclusion

The incidence of RV infections was exceptionally low in the Netherlands in the winter of 2013/14 in the absence of RV vaccination. This finding is relevant to countries assessing the impact of RV vaccination. Data from other European countries with and without RV vaccination and mathematical modelling are needed to provide further insight in determinants of low and high epidemic years. The risk of a compensatory hyperepidemic RV season in the coming year(s) needs to be urgently assessed, to allow adequate hospital bed capacity management and to inform RV vaccination policy [13].

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Conflict of interest

None declared.

Authors' contributions

Designed the study: SH, PB-V, WvP. Collected, synthesised and analysed data: SH, PB-V, MH, AvG. Wrote the first draft: SH, PB-V. Interpreted the results and revised the article: SH, MH, PB-V, JW, AvG, WvP, HV, HdM. All authors read and approved the final manuscript.

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Identification of verocytotoxin-producing *Escherichia coli* O117:H7 in men who have sex with men, England, November 2013 to August 2014

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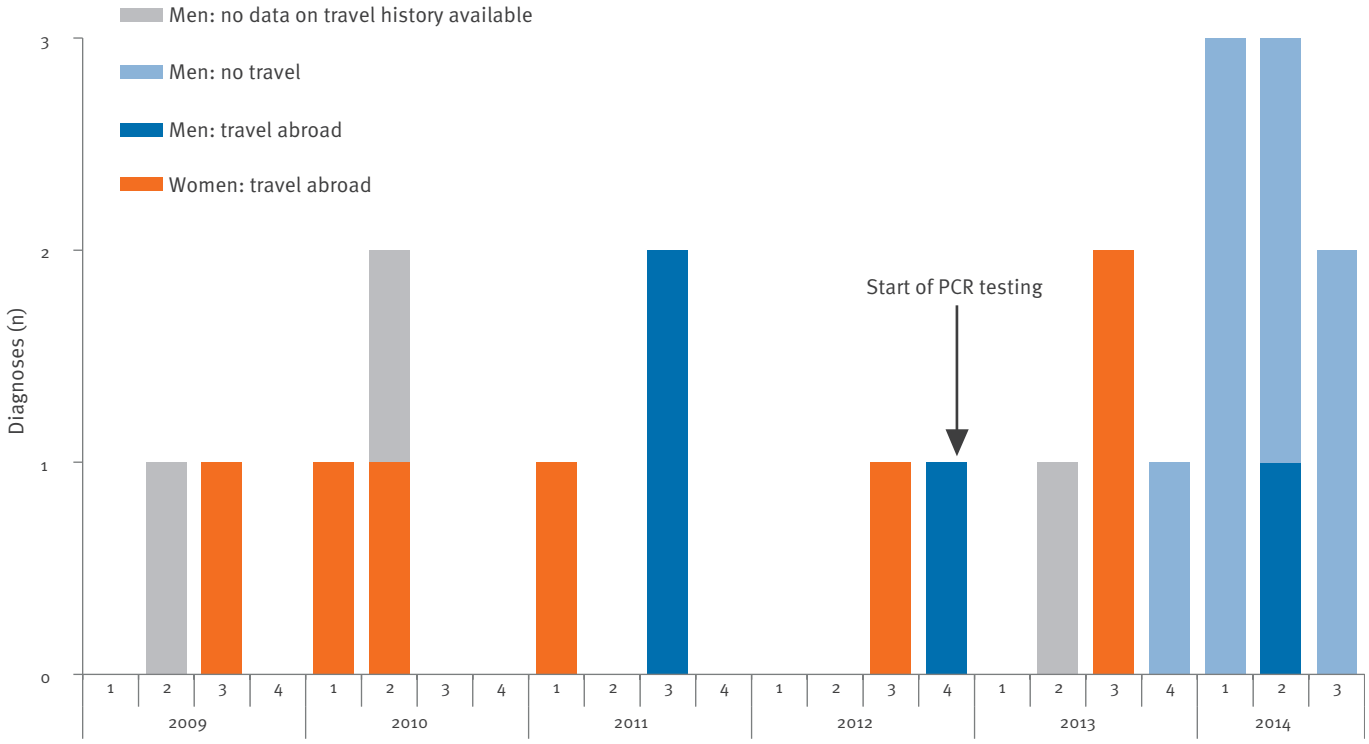
Between November 2013 and August 2014, nine cases of verocytotoxin-producing *Escherichia coli* O117:H7 VT1 were confirmed in adult men. Further investigation using semi-structured interviews revealed that eight cases were United Kingdom (UK)-born men who have sex with men (MSM) who had sexually acquired infection in the UK. Most were HIV-positive with high numbers of sexual partners. This behavioural profile resembles that associated with the recent rapid increase in other sexually acquired infections in MSM.

Few diagnoses of verocytotoxin-producing *Escherichia coli* (VTEC) O117:H7 VTI are reported each year in England. Between 1 January 2009 and 20 November 2013, Public Health England's (PHE) Gastrointestinal Bacteria Reference Unit (GBRU) confirmed just 13 cases of VTEC O117:H7 VT1 (Figure) compared with 4,050 cases of VTEC O157 in the same period. Travel history was available for 10 of the 13 cases and all reported recent travel to countries with a high prevalence of VTEC O117:H7 [1,2].

Figure

Diagnoses of verocytotoxin-producing *Escherichia coli* O117:H7 VT1, by sex and history of travel abroad, England, 2009 to 2014 (n=22)



In June 2014, GBRU detected an increase in VTEC O117:H7 VT1 diagnoses with nine cases confirmed since 21 November 2013 (Figure). All were adult men, of whom three were referred from genitourinary clinics and only one reported recent travel. An incident control team was established on 4 July 2014 to investigate this increase, the clinical presentation of cases and possible links with the ongoing *Shigella flexneri* 3a outbreak in men who have sex with men (MSM) [3-5].

Incident investigation

On 19 June, 204 laboratories in England were alerted to the increase in VTEC O117:H7 VT1 and asked to refer all presumptive *S. sonnei* isolates to the GBRU. Cases of reported VTEC infection were investigated in line with PHE standard operating procedures [6]. In addition to the routine enhanced surveillance questionnaire for VTEC, all men older than 18 years diagnosed with VTEC O117:H7 VT1 after 1 November 2013 were invited to take part in confidential semi-structured face-to-face interviews with a sexual health advisor after patient consent had been obtained by the local Public Health Office. The interviews lasted 1.5 hours and explored the men's lifestyle and sexual behaviour, focusing on the two weeks before the onset of symptoms, i.e. the period within which infection had been acquired. Demographic data and information on sexual behaviour were collected, together with details of where they met sexual partners, recreational drug use, engagement with health services, previous history of sexually transmitted infections (STI) and testing for human immunodeficiency virus (HIV). For HIV-positive men, the most recent CD4 T-cell count, viral load and current treatment regime were also discussed.

Results

Between 21 November 2013 and 21 August 2014, nine cases of VTEC O117:H7 VT1 were identified, none of which had evident common food, water or animal exposures. One of them was a heterosexual man who had recently travelled to South America. The remaining eight were MSM, seven of whom consented to be interviewed. The median age of the seven men was 46 years (range: 33–50 years), most of whom (6/7) were of white ethnicity. Six men were in a relationship and three of whom cohabited with their male partner. All had open relationships and were reported from London and Brighton, cities that have large, vibrant gay communities.

In the two weeks before onset of illness, the seven men reported a median of five partners (range: 2–15) and in the previous year, a median of 40 partners (range: 5–480). There was no evidence that any of the sexual contacts were cases. For the two weeks before onset, all seven men reported unprotected oral-anal contact with casual partners, they also reported receptive fisting (2/7), insertive fisting (3/7) and scat play (1/7). Four men had met sexual partners through social media using geo-spatial networking applications that allow users to locate and meet men with similar sexual interests within close proximity. Two men had attended sex parties during that time. Three of the seven men reported chemsex, i.e. having sex under the influence of one or more of the following drugs: mephedrone, crystal methamphetamine (crystal meth), gamma-hydroxybutrate (GHB) and gamma-butyrolactone (GBL). These drugs, which are taken immediately before and/or during sex, facilitate sexually disinhibiting behaviour and increased sex drive. Although the drugs can be injected, none of the men reported this practice [7].

Three men were HIV-positive and on therapy. A history of a range of STIs including lymphogranuloma venereum (LGV) (n=3), gonorrhoea (n=4), syphilis (n=1) and chlamydia (n=6) was reported within the past five years. All seven men had heard of *E. coli* but only three had seen the recent shigellosis information campaigns by PHE and Terrence Higgins Trust (www.tht.org.uk). Although one man experienced acute infection with bloody diarrhoea, others described more chronic illness with symptoms of mild stomach cramps and fatigue which generally lingered for several weeks, one man seeking medical advice after a month. The men sought medical advice from their general practitioner or genitourinary medicine clinic. One man was subsequently admitted to hospital.

Discussion

Initial findings from this ongoing investigation detail the first cluster of VTEC O117:H7 VT1 to be described in MSM. There was no evidence that this was part of a generalised epidemic or that the observations were the result of sampling bias. VTEC O117:H7 may be misidentified as *S. sonnei* by local diagnostic laboratories as there are similarities in colony appearance and biochemical profile. Such misidentifications are detected when strains are referred for confirmation and typing at the GBRU. Since December 2012, a small number of laboratories, including services in Brighton and central London have adopted polymerase chain reaction (PCR) techniques to detect verocytotoxin genes in stool samples, resulting in an increased number of diagnoses of non-O157 VTEC, including serogroup O117 [3].

This strain does not possess the pathogenicity factor intimin and the stx2a shiga-toxin subtype which are known to be associated with more severe disease and progression to haemolytic-uraemic syndrome (HUS) [3]. Previous clusters have mainly been travel-associated and limited to around three cases [1]. Although the small sample size restricts interpretation, we have shown that recent diagnoses of VTEC O117:H7 VT1 in England have been sexually acquired by highly sexually active MSM born in the United Kingdom, some of whom were HIV-positive and took chemsex drugs. This behavioural profile resembles that of LGV, infectious syphilis and shigellosis epidemics in England [4,8,9]. Such overlapping epidemics, sustained by closely related sexual networks facilitated by geo-spatial social networking applications, allow hyperefficient transmission, an environment in which infection

control has been difficult to achieve. Infections have become endemic despite increased case finding and proactive campaigns (www.tht.org.uk/shigella) to raise awareness about *Shigella* and provide hygiene advice through advertisement in the gay press, magazines, leaflets in clinics and general practitioners' surgeries as well as pop-up banners on internet sites.

