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Postsurgical wound infections due to rapidly growing mycobacteria in Swiss medical tourists following cosmetic surgery in Latin America between 2012 and 2014

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Between October 2012 and August 2014, several Swiss patients developed severe soft tissue infections due to rapidly growing mycobacteria following cosmetic surgery in the Dominican Republic, Ecuador and Mexico. Infections were caused by *Mycobacterium abscessus* (n=5), *Mycobacterium* sp. JAN1 (n=1) and *M. conceptionense* (n=1). Similar cases may have remained unrecognised due to a lack of notification requirements. Microbiological work-up of medical tourists with infections following cosmetic surgery should include rapidly growing mycobacteria.

Between October 2012 and August 2014, the Swiss National Reference Centre for Mycobacteria identified a series of severe healthcare-associated soft-tissue infections caused by rapidly growing mycobacteria (RGM) in seven female Swiss citizens who had undergone cosmetic surgery in the Dominican Republic, Ecuador and Mexico. Here we report the clinical presentation and microbiological findings and discuss possible implications for medical tourists and healthcare providers.

Case series

Between October 2012 and August 2014, seven previously healthy female patients sought medical advice at different hospitals in the German-, French- and Italian-speaking parts of Switzerland due to severe healthcare-associated infections following cosmetic surgery. All patients were Swiss citizens of Latin American descent between 19 and 52 years of age. The patients were not related to each other and there was no history of contact between them. All patients had recently undergone plastic surgery as medical tourists in the Dominican Republic (five patients), Ecuador (one patient) and Mexico (one patient). Surgical procedures performed were abdominal liposuction (two patients), breast augmentation (two patients) and breast reduction with or without simultaneous abdominoplasty (three patients). The patients developed post-surgical wound infections with symptoms ranging from local inflammation to painful subcutaneous and severe deep tissue abscesses that failed to respond to initial antibiotic chemotherapy.

Microbiological investigations

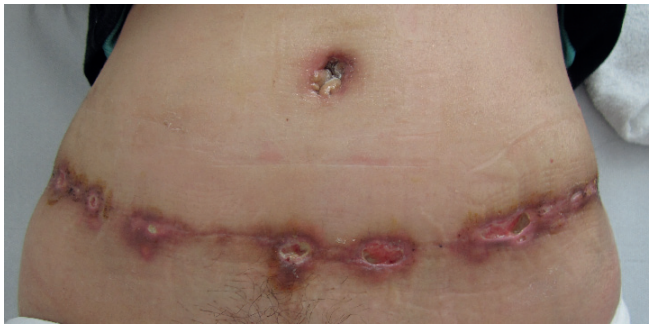
Microbiological cultures and 16S rRNA gene analyses performed on tissue biopsies or drainage fluid from the infected sites repeatedly identified RGM as the infectious agent, namely *Mycobacterium abscessus* subsp. *abscessus* (four patients), *M. abscessus* subsp. *massiliense* (one patient), *Mycobacterium* sp. JAN1 (closely related to *M. abscessus*, one patient) and *M. conceptionense* (*M. fortuitum* group, one patient) [1,2]. Drug susceptibility testing was performed according to standard procedures [3]. Minimal inhibitory concentrations (MICs) were as follows: amikacin 0.5–8.0 mg/L; clarithromycin ‹0.5 mg/L for *M. abscessus* subsp. *massiliense*, *Mycobacterium* sp. JAN1, and *M. conceptionense*, inducible resistance due to a functional Erm(41) methylase for three of the four *M. abscessus* subsp. *abscessus* isolates [4]; linezolid 1.0–16.0 mg/L; moxifloxacin 2.0–8.0 mg/L for *M. abscessus* spp. and *Mycobacterium* sp. JAN1, and 0.125 mg/L for *M. conceptionense*; doxycycline 64–›256 mg/L for *M. abscessus* spp. and *Mycobacterium* sp. JAN1, and ‹0.5 mg/L for *M. conceptionense*; tigecycline 0.5–8.0 mg/L.

Treatment

All infections required surgical revision in combination with multidrug antibiotic chemotherapy, and removal of breast implants in two patients. As only mycobacterial cultures isolated in different laboratories were referred to us and medical records were not available,

Figure 1

Purulent lesions following abdominoplasty at a medical centre in Ecuador, caused by *Mycobacterium abscessus* subsp. *abscessus*, Switzerland, October 2013



further details on administered antimicrobials and on outcome could not be obtained for six patients. One patient (Figures 1 and 2), for whom complete follow-up information was available, was treated with a combination of amikacin, linezolid and moxifloxacin following surgical resection and debridement. Macrolides were not administered because of an inducible resistance phenotype. Due to serious side effects, amikacin and linezolid were stopped after four and five weeks, respectively. Moxifloxacin was given for an additional week but was stopped thereafter, because of the risk of high-level resistance when given as monotherapy. Ten months later, the patient still had transitory nodular skin lesions. Histopathological analyses of three lesions showed granulomatous inflammation. However, microbiological cultures and PCR from corresponding specimens remained negative.

Transmission history

In order to identify possible transmission links between the four patients with confirmed *M. abscessus* subsp. *abscessus* infection, molecular typing was performed using both randomly amplified polymorphic DNA (RAPD) PCR and multilocus sequence typing (based on partial sequences of the *argH*, *cya*, *glpK*, *gnd*, *murC*, *pta*, and *purH* genes) [5,6]. A clonal relationship between the four *M. abscessus* subsp. *abscessus* isolates was excluded. In addition, available information did not indicate an association of the infections with one particular clinic. Several sources can be considered that may have been responsible for the infections such as contaminated rinsing fluids, gentian violet for marking skin incisions, injectable medications, antiseptic solutions, unsterile surgical instruments or poor wound aftercare, e.g. by using contaminated tap water to irrigate postoperative wounds [7-10]. The Swiss health authorities have been informed in order to conduct further epidemiological investigations and to contact the health authorities in the affected countries.

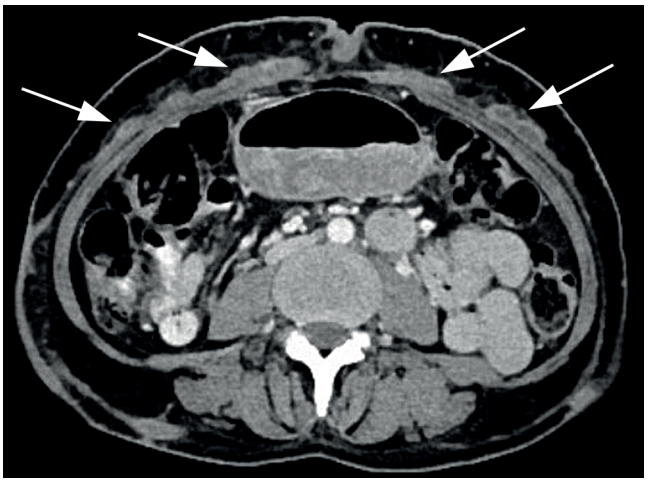
Background

Similar to other RGM, *M. abscessus* can be isolated from a wide variety of environmental sources including water and soil [11,12]. *M. abscessus* is considered an emerging pathogen causing severe infections in patients suffering from chronic pulmonary diseases, e.g. bronchiectasis and cystic fibrosis [13]. It has also been associated with infections following cosmetic procedures, e.g. tattooing [14], and with surgical wound infections, post-injection abscesses and healthcare-related outbreaks [15,16]. Antibiotic therapy of *M. abscessus* infections is challenging due to the organism