Conclusion

This new cluster further highlights the importance of enhancing and strengthening measures to decrease faecal-oral transmission of infection and increase awareness amongst MSM. This is of particular importance and a cause for concern should a more pathogenic VTEC be introduced into this high-risk group. The public health response to VTEC O117:H7 VT1 has been combined with ongoing initiatives aimed at improving the health and wellbeing of MSM and promoting access to health services [10].

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Conflict of interest

None declared.

Authors' contributions

All the authors contributed to the initiation of the project, designing the methods used as well as the data analysis and interpretation. All the authors contributed to writing the manuscript and have all read and agreed the final draft.

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Control of carbapenemase-producing *Klebsiella pneumoniae*: a region-wide intervention

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Starting in 2010, there was a sharp increase in infections caused by *Klebsiella pneumoniae* resistant to carbapenems in the Emilia-Romagna region in Italy. A region-wide intervention to control the spread of carbapenemase-producing *K. pneumoniae* (CPKP) in Emilia-Romagna was carried out, based on a regional guideline issued in July 2011. The infection control measures recommended to the Health Trusts (HTs) were: phenotypic confirmation of carbapenemase production, active surveillance of asymptomatic carriers and contact isolation precautions for carriers. A specific surveillance system was activated and the implementation of control measures in HTs was followed up. A significant linear increase of incident CPKP cases over time (p<0.001) was observed at regional level in Emilia-Romagna in the pre-intervention period, while the number of cases remained stable after the launch of the intervention (p=0.48). Considering the patients hospitalised in five HTs that provided detailed data on incident cases, a downward trend was observed in incidence after the release of the regional guidelines (from 32 to 15 cases per 100,000 hospital patient days). The spread of CPKP in Emilia-Romagna was contained by a centrally-coordinated intervention. A further reduction in CPKP rates might be achieved by increased compliance with guidelines and specific activities of antibiotic stewardship.

Introduction

The emergence and spread of carbapenemase-producing Enterobacteriaceae (CPE) has been observed throughout the world [1–6]. Infections caused by CPE, a group of organisms characterised by extensive resistance to antimicrobials, are very difficult to treat, with limited therapy options [2,3,6,7]. The first case of carbapenemase-producing *Klebsiella pneumoniae* (CPKP) in Italy was detected in October 2008 [8]. In 2011, the European Antimicrobial Resistance Surveillance Network (EARS-Net) reported that Italy was one of the most affected countries in Europe, with a worrisome increasing trend in CPKP [9].

Several studies have convincingly demonstrated that aggressive control measures are effective in halting the spread of CPE in healthcare institutions [2,3,10-14]. So far, Israel is the only setting with high transmission rate of CPKP where a nationwide intervention has been effectively implemented. The Israeli control activities were based on cohorting CPE carriers and creating dedicated staffing in hospital; the intervention was monitored at national level by a central authority and a task force was created to collect data from hospitals and to participate locally to the outbreak control [14]. This paper describes the impact of a regional intervention to control CPKP and other CPE implemented in Emilia-Romagna, Italy.

The regional setting

Emilia-Romagna is a northern Italian region of 4.4 million inhabitants. The Regional Public Health System includes 17 Health Trusts (HTs) with 60 hospitals and about 550,000 hospital admissions per year. Isolation facilities for infected or colonised patients are unevenly distributed in the HTs of Emilia-Romagna, with some older hospitals having few single rooms.

A regional antimicrobial resistance surveillance system, established in 2003, is based on twice-yearly electronic transfer of microbiological tests performed in public hospital laboratories [15]. The representativeness of the system was estimated to be about 90% of bacterial cultures performed in public hospitals, covering the vast majority of cultures for diagnosis of invasive infections, both in hospital and the community [15]. The proportion of carbapenem-non-susceptible *K.*

pneumoniae isolates from blood increased from 2% in 2009 to 21% in 2011 [15].

Methods

Infection control measures

In July 2011, a regional guideline for the control of CPKP and other CPE was issued (subsequently updated in January 2013) [16]. The guideline was supplemented by a leaflet that informed hospital patients colonised with CPE and their caregivers of the actions to be taken to prevent transmission, as well as specific recommendations for the management of CPE-infected or colonised patients in the community and in long-term care facilities (LTCFs) [17].

The documents were issued after two months of consultations involving all representatives of infection control (IC) teams in the 17 HTs in the region, and other experts in the fields of microbiology, infectious diseases and risk management.

The following infection control measures were recommended:

- CPE diagnosis by phenotypic confirmation of carbapenemase production, by modified Hodge test or by a disk-diffusion synergy test including meropenem and two carbapenemase-inhibiting compounds (dipicolinic acid and boronic acid) [18,19];
- active surveillance of CPE asymptomatic carriers by rectal swabs for close contacts of CPE hospitalised patients (patients staying in the same hospital unit), high-risk patients at hospital admission (i.e. patients transferred from other acute hospitals and LTCFs or coming from endemic countries), and, only for hospitals where CPE were endemic (with sustained intra-facility transmission) or where epidemic clusters were detected the previous year, patients admitted to intensive care units, spinal units, transplant units, oncology and hematology units. CPE screening of carriers was not recommended in LTCFs;
- contact isolation precautions for all CPE infected patients and asymptomatic carriers, during their stay in hospital. It was strongly recommended, where possible, to place these patients in a single room or to cohort them with other CPE-infected patients or asymptomatic carriers; otherwise, they were accommodated in a room with non-carriers and contact precautions were applied. Staff cohorting was recommended and, if this was not feasible, it was recommended that each case be assigned a manager nurse responsible for checking that all health care workers and visitors applied contact precautions. Health Trust general directors were requested to assure the monitoring of compliance with standard and contact precautions and the scheduling of educational activities for health workers aimed at improving infection control skills;
- communication of CPE presence at the time of patient transfer (to receiving institution for patients transferred to other hospitals or to LTCFs, and to the general practitioner for patients returning home).

The guideline, although not explicitly promoting antimicrobial stewardship, recommended using antibiotics sparingly and encouraged laboratories to attach notes to the microbiological results inviting clinicians to carefully evaluate the need for antibiotic treatment.

Specific surveillance

From June 2011, all HTs were asked to send a monthly report to the Regional Agency for Health and Social Care, with the aggregated number of prevalent cases stratified by patients with bacteraemia, patients with other infections and asymptomatic carriers identified by rectal swabs. Data, referring to all patients (hospitalised or not) diagnosed in the area of competence of the HT, were reported separately for public hospitals, private hospitals, LTCFs and other community settings. A report was returned monthly to all HTs. Five HTs, those where CPE were endemic or placed in the same area of an endemic hospital, were asked to provide more detailed monthly reports that differentiated, for hospitalised patients, CPKP incident cases from already known CPKP cases.

Follow-up of implementation of regional recommendations

In the period August-September 2011, after the release of regional guidelines for CPE control, all HTs were requested to confirm through an official written statement that they had produced local operational protocols based on the recommendations of the regional guidelines. A questionnaire-based survey was conducted in May and June 2012 to evaluate the actual implementation of regional guidelines. Individualised feedback, based on the questionnaire results and observed epidemiological trends, was delivered in October 2012 to the directors of the eight HTs which had an average of 2 or more cases of CPE infections per month. The feedback reports pointed out potential failures in the implementation of control measures and provided suggestions for improvement. The HT directors were asked to check for actual implementation of screening activities and of contact precautions in hospitals under their responsibility, by using available data or performing ad hoc audit.

Statistical Analysis

Stata 10.1 (Stata Corporation, College Station, Texas) was used for statistical analysis. The weekly trend line of incident cases of carbapenem-non-susceptible *Klebsiella pneumoniae* (CRKP) in the period 2009–2012 was smoothed by the moving average method (13-period moving average: each point representing the same week and the previous 12). An incident case was defined as the first-ever CRKP isolated in a subject. Analysis of covariance (ANCOVA) was used to compare the difference between slopes of linear

regression of incident cases over time. The time frame up to the 22nd week of 2011 was considered to be the pre-intervention period, while the period starting from the 31st week of 2012 was considered to be the post-intervention period; a window of eight weeks (from the 23rd to the 30th week), during which the HTs produced and implemented local protocols based on the regional guidelines, was removed from this particular analysis.

Multivariate linear regression was performed to evaluate temporal trends and the correlation among independent variables and the monthly CPKP incidence rate in five HTs. The unit of observation was HT-month and a significance level of 0.05 was used. In order to account for the likely correlation among observations coming from the same HT, a multilevel linear regression was also performed, introducing the HT as a random effect variable. However, since the results thus obtained were similar to those yielded by the simpler model, the former is presented here.

Results

Infection trends

The regional system for surveillance of antimicrobial resistance displayed a swift upward incidence trend in the weekly number of patients with carbapenem-resistant *Klebsiella pneumoniae* (CRKP), including isolates from all cultures other than gastrointestinal ones, during 2010 and the first half of 2011. The trend remained stable in the second half of 2011 and the first quarter of 2012, then showed a slight increase in the mid-2012 with a subsequent return to the previous rate (Figure 1). Running two separate linear regressions of incident cases over time, a slope significantly higher than zero ($p<0.001$) was obtained for the pre-intervention period (estimate: 0.10, 95% CI: 0.09–0.12), and a slope not significantly different than zero ($p=0.48$) for the post-intervention period (estimate: 0.02, 95% C.I.: -0.04; 0.08). An analysis of covariance indicated a significant ($p<0.01$) difference in the magnitude of the two slopes. Considering hospitalised patients, the incidence rates

Figure 1

Incident cases of carbapenemase-producing *Klebsiella pneumoniae*, Emilia-Romagna, Italy, 2009–2012

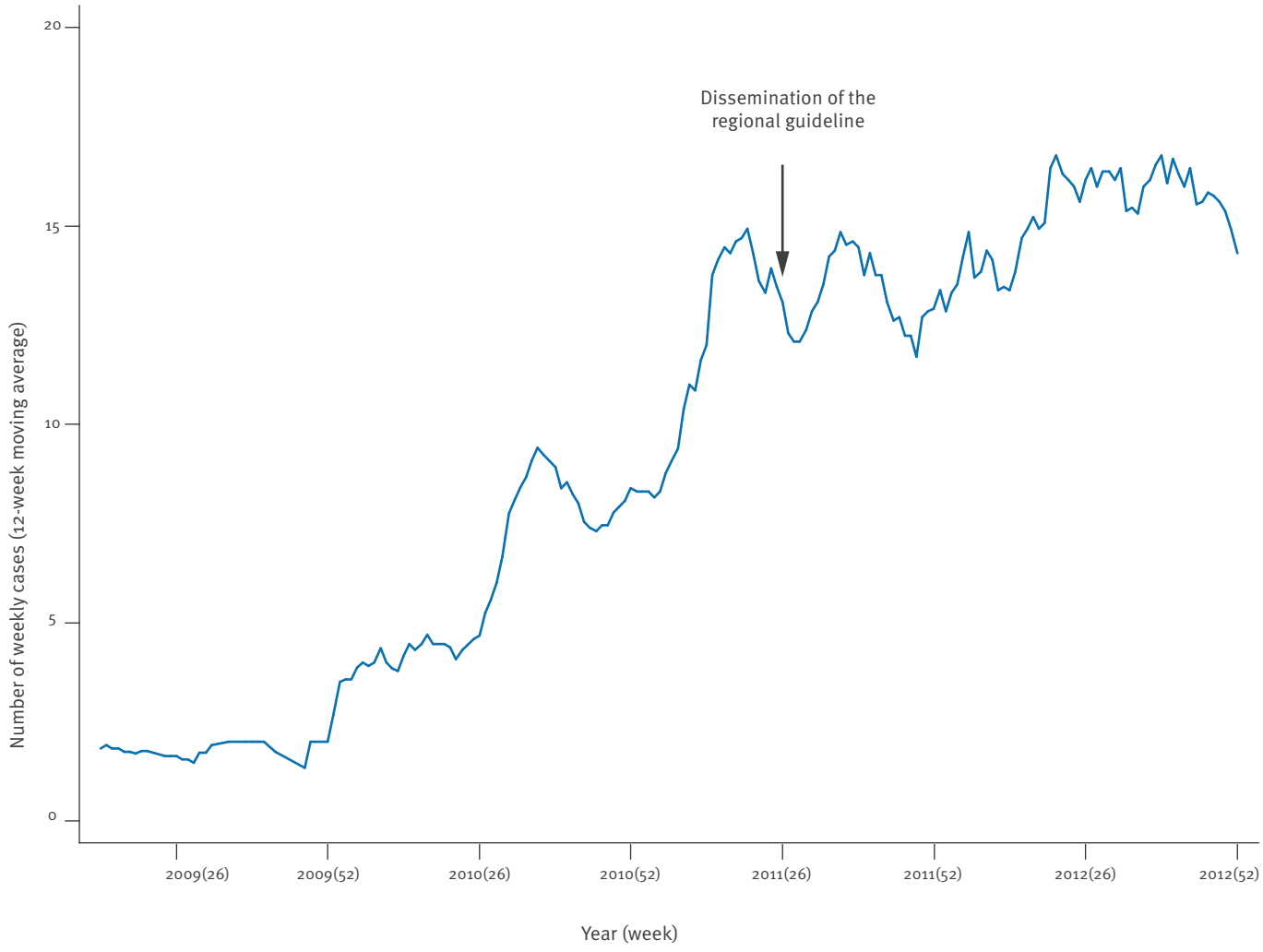


Figure includes all non-gastrointestinal cultures positive for carbapenem-resistant *Klebsiella pneumoniae* reported to the regional antimicrobial resistance surveillance system.

Figure 2

Prevalent cases of carbapenemase-producing *Klebsiella pneumoniae* by sample type, Emilia-Romagna, Italy, July 2011–March 2013

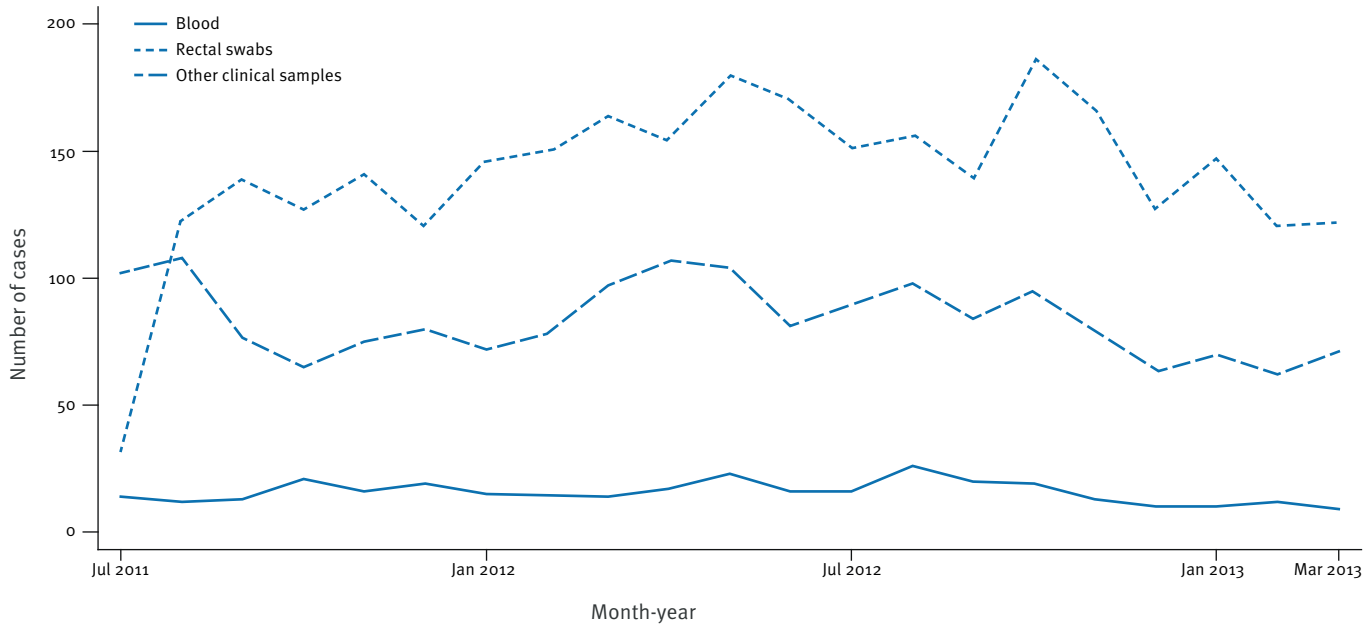


Figure shows all cultures positive for carbapenemase-producing *Klebsiella pneumoniae* reported to the regional carbapenemase-specific surveillance system.

of CRKP isolation from all non-gastrointestinal samples were 16 and 15 cases per 100,000 hospital patient days, after guideline implementation, in the third quarter of 2011 and in the fourth quarter of 2012, respectively, compared to an incidence rate of 7 cases per 100,000 hospital patient days in 2010.

The specific surveillance system for CPE, covering the entire region and providing monthly prevalence data starting from July 2011, showed CPKP as the most prevalent CPE, representing 95% of all cases while *Escherichia coli* and other Enterobacteriaceae accounted for 2% and 3% of total prevalent CPE cases, respectively. Before implementation of regional guidelines, no HT was performing an active search of asymptomatic carriers. Starting from July 2013 the number of CPKP isolated by rectal swab increased (Figure 2) and in September it overtook the number of isolates from clinical samples: the ratio of CPKP isolates from rectal swabs and isolates from clinical samples was 0.3 in July 2011, reached 1.7 in September 2011 and remained quite stable in the subsequent months with a peak of 2 in November and December 2012. Clinical isolates decreased in the period between September 2011 and February 2012, but registered a subsequent growth between March and October 2012 and a further decrease starting from November 2012 (Figure 2). Patients admitted to public hospitals constituted the vast majority of CPKP prevalent cases, accounting for 97% of bacteraemia cases, 93% of asymptomatic

Table

Multivariate linear regression of covariates affecting monthly incidence of carbapenemase-producing *Klebsiella pneumoniae* in five Health Trusts, Emilia-Romagna, Italy, July 2011–March 2013

Variable	Regression coefficient	95% CI	p value
Months since guideline release	-0. 73	-1.21 to -0.25	0.003
Monthly prevalence rate[a]	0.14	0.08 to 0.21	‹0.001
Isolation of identified cases in a single room	-19.30	-26.17 to -12.42	‹0.001
Audit of compliance with contact precautions	-7.58	-13.66 to -1.50	0.015
Intercept	31.06	20.42 to 41.70	‹0.001

CI: confidence intervals.

Incidence defined as new cases per 100,000 patient days: all cultures other than rectal swabs included.

[a] Total cases per 100,000 patient days: all cultures included.

carriers identified by rectal swabs, and 82% of other cases.

Reports from the five HTs providing monthly data on incident in-hospital cases showed a downward incidence rate trend of CPKP cases (isolates from rectal swabs were excluded) after the release of the regional guidelines. The incidence rates observed in these HTs, were 32 and 15 cases per 100,000 hospital patient days in the third quarter of 2011 and in the first quarter of 2013, respectively. In the period between July 2011 and March 2013, the monthly incidence rates showed a significant positive correlation with the monthly prevalence rates and a negative correlation with placement in a single room as main isolation method and with performing observational audit of compliance with contact precautions (Table). Both measures were implemented in one of the five HTs, while one of the two measures was implemented in two HTs. Evaluating the linear trend by HT, we observed a significant reduction in two of the five HTs, an increase in one HT and a non-significant trend in the remaining two (Figure 3). The HT with an increasing trend had incidence rates of seven and 19 cases per 100,000 hospital patient days in the third quarter of 2011 and in the first quarter of 2013, respectively.

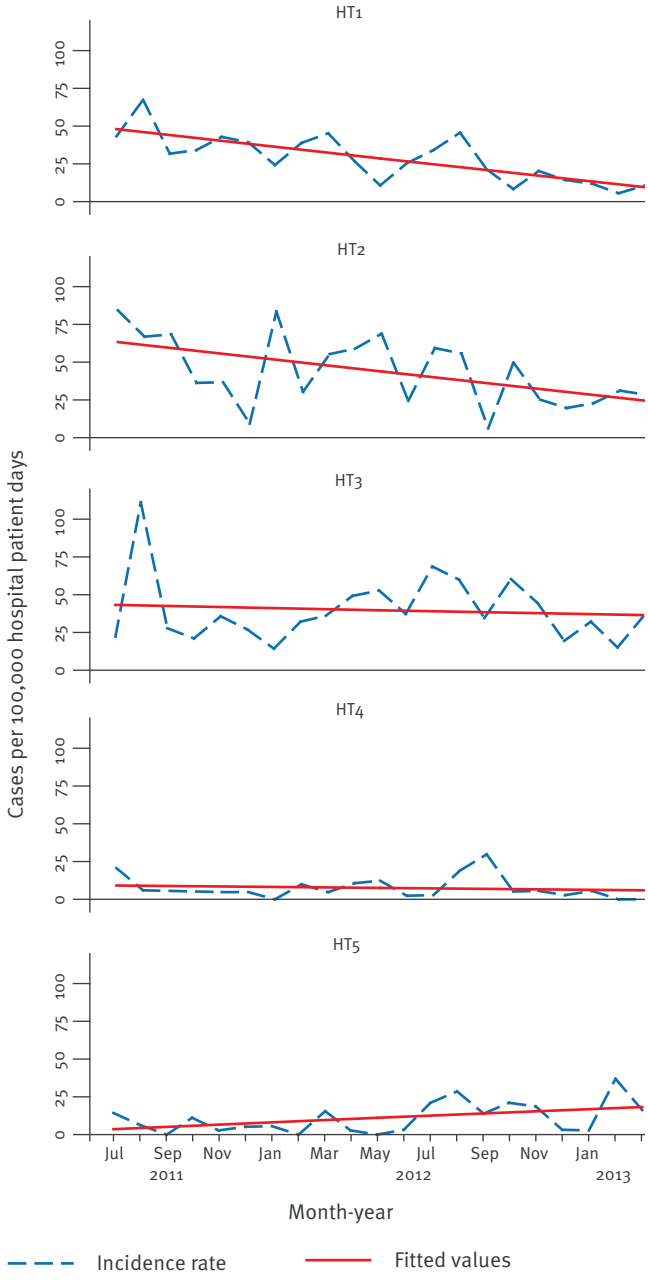
Survey of implementation of regional recommendations to contain CPE (May–June 2012)

All HTs in Emilia-Romagna participated in the questionnaire-based survey. All HTs implemented the appropriate tests for screening and phenotypic confirmation of carbapanemase production. In 16 of the 17 HTs, close contacts of hospitalised patients with CPE were actively screened, while in all HTs at least one of the following groups at risk was screened at hospital admission: (i) patients transferred from other hospitals or from LTCFs; (ii) patients discharged from hospital in the previous 60 days; (iii) patients coming from countries endemic for CPE; and/or (iv) patients admitted to intensive care units, spinal units, transplant units, oncology and haematology units. Isolation in a single room was the main isolation method in eight HTs and cohorting of patients in a dedicated area of the hospital was the main method in one HT, while four HTs mainly applied contact precautions, placing CPE cases in a room shared with non-CPE carriers; the remaining four HTs implemented a mixed approach consisting of two or more methods of physical isolation. In seven HTs, staff cohorting and/or appointment of a unit case-manager nurse was adopted, while in the other 10 HTs neither of the two was adopted. Eight HTs out of 17 reported repeated observational audits of compliance with contact precautions based on a planned schedule, while seven HTs reported random audit activities and two HTs reported no audit activity.

Data on use of hand hygiene products were also requested from the HT hospital pharmacies. The consumption rate of these products significantly increased

Figure 3

Incidence rate of carbapenemase-producing *Klebsiella pneumoniae* in five Health Trusts, Emilia-Romagna, Italy, July 2011-March 2013



HT: Health trust.

Figure shows all cultures positive for carbapenemase-producing *Klebsiella pneumoniae* reported to the carbapenemase-specific surveillance system by five Health Trusts in Emilia-Romagna. The incidence trend over time is plotted by a linear regression line.

during the implementation of regional guidelines for CPE control, and the mean regional rates (litres/1,000 hospital days) were 5.1, 6.8 and 9.4 in 2010, 2011 and 2012, respectively.

Discussion

The intervention implemented in Emilia-Romagna was characterised by a rapid slowdown of the earlier upward CRKP trend, and for nine months, incidence remained stable at to the pre-intervention rate but did not show a dramatic drop, as observed in other contexts (e.g. Israel) [10-14]. After a slight increase observed in the second and third quarters of 2012, a return to the pre-intervention rate occurred, starting in November 2012, with a more noticeable reduction in some hospitals with a high frequency of cases. Considering the five HTs that provided monthly data on incident cases, we observed that the incidence of CPKP infections correlated with prevalence, the availability of single rooms for isolation and the implementation of observational audits for monitoring of adherence to contact precautions (Table). Two out of five HTs considered in this analysis showed significantly decreasing trends, while one had an increasing incidence of CPKP cases (Figure 3). The latter HT was selected to provide monthly incidence data because, even with a low frequency of infection when the regional intervention started, it was located in the same area as a highly affected hospital. This HT had the lowest rate of the five HTs at the beginning of the observation and a rate slightly over the average in the first quarter of 2013. These results highlight how local infection trends can differ significantly from the average observed in a wider setting, such as a region, because of discrepancies in exposure to specific risk factors or in compliance with control measures.

The different response to the activities of control in Emilia-Romagna compared to what was observed in Israel probably depends on the epidemiological context and the type of intervention. In Israel, the incidence observed at the time of the introduction of control activities (55.5 cases per 100,000 hospital patientdays) was significantly higher than that observed in Emilia-Romagna (16 cases per 100,000 hospital patient days). On the contrary, the post-intervention rates observed in the two settings were quite similar: 11.7 and 15 per 100,000 hospital patient days in Israel and Emilia-Romagna, respectively [14]. In Israel, the activities were implemented with very strict systematic cohorting of colonised patients and care staff [12,14]. This approach, while highly effective, was considered unsuitable for Emilia-Romagna, due to the organisation of the regional health system and to high costs. In Emilia-Romagna, contact precautions were implemented without cohorting staff and patients in most HTs. In four HTs, due to scarcity of single rooms, CPE cases were mainly placed in rooms with non-CPE carriers, posing additional difficulties for the correct implementation of contact precautions. Moreover, only eight HTs out of 17 implemented strict monitoring of compliance with contact precautions by observational audit. Still, the control activities implemented in the Region achieved a slowdown of CPKP spread. Incidence remained stable for three consecutive quarters after the launch of the regional intervention and, after a slight increase between April and October 2012, showed a return to the initial rate.

The present study has several limitations. First of all, no control group was available to compare the effect of the intervention, because the study was launched simultaneously across the region. Moreover, the observed results showing a slowdown of CPKP transmission at regional level hide a more heterogeneous result at local level depending on the pre-intervention incidence and on compliance with recommendations achieved during the intervention period [19,20]. The implementation of the regional recommendations at HT level has been monitored through a questionnaire-based survey but specific data on the actual degree of compliance with each of the proposed measures are not available. This an important limitation of the study, but, on the other hand, the increase of asymptomatic carriers identified by the active surveillance and the upward trend of hand hygiene products use are proxies of improvements in compliance with control measures after the start of the regional intervention for CPE containment. There is a consistent and clear temporality between the introduction of the control activities and the change in the slope of the CPKP trend line. Moreover, the reduction of incidence in the hospitals of five HTs correlates negatively with prevalence rate and positively with availability of single rooms for isolation and with monitoring of compliance with contact precautions, showing how control activities can drive the outcome in the expected direction.

Another important issue is the evaluation of the infection trend before the implementation of the regional intervention, which is based on routine data collected through the antimicrobial resistance surveillance system of Emilia-Romagna. This system has the limitation of not including the results of the phenotypic confirmation of carbapenemase production, yet it is a reliable tool for monitoring the trends of antimicrobial resistance, including CRKP before and during the implementation of CPE control measures. Finally, the specific surveillance implemented in Emilia-Romagna did not provide a systematic genotyping of CPKP, though available results indicate that CPKP, which was isolated in this region and more generally in Italy, mainly produces *K. pneumoniae* carbapenemases (KPC) [4,8,21,22].

According to these findings, but in the absence of a controlled study to assess the effectiveness of the intervention, the measures recommended in Emilia-Romagna appear to have contained the spread of CPKP. The intervention in Emilia-Romagna succeeded in curbing CPKP transmission although the activities were hospital-centered and did not include the widespread use of staff and patient cohorting as in Israel. These characteristics of the intervention along with

the incidence, which was lower than in Israel, probably explain why, after stabilising the rate, no evident decrease was observed at regional level, despite an encouraging trend towards reduction in some hospitals (Table and Figure 3). Further reduction might result from increased compliance with standard and contact precautions and from a more effective implementation of antibiotic stewardship. Moreover, the results of this study appear to be of particular interest as they may have direct and indirect effects on the epidemiology of CPE in other Italian regions. In particular, the containment of the spread in Emilia-Romagna reduces the probability of transfer to other regions through colonised patients. In addition, the organisational model can be implemented in other regional contexts. Finally, given the possibility of European citizens to receive free cross-border healthcare, as defined in a recent directive of the European Parliament and of the council [23], there would be also a reduction in the probability of transfer of CPE cases into other European countries.

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Conflict of interest

None declared.

Authors' contributions

C Gagliotti and ML Moro wrote the manuscript. C Gagliotti, V Cappelli, E Carretto, M Marchi, A Pan, P Ragni, M Sarti, R Suzzi, GA Tura, ML Moro and The Emilia-Romagna Group for CPE Control provided feedback, contributed with comments and reviewed the manuscript. C Gagliotti, V Cappelli, E Carretto, M Marchi, A Pan, P Ragni, M Sarti, R Suzzi, GA Tura and ML Moro contributed to implementation of the specific surveillance system for carbapenemase-producing Klebsiella pneumoniae. C Gagliotti and M Marchi performed the data analysis. The Emilia-Romagna Group for CPE Control provided the specific surveillance data.

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Outbreak of hepatitis A infection associated with the consumption of frozen berries, Ireland, 2013 - linked to an international outbreak

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In May 2013, a European alert was issued regarding a hepatitis A virus (HAV) outbreak in Italy. In June 2013, HAV subgenotype IA with an identical sequence was identified in Ireland in three cases who had not travelled to Italy. The investigation consisted of descriptive epidemiology, a case–control study, microbiological testing of human and food specimens, molecular typing of positive specimens and food traceback. We identified 21 outbreak cases (14 confirmed primary cases) with symptom onset between 31 January and 11 October 2013. For the case–control study, we recruited 11 confirmed primary cases and 42 matched controls. Cases were more likely than controls to have eaten berry cheesecake (matched odds ratio (mOR): 12; 95% confidence interval (CI): 1.3–114), whole frozen berries (mOR: 9.5; 95% CI: 1.0–89), yoghurt containing frozen berries (mOR: 6.6, 95% CI: 1.2–37) or raw celery (mOR: 4; 95% CI: 1.2–16). Among cases, 91% had consumed at least one of four products containing frozen berries (mOR: 12; 95% CI: 1.5–94). Sixteen food samples tested were all negative for HAV. As products containing frozen berries were implicated in the outbreak, the public were advised to heat-treat frozen berries before consumption.

Background

Hepatitis A is a vaccine-preventable, self-limiting infection of the liver caused by hepatitis A virus (HAV). Infection in children is asymptomatic or associated with mild illness. In adults, the most common symptoms are fever, loss of appetite, nausea, fatigue and abdominal pain, followed within a few days by jaundice. Clinical severity varies from a relatively mild illness lasting one to two weeks to a severely disabling illness lasting months [1]. Transmission is primarily by the faecal–oral route, including person-to-person spread and contaminated food or water. The average incubation period is 28 to 30 days (range: 15–50 days), with maximum infectivity occurring during the latter half of the incubation period and for a few days after onset of jaundice [1]. In developed countries, HAV infection rates are low and decreasing. In the European Union, the overall incidence of HAV decreased from 15.1 per 100,000 in 1996 to 3.9 per 100,000 in 2006, attributable to improved living and sanitary conditions [2]. In 2011, the incidence rate in Europe was 2.5 per 100,000 [3]. However, a reduction in the circulation of HAV leads to an accumulation of susceptible individuals in a population and allows for outbreaks to occur [2]. Foodborne transmission of HAV has been associated with several outbreaks in recent years in Europe, Australia and the United States (US); the implicated foods included fish and seafood products, vegetables, juices, semi-dried tomatoes, berries [4] and pomegranate seeds [5,6].

Since 1981, HAV infections have been notifiable in Ireland by clinicians. Since 2004, laboratories have also been required to notify. The incidence of HAV in Ireland has fallen substantially from a peak of 16 per 100,000 population (564 cases) in 1989 to 0.7 per 100,000 population (30 cases) in 2012 [7,8]. The HAV

vaccine is not included in the Irish childhood immunisation programme. From 2004 (when outbreaks of infectious diseases became notifiable) to the end of 2012, no foodborne outbreaks due to HAV were reported in Ireland. Before 2013, molecular investigation of HAV cases was not routinely available in Ireland.

In April 2013, a European alert was issued relating to an outbreak of HAV subgenotype IB in four Nordic countries [9]. Frozen berries were later found to be the likely vehicle of infection [10]. A second alert due to an outbreak of HAV subgenotype IA associated with travel to Italy was issued in May 2013 [11]. Between January and May 2013, 352 cases of HAV infection were notified in Italy corresponding to a 70% increase compared to the same period in 2012, and preliminary investigations indicated mixed frozen berries as the most likely vehicle of infection [12]. Following these alerts, a decision was taken in Ireland to retrospectively and prospectively genotype and sequence all available samples from serologically confirmed cases of HAV infection since the beginning of 2013. In June 2013, three HAV cases from Ireland were identified as subgenotype IA, a strain identical to the one occurring in the Italian outbreak (GenBank accession number: KF182323) [12]. None of the cases had a history of travel to Italy. An outbreak investigation commenced and a multidisciplinary outbreak control team was established.

The objectives of our investigation were to describe the outbreak in order to determine its extent and to identify the vehicle and source in order to implement control measures.

Methods

Case definition

A confirmed outbreak case was defined as a person living in Ireland with a laboratory-confirmed (HAV IgM and HAV RNA PCR-positive) infection of HAV subgenotype IA with a sequence identified by GenBank accession number KF182323 and with onset of symptoms from 1 January 2013. Cases who had travelled to Italy were excluded. A possible case was the same as a confirmed case, except the genotype and sequence type were unknown and the person had not travelled outside Europe to a country of high endemicity.

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

Case finding and descriptive epidemiology

Cases were identified using the mandatory notification system in conjunction with the genotyping and sequencing results reported by the National Virus Reference Laboratory (NVRL). We sent alerts to Departments of Public Health, general practitioners, hepatologists, emergency department physicians, infectious diseases consultants and microbiologists, informing them of the outbreak and reminding them to notify HAV cases.

Case–control study

Public health personnel undertook telephone interviews with the first 10 cases using a detailed trawling questionnaire which included questions on exposures previously associated with HAV infections. We generated hypotheses based on data obtained from the trawling questionnaires. Our resulting primary hypothesis was that illness was associated with the consumption of frozen berries. Other hypotheses were also considered, including illness being associated with the consumption of fresh berries, or specifically fresh blueberries.

We conducted a matched case–control study to test these hypotheses. Only confirmed primary cases were eligible. Controls were randomly selected using random digit dialling of landline and mobile telephone numbers, matched on age (±5 years), sex and county of residence. A market research company recruited the controls for the study according to an agreed protocol. Investigators from the Departments of Public Health and Health Protection Surveillance Centre (HPSC) interviewed study participants by telephone using a pre-tested questionnaire, which included questions on the consumption of fresh berries, frozen berries, various frozen-berry products, pomegranate, salad items, shellfish and raw seafood, and details on the place of purchase and brands of these items. Cases were asked about their exposures in the seven weeks before onset of symptoms and controls were asked about their exposures in the same time period as their matched case. Controls were excluded if they were vaccinated against HAV, had previously been diagnosed with HAV (self-reported) or lived in the same household as an HAV case in 2013. Interviews were completed on three or four controls per case.

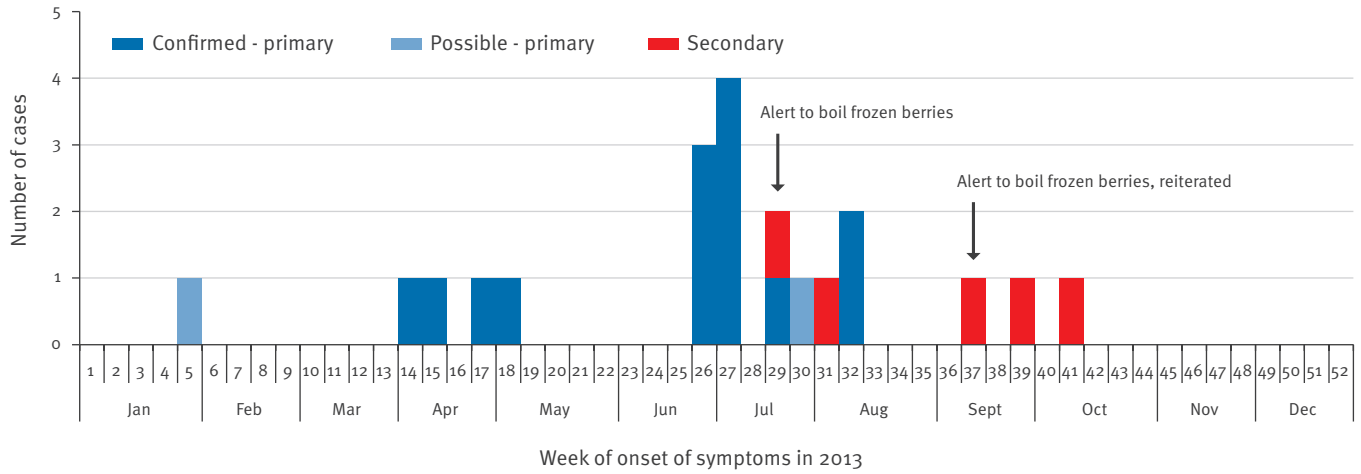
Data analysis

We described the distribution of cases notified on the national Computerised Infectious Diseases Reporting (CIDR) system for notifiable infectious diseases by time, place, person and case classification. We entered the questionnaire responses from the case–control study into an EpiData database (Epidata association, Denmark, version 3.1) and analysed them using Stata 11.2 (Stata Corporation,Texas, US).

The association between illness and food items consumed was estimated by calculating crude matched odds ratios (mOR) and 95% confidence intervals (CI). Subsequently, we conducted conditional logistic regression to identify independent risk factors for the disease. We constructed initial models including variables with a p value ≤ 0.25 in the univariate analysis. To simplify the model, variables were removed one at a time depending on the significance testing, using likelihood ratio tests. An association was considered statistically significant when $p \leq 0.05$.

Figure

Distribution of hepatitis A outbreak cases by week of onset of symptoms, Ireland, 2013 (n=21)



Microbiological investigation

The diagnosis of acute HAV infection was established by serological testing for anti-HAV IgM at local laboratory level. Additional serological and molecular investigations using an in-house real-time PCR assay for HAV RNA was performed at the NVRL. Following detection of HAV RNA in serum, molecular sequencing was performed. In the initial stages of the outbreak, HAV RNA sequence analysis was not available in Ireland and therefore the Virus Reference Department, Public Health England (PHE) performed these investigations. Subsequently, HAV molecular sequencing was introduced in the NVRL, consisting of nested reverse transcriptase PCR (RT-PCR) to amplify a ca 400 bp region in the VP1/2PA region of the HAV genome [13]. Sanger sequencing and phylogenetic analysis was performed to characterise HAV sequences.

Selected food samples from food companies and cases' freezers were sent to the Istituto Zooprofilattico Sperimentale in Italy for analysis as no laboratory in Ireland currently conducts testing for HAV in berries. The samples were analysed using a nested PCR method.

Trace-back investigation

Suspect foods identified in the trawling questionnaires and the case–control study were investigated by Health Service Executive (HSE) Environmental Health Officers, the Department of Agriculture Food and Marine (DAFM) and the Food Safety Authority of Ireland (FSAI). Traceback and traceforward information was obtained from retailers, manufacturers, distributors and importers. Traceability information of foods imported into Ireland was obtained using the European Commission's Rapid Alert System for Food and Feed (RASFF).

Results

Descriptive epidemiology

In total, 50 cases of HAV infection were notified in Ireland in 2013 and 21 cases met the outbreak case definition (14 confirmed primary, two possible primary and five secondary). Cases were distributed nationally, occurring in five of the eight health regions. Cases ranged in age from 25 to 64 years (median age 35 years) and 12 of 21 were female. Twelve cases were hospitalised and the median length of hospital stay was five days (range: 1–9 days). There were no deaths. Among the 14 confirmed primary cases, there were two clusters in time; one cluster of four cases with onset of symptoms in April and a second cluster of 10 cases with onset of symptoms between 24 June 2013 and 9 August 2013 (Figure).

Case–control study

Of the 14 confirmed primary cases eligible for the case–control study, 11 participated and three declined. A total of 42 controls were included, with three or four matched controls per case. Cases were significantly more likely than controls to have consumed cheesecake containing frozen berries (mOR=12; 95% CI: 1.3–115), whole frozen berries (mOR=9.5; 95% CI: 1.0–89), a particular yoghurt (yoghurt A) containing frozen berries (mOR=6.6; 95% CI: 1.2–37) and raw celery (mOR=4, 95% CI: 1.2–16) (Table 1). The cheesecakes containing frozen berries consumed by the cases and controls were store-bought. Yoghurt A is a commercial product available in stores nationwide. The consumption of fresh berries or fresh blueberries was not significantly associated with illness. In the conditional regression analysis, only cheesecake containing frozen berries remained significant in the final model. However, four of 10 cases for whom this information was available, had consumed this item (Table 1).

Table 1

Food exposures among hepatitis A subgenotype IA cases (n = 11) and controls (n = 42), Ireland, 2013

Exposure Total	Cases			Controls			Crude mOR	95% CI	p value
	Total[a]	Exposed	%	Total	Exposed	%			
Individual products									
Cheesecake[b]	10	4	40	42	4	10	12	1.3–114	0.026
Celery	11	6	55	42	7	17	4.0	1.2–16	0.026
Whole frozen berries	10	4	40	42	4	10	9.5	1.0–89	0.031
Yoghurt A[b]	10	4	40	42	3	7	6.6	1.2–37	0.048
Fresh blueberries	11	9	82	42	22	52	4.1	0.79–21	0.093
Ice cream[b]	11	2	18	42	1	2	7.3	0.66–81	0.105
Yoghurts[b,c]	10	5	50	42	9	21	3.1	0.77–12	0.112
Smoothies[b]	9	4	44	42	9	21	3.1	0.67–14	0.151
Fresh berries	11	10	91	42	33	79	3.4	0.30–38	0.321
Grouped products									
Cheesecake or yoghurt A	10	6	60	42	7	17	5.3	1.3–22	0.023
Whole frozen berries or yoghurt A	10	6	60	42	7	17	7.0	1.4–37	0.02
Cheesecake or whole frozen berries	10	7	70	42	7	17	14	1.7–122	0.014
Cheesecake or whole frozen berries or yoghurt A	10	8	80	42	10	24	8.1	1.6–40	0.011
Cheesecake or whole frozen berries or yoghurt A or smoothies[d]	11	10	91	42	16	38	12	1.5–94	0.02

CI: confidence interval;.mOR: matched odds ratio.

[a] Cases with available information.

[b] These products contained frozen berries.

[c] Includes yoghurt A and other yoghurts containing frozen berries.

[d] No change when ice-cream was added to this combination.

When products containing frozen berries (i.e. whole frozen berries or cheesecake or yoghurt A or smoothies) were grouped together, cases were more likely than controls to have consumed at least one of the products within the group (mOR = 12; 95% CI: 1.5–94), with 10 of 11 cases exposed (Table 1).

The odds of the illness increased with increasing frequency of consumption of yoghurt A and smoothies (Table 2). No frequency response could be calculated for cheesecake, while a frequency response effect was not demonstrated with celery (Table 2).

Microbiological analysis

Of the 21 HAV cases, 18 were sequenced and genotyped (14 primary and four secondary). HAV RNA sequences from these cases had a 100% nucleotide homology to the HAV IA outbreak strain over the region sequenced.

In total, 16 food items which included frozen berries, products containing frozen berries and fresh berries were tested. HAV RNA was not detected in any of these food samples. Samples included two batches of frozen mixed berries used in batches of yoghurt A potentially consumed by some cases and a third batch to which cases could not have been exposed.

Traceback analysis

The Italian authorities had detected HAV in frozen berries imported to Italy with a sequence identical to the Irish outbreak strain [4]. Therefore, the traceback investigations in Ireland focused on the imported frozen berry supply chain, but fresh berries and other foods consumed by cases were also investigated.

The investigations found that products from a single Irish distributor of imported frozen berries had been used in foods consumed by nine of the 14 primary cases eligible for the case–control study. A comparison with the Italian traceability investigation, however, has not to date identified an overlap in the supply chain that could explain the source of the outbreak in both countries.

The traceback investigation on fresh berries did not point to a common point source.

Control measures

Prophylactic human normal immunoglobulin and/or HAV vaccine were offered to close contacts of cases as necessary. Advice about hygiene and exclusion from schools or childcare facilities and certain workplaces was also provided to cases and their contacts.

On 4 June 2013, the FSAI issued an information note to food business operators in Ireland regarding the

Table 2

Frequency of consumption of selected food items among hepatitis A subgenotype IA cases (n = 11) and controls (n = 42), Ireland, 2013

Exposure / frequency	Cases			Controls			Crude mOR	95% CI	p value
	Total	Exposed	%	Total	Exposed	%			
Yoghurt A[a]									
Never	10	6	60	42	39	93	Ref	-	-
<1 weekly[b]	10	1	10	42	2	5	2.9	0.23–36	0.406
≥1 weekly[c]	10	3	30	42	1	2	12	1.2–125	0.032
Smoothies[a]									
Never	9	4	45	42	33	79	Ref	-	-
<1 weekly[b]	9	2	22	42	5	12	2.7	0.40–19	0.307
≥1 weekly[c]	9	3	33	42	4	9	5.9	0.90–38	0.064
Whole frozen berries									
Never	10	6	60	42	38	90	Ref	-	-
<1 weekly[b]	10	2	20	42	2	5	9.5	0.61–148	0.107
≥1 weekly[c]	10	2	20	42	2	5	9.5	0.61–148	0.107
Cheesecake[a]									
Never	10	6	60	42	38	91	Ref	-	-
<1 weekly[b]	10	3	30	42	4	9	8.6	0.85–86	0.068
≥1 weekly[c]	10	1	10	42	0	0	NC	NC	NC
Celery									
Never	10	5	50	42	35	83	Ref	-	-
<1 weekly[b]	10	2	20	42	1	3	8.1	0.72–92	0.091
≥1 weekly[c]	10	3	30	42	6	14	2.4	0.51–12	0.267

CI: confidence interval;.mOR: matched odds ratio; NC: non calculable.

[a] These products contained frozen berries.

[b] Ranged from once in seven week period to two to three times per month.

[c] Ranged from one to two times per week to five or more times per week.

potential risk posed by imported frozen berries. This was based on information from the Nordic and the Italian outbreak investigations. On 19 July 2013, following the identification of HAV cases in Ireland with a strain identical to the Italian outbreak strain, the FSAI issued a press release advising consumers and food business operators, as a precautionary measure, to boil imported frozen berries for one minute before consumption[14]. At the same time, the FSAI issued a second information note to food business operators reminding them to source berries from reputable suppliers with efficient and comprehensive traceability systems and effective food safety management systems. Furthermore, food business operators were advised that if they had any concerns regarding the source of the berries, they should boil the berries before inclusion in uncooked products. The advice to consumers and food business operators was reiterated by the FSAI on 8 September 2013.

Discussion

We report on a foodborne outbreak of HAV subgenotype IA infection in Ireland. Nucleic acid sequences of HAV RNA were identical, indicating that cases were likely to have been infected from a common source. The outbreak strain was identical to the one in a concurrent but separate foodborne outbreak in Italy. We present epidemiological evidence that the HAV subgenotype IA outbreak in Ireland was associated with the consumption of products containing frozen berries. Traceback investigations at an international level, coordinated by the European Food Safety Authority, to identify the origins of the imported frozen berries failed to identify a single point source of contamination [15].

Our case–control study revealed that no single product explained the majority of the cases. Although the consumption of cheesecake containing frozen berries remained significantly associated with illness in the multivariable analysis, the product was consumed by a relatively small proportion (40%) of the cases. Yoghurt A and smoothies were found not to be individually associated with illness in the final multivariable model; however, a frequency response effect was demonstrated for both of these products. When frozen berry products were grouped together in the analysis, a strong and significant association was shown between the consumption of the grouped products and HAV subgenotype IA infection of the same sequence type. All but one of the 11 cases had consumed at least one of the frozen berry-containing products in the group: cheesecake, yoghurt A, smoothies or whole frozen berries.

Mixed frozen berries were also identified as the potential vehicle of infection in the related Italian outbreak [12]. Furthermore, specific frozen berries were implicated in several other HAV outbreaks including frozen

strawberries in Nordic countries in 2013 [16] and in the US in 1997 and 1992 [17,18] and frozen raspberries in Scotland in 1987 and 1983 [19,20]. Pomegranate seeds were implicated in HAV outbreaks in the US in 2013 and Canada in 2012 [5,6]. Fresh berries have also been associated with hepatitis A infections, including an outbreak in New Zealand linked to the consumption of blueberries [21].

Raw celery consumption was also significantly associated with being a case in the univariate analysis, but did not remain significant in the multivariable analysis. Six of the cases had consumed raw celery over a five-month period. However, since celery is a fresh product with a short shelf life, it is highly unlikely to be the source such a prolonged outbreak or indeed an embedded secondary outbreak. In contrast a frozen product such as frozen berries, with a long shelf life explains a long outbreak such as this one very well.

The selection and recruitment of controls for case–control studies can be difficult and resource-intensive, particularly as Ireland does not have a national population registry. In this analytical study, the novel use of a market research company to recruit by random digit dialling proved efficient and effective, although matching of controls to the county of residence of the cases prolonged the process (personal communication: Sonya McGuirl, Millward Brown, Dublin, October 2013). Almost 1,900 calls were made to recruit sufficient controls, which equated on average to one hour of calls per control recruited.

The sequencing and genotyping of HAV RNA from the cases was crucial in identifying a national outbreak and in establishing a link with the Italian outbreak. Rapid and comprehensive referral of samples from local laboratories to the NVRL and PHE for HAV RNA PCR and molecular sequencing as well as international collaboration with laboratory colleagues in PHE, Colindale and the Hepatitis A Laboratory Network (HAV-NET) group played a vital role in the identification and investigation of this outbreak against the backdrop of an increasingly globalised food production industry.

We did not obtain confirmatory microbiological evidence from the food to support the epidemiological findings; in many cases the implicated batch was no longer available for testing, due to the long incubation period for HAV. The microbiological investigation was further complicated by limitations in food testing capability in Ireland. Consequently, only a limited number of food samples could be tested by an external laboratory. Despite the lack of confirmatory microbiological evidence from the food in Ireland, indirect evidence was available. HAV was identified in a number of frozen berry samples in connection with the outbreak in Italy and the identical sequence to the outbreak strain was confirmed in one sample [4].

The long incubation period for HAV infection also hampered the traceback investigations. In most cases the traceback focused on food batches delivered over several weeks rather than on specific batches consumed by cases. In addition, the frozen berry supply chain is complex, further challenging the traceability investigation. An overlap in the supply chain between Ireland and Italy has not been found, although the outbreak strains were identical and epidemiological evidence had been obtained in both countries for a link with imported frozen berries. In autumn 2013, the Netherlands reported an outbreak with the identical strain and at the request of the European Commission, the European Food Safety Authority (EFSA) co-ordinated an investigation, in collaboration with the European Centre for Disease Prevention and Control (ECDC) and European Union (EU) Member States in order to help identify the origin of these HAV subgenotype IA outbreaks [15]. Between January 2013 and June 2014, 1,444 cases of HAV potentially linked to the outbreak were reported by 12 Member States; Italy reported 90% of the cases [15]. Apart from Ireland, seven other Member States reported cases with no travel history to Italy but with a sequence identical to the GenBank accession number KF182323 strain [15], including an outbreak in Norway where the likely vehicle was a cake containing a frozen berry mix [22]. No single point source of contamination linking all cases and contaminated batches identified during the multinational outbreak could be determined [15].

When the Irish outbreak was declared over in late October 2013, the advice to boil berries before consumption was reviewed. Due to ongoing outbreaks in other EU countries indicating that contaminated product was still available, the FSAI's precautionary advice to boil imported frozen berries remained in place. A fourth information note was issued on 8 November 2013, reiterating advice to food business operators that they should boil the berries before inclusion in uncooked products if they had any concerns regarding the source of the berries.

There are a number of limitations in this investigation. One limitation of the analytical study was participant recall because of the long incubation period for HAV infection. In addition, because sequencing was carried out retrospectively on specimens from the earlier cases, there was an additional time lag before these cases and their matched controls were interviewed. Another limitation was that public health warnings were issued before the case–control study was conducted and may have introduced some recall bias. A further limitation of this investigation was that whole genome sequencing of the HAV isolates was not performed. This service is currently not available in Ireland. It is acknowledged that using sequence information from subgenomic regions to track HAV transmission may be misleading when applied to HAV strains with unknown epidemiological association [23]. As whole genome sequencing provides a more complete genetic characterisation of

HAV strains, its use in investigating suspected foodborne outbreaks has been recommended [23,24].

Despite excluding vaccinated controls or those with a history of HAV disease, immune controls as a result of infection in early childhood may have been included, which would have resulted in a bias towards the null hypothesis. Misclassification of belonging to the outbreak was minimised as all cases included in the analytical study were infected with HAV of the same sequence and genotype, and several exclusion criteria were used for the controls.

Conclusion

In conclusion, this outbreak and other HAV outbreaks in Europe in 2013-14 have highlighted the risk of HAV infection associated with the consumption of imported frozen berries. Owing to the complex supply chain it was not possible to identify the origin of the contaminated berries despite a concerted action at European level to comprehensively investigate the supply chain. However, the investigation identified critical risk factors for contamination by HAV and recommended preventative measures to be taken to minimise the risk and to protect consumers in Europe from the risks posed by contaminated frozen berries. Consumers can also protect themselves by boiling frozen berries, and food businesses that are unable to use boiled frozen berries in their products should take measures to ensure that the berries are sourced from suppliers where strict hygiene is implemented at harvest and during processing.

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Conflict of interest

None declared.

Authors' contributions

All authors contributed to the writing of this manuscript and approved the final version. MF led the case–control study, analysed the data and drafted the manuscript as lead writer. LT led and chaired the Outbreak Control Team, coordinated the investigation at national level, contributed to the design of the case–control study, contributed to and advised on the manuscript. JOG was in charge of the laboratory typing of human cases, contributed to the design of the case–control study and contributed to and advised on the manuscript. LOC was in charge of the traceback, contributed to the design of the case-control study and contributed to and advised on the manuscript. PG was responsible for the descriptive epidemiology, contributed to the design of the case–control study and advised on the manuscript.
JR contributed to the design of the case-control study, designed the database and advised on the manuscript. AMP managed the environmental investigations and advised on the manuscript. MB, JMcD, HC, JH, MC and PF coordinated the investigation at local level, contributed to the design of the case–control study and advised on the manuscript. JM was responsible for the laboratory reporting of cases, contributed to the descriptive epidemiology and advised on the manuscript. DOF chaired the Outbreak Control Team (in LT's absence) and advised on the manuscript.

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Three simultaneous, food-borne, multi-country outbreaks of hepatitis A virus infection reported in EPIS-FWD in 2013: what does it mean for the European Union?

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Between March and May 2013, three multi-country outbreaks of hepatitis A virus (HAV) infection were reported through the Epidemic Intelligence Information System for Food- and Water-borne diseases (EPIS-FWD) of the European Centre for Disease Prevention and Control (ECDC). The aim of this work is to put these outbreaks into a European Union (EU) and European Economic Area (EEA) perspective and highlight opportunities for improving detection and investigation of such outbreaks. Although HAV outbreaks are not unusual in the EU/EEA, having three large food-borne multi-country outbreaks declared within three months is an unexpected event, particularly when at least two of these outbreaks are associated with frozen berries. Factors influencing the occurrence of these events include the increased number of susceptible Europeans, the limited coverage of HAV vaccination, the global trade of potentially contaminated products introduced in the EU/EEA, and the 'awareness chain effect' leading to a wave of notifications. Further studies should be conducted to understand the risk posed by frozen berries. Laboratory capacity and surveillance of viral infections in the EU/EEA, as well as HAV vaccination recommendations to travellers to endemic countries should be strengthened. Finally, timely reporting food-borne events through EPIS-FWD, to ensure timely response.

Surveillance and early warning for hepatitis A virus infection in the European Union and countries of the European Economic Area

Hepatitis A is a self-limiting viral disease caused by hepatitis A virus (HAV). HAV has low to very low endemicity in northern and western Europe and intermediate to low endemicity in eastern and southern Europe [1]. Infection takes place mainly via the faecal–oral route through person-to-person contact but food- and waterborne transmission is also common. Groups at increased risk for HAV infection include travellers to endemic areas, men who have sex with men, people who inject drugs, recipients of blood and blood product and close contacts of infected individuals [1].

Hepatitis A is a notifiable disease at the European Union (EU)/European Economic Area (EEA) level. In 2011, the overall annual disease incidence in EU/EEA was 3 per 100,000 inhabitants; of 28 countries for which incidence is available, 19 reported HAV incidence below one per 100,000 inhabitants and three reported more than ten per 100,000 inhabitants, the highest incidence being 74 per 100,000 inhabitants in Bulgaria [2]. Cases are usually confirmed through serological analysis. Only a few EU countries perform routine molecular characterisation of the viral isolates of the cases. Timely collection and analysis of surveillance information is essential to monitor hepatitis A trends over time and early detect increases in disease incidence. Molecular characterisation of the collected HAV isolates is then helpful to understand whether reported cases are linked. While EU/EEA countries report individual cases of HAV infection on an annual basis to The European Surveillance System (TESSy) of the European Centre for Disease Prevention and Control (ECDC), outbreaks with a potential international dimension are reported in real time through ECDC Epidemic Intelligence Information System for Food- and Water-borne Diseases [3]. EPIS-FWD was established in March 2010 and is a communication platform for early detection and assessment of food- and waterborne threats with potential international dimension. The system gathers epidemiologists and microbiologists from all EU/EEA countries, plus Australia, Canada, Japan, New Zealand, South Africa, Switzerland, Turkey and the United States. In 2010 and 2011, three outbreaks of HAV infections were reported via EPIS-FWD. Two of these outbreaks, involving Australia, France, the Netherlands and the United Kingdom, were due to the consumption of semi-dried tomatoes [4–6]; the source of infection of the third outbreak in Estonia was not identified, but a food-borne

Table

Description of the food-borne multi-country hepatitis A outbreaks in the European Union/European Economic Area, 2013

	1 March 2013 Demark reports a national outbreak in EPIS-FWD	17 April 2013 Norway reports an outbreak in travellers returning from Egypt in EPIS-FWD	8 May 2013 Germany reports an outbreak in travellers returning from Italy in EPIS-FWD
Countries reporting associated cases	Denmark, Finland, Norway and Sweden.	Denmark, Estonia, Finland, France, Germany, Ireland, Latvia, Lithuania, Norway, Slovakia, Sweden, Switzerland, the Netherlands and the United Kingdom.	Italy, Ireland, Germany, the Netherlands and Poland.
Epidemiological investigations	As of 6 August 2013, there were 106 cases reported since 1 October 2012. 61% female, median age 23 years old. Trawling questionnaire and case control studies in the affected countries pointed towards frozen strawberries as vehicle of infection.	As of 20 August 2013, there were 107 cases reported since 1 November 2012. 50% female, median age 36 years old. Trawling questionnaire and case control study in the affected countries pointed to foodborne transmission. Strawberries were suspected among other fruits.	As of 27 July, there were more than 200 reported cases in 2013, the majority in Italy. Trawling questionnaire and case–control study in the affected countries pointed towards frozen mixed berries.
Microbiological information	Subgenotype 1B. Two RNA sequences (sequence 1 and sequence 2) which differed by 1.7% over 847 bp.	Subgenotype 1B. One RNA sequence which differed by 1.22% over 1,233 bp and by 1.26% over 397 bp from sequence 1 and 2 of the Nordic countries outbreak.	Subgenotype 1A. One RNA sequence.
Food investigations	Trace-back analysis pointed to strawberries from Egypt and Morocco. No food sample was found positive. One RASFF notification issued.	No information available about possible food investigations in Egypt.	HAV isolated in frozen mixed berries of various origins, mostly from Eastern European countries. One isolate from the food samples had an identical sequence to the outbreak strain. Eleven RASFF notifications issued.

EPIS-FWD: Epidemic Intelligence Information System for Food- and Water-borne diseases; RASFF: Rapid Alert System for Food and Feed

origin was suspected. No HAV outbreak was reported in EPIS-FWD in 2012.

Between March and May 2013, three multi-country outbreaks of hepatitis A virus infection were reported through EPIS-FWD. Prompt analysis of surveillance information and timely reports triggered rapid and coordinated response among affected countries and ECDC.

The aim of this work is to put these recent outbreaks into an EU perspective and highlight opportunities for improving detection and investigation of future multi-national HAV outbreaks.

The three outbreaks of HAV infection reported on EPIS-FWD between March and May 2013 involved over 400 cases from 15 EU/EEA countries and Switzerland. The first outbreak was initially reported by Denmark on 1 March, with cases subsequently reported by Finland, Norway and Sweden. As of 6 August, 106 cases had been reported by the four Nordic countries of Denmark, Sweden, Norway and Finland [7]. Two closely related strains with subgenotype IB were associated to this outbreak [8]. Epidemiological investigations, including case interviews and case control study, and purchase history investigations pointed towards frozen strawberries from Egypt and Morocco as the most likely source of infection [9,10]. Although strawberries are not botanical berries, they are treated as berries in this article. Despite extensive food sampling and testing of frozen strawberries, no HAV could be isolated.

The second outbreak was initially notified by Norway on 17 April and subsequently an additional 13 countries reported associated cases (see Table). As of 20 August, 107 travellers returning from different locations in the Red Sea region, Egypt, were reported infected. The outbreak strain was subgenotype IB as well, but with a different sequence from the Nordic countries outbreak mentioned above. Multi-country epidemiological investigations, including case interviews and case control study, suggested that the implicated vehicle of infection was a food item distributed to different hotels in Egypt, with strawberries suspected among other fruits [11,12].

The third outbreak, reported on 8 May on EPIS-FWD, was thought to have affected about 200 Italian residents as of August 2013, although it was initially reported by Germany following identification of nine HAV infections in travellers returning from northern Italy [13]. A Dutch traveller and five Polish travellers to Italy were also part of this outbreak [14]. In addition, 21 people living in Ireland with no travel history to Italy were infected with an HAV strain with identical sequence [15]. The outbreak strain was subgenotype IA. Case interviews and a case control study in Italy, including the Dutch

and Polish cases, identified imported frozen mixed berries as the vehicle of infection [14,16]. Subsequently, a case–control study in Ireland led to the same conclusion [15]. Isolation of HAV in frozen mixed berries in Italy led to eleven notifications through the Rapid Alert System for Food and Feed (RASFF), which is the EU notification system to exchange information on measures taken on risks related to food and feed. Berries forming the mix mentioned in the RASFF notifications originated mostly from east European countries [17]. Several isolates from the food samples had a sequence identical to the outbreak strain [16,18].

Is there a link between these outbreaks?

The epidemiological and microbiological information available suggests no direct link between these simultaneous HAV outbreaks. All outbreaks were caused by a different persistent source of exposure: two were confirmed to be associated with the consumption of berries, while strawberries were one of the suspected vehicles of infection in the third outbreak. In the Nordic countries outbreak, having two closely related sequences co-circulating may suggest an environmental contamination of the berries, most likely through sewage water [19,20], or that the berries have geographically close origins.

Three of the outbreaks strains belong to subgenotype IB (two from the Nordic countries outbreak, one from cases with travel history to Egypt). The fourth outbreak strain, associated with berries in Italy, belongs to subgenotype IA, which excludes any link between the Italian outbreak and the other two outbreaks. Based on overlapping RNA fragments in the VP1 2A region, it was established that the three subgenotype IB sequences differed from each other by less than 2% [8]. Considering that the rate of mutation of the HAV RNA sequence is low [21], a 2% difference between sequences is a marker of a relatively long phylogenetic evolution. This suggests that it is unlikely that it would be one strain that would have rapidly mutated and spread but rather that the strains involved in the two IB subgenotype outbreaks would have a common geographical origin.

Is this situation unusual or unexpected?

Having three multi-country outbreaks declared within three months is an unexpected situation. Several HAV outbreaks in European travellers returning from HAV endemic countries such as Egypt were described in the past decade [22,23]. Food-borne HAV outbreaks due to the consumption of fruit including berries have previously been reported. Such outbreaks have involved vehicles like raspberries [24], strawberries [25], blueberries [26] and semi-dried tomatoes [4]. Also the simultaneous occurrence of HAV outbreaks in the EU has been previously observed, as in 2008 when three outbreaks in the Czech Republic, Latvia and Slovakia occurred. However, for these later outbreaks, transmission was mostly human-to-human [27].

Several factors have most likely drove toward this peculiar situation: first, the decreased incidence of HAV infections in the past decade, coupled with the fact that HAV was not included in the vaccination schedule of most of the countries of the EU/EEA, led to an increase in the number of susceptible European citizens, leaving the opportunity for large outbreaks to occur [1]; second, the limited coverage of HAV vaccination among European travellers to HAV endemic countries, particularly when staying in all-inclusive resorts [22,28], together with the increase in the number of travellers [29], explains the pool of cases among travellers to endemic areas; third, the large amount of fruit and vegetables and other food items imported into the EU and their extensive redistribution within the EU [30] may facilitate the introduction of HAV-contaminated products, leading to multi-country outbreaks. Contamination of the berries early in the food production chain seems most likely for the outbreaks in the Nordic countries and in Italy, allowing wide distribution of the contaminated fruit. Several pathways of contamination of berries can be suggested: irrigation with faecally-contaminated water prior to harvesting, infected field workers during the harvest or processing at the factory, and spraying with contaminated water before distribution [31].

Since the first RASFF notification in 1979, and as of 15 September 2013, over 37,100 notifications have been issued, and in the past five years, there has been an average of 3,400 notifications per year. So far, 35 notifications related to food-borne viruses and berries have been issued, which represents 7.4% (35/474) of the notifications related to pathogenic microorganisms in fruits and vegetables. Both notifications related to berries contaminated with food-borne viruses and notifications of food-borne virus outbreaks implicating berries have increased in recent years. Thirty-nine notifications related to berries, of which 30 (77%) were reported since 2009 and twelve (31%) between 1 January and 15 September 2013. The most frequently reported pathogenic microorganism in berries was norovirus (23 notifications, 59%) and HAV (nine notifications, 23%). All nine HAV notifications in berries are since November 2012, which suggests that more berries have been found to be contaminated recently than in previous years. In addition, there were 30 notifications about food items contaminated with HAV, of which 16 (53%) were made between 1 January 2012 and 15 September 2013. While notifications before 2012 on HAV findings in food and HAV outbreaks were mostly related to crustaceans and bivalve molluscs (10/14), since January 2012, the majority of HAV notifications have been related to fruit and vegetables (12/16), among which 9/12 are berries. This may indicate that fruit and vegetables, particularly berries, have become more frequently contaminated in the recent years. It should be emphasised that these could also be an effect of increased frequency of samplings and improvement of the sensitivity of analytical methods.

Finally, the first outbreak in the Nordic countries, initially reported by Denmark, may have indirectly facilitated the reporting of the following two outbreaks. In fact, the investigation of the Nordic countries outbreak may have encouraged Norway to increase the sequencing of HAV isolates from reported cases and therefore to detect and report the second outbreak through EPIS-FWD. In the same way, these first two outbreaks may have facilitated the detection of the cases related to travel to Italy and prompted Germany to report the outbreak through EPIS-FWD. Finally, the Italian public health authorities were alerted to the travel-related cases and immediately acknowledged the occurrence of a local outbreak. In the absence of a direct link between the outbreaks, the hypothesis of an 'awareness chain effect' might explain the quasi-simultaneous notifications of these outbreaks.

Avenues to prevent recurrence of similar HAV outbreaks

The outbreaks described have shown that frozen berries are efficient vehicles of HAV infection; to this extent, the risk posed by berries should be studied further. Such study could include the analysis of the pathways of berry contamination and the likelihood of being exposed to HAV-contaminated berries in the EU considering the intensive intra- and extra-EU trade of berries.

EPIS-FWD allowed the early detection of the multinational dimension of these outbreaks. The system supported the rapid exchange of information among the network's experts and easy access to up-to-date epidemiological and microbiological results. EPIS-FWD was also used as a document repository for the line listings, questionnaires and protocols, and rapid risk assessments prepared by ECDC. Although the system currently extends beyond EU/EEA borders, it is limited to very few non-EU/EEA countries. To fill the gap, the new version of EPIS-FWD, launched in July 2013, allows inviting non-network countries to participate in a discussion if the need arises, aiming to facilitate the exchange of information.

The development of molecular characterisation, and particularly RNA sequencing, has allowed the three simultaneous outbreaks to be identified and defined, and has allowed dispersed cases to be either linked or individuated within the outbreaks. ECDC and the European Commission should play a role in ensuring that adequate capacity to isolate and sequence HAV in food and human samples is available at the EU level, through promoting common protocols and sharing expertise.

The ECDC food- and waterborne toolbox for outbreak investigation [32] could be further developed beyond the standard trawling questionnaires already present to include the necessary protocols for HAV detection and sequencing.

Timely coordination of the control actions by the European Commission, including coordination of the trace-back and trace-forward activities at the EU/EEA level, is crucial during multi-country investigations. To minimise the risk of contamination of berries at farm and processing plant level, and therefore to minimise the risk of importing contaminated berries into the EU, food safety agencies and private food industries in the importing and exporting countries should work closely together, ensuring that best practices are applied. Good intersectoral cooperation is paramount during outbreak investigation to timely receive information about distribution of the product and eventual breaches in production practices.

These outbreaks have shown the complexity of viral epidemiology and microbiology. ECDC has initiated in 2013 the nomination of food-borne viral infections experts (microbiologists and epidemiologists) from the EU/EEA countries to contribute in providing data and expertise through TESSy and EPIS-FWD. Strong collaboration with existing international networks such as the International HAV laboratory network, HAVNET (www.havnet.nl), managed by the Dutch National Institute for Public Health and the Environment (RIVM) should be ensured.

The outbreak in travellers to Egypt also highlights the importance of vaccination in travellers to endemic areas in a time of increasing tourism to endemic destinations.

More HAV outbreaks are expected to occur in the EU. The 2013 experience demonstrates the absolute necessity for extensive collaboration between countries and between the public health and food sectors to identify as quickly as possible the vehicle of infection and, ideally, to control the outbreak in an timely fashion.

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Conflict of interest

None declared.

Authors' contributions

Both authors worked on the EU coordination of these outbreaks and wrote this manuscript.

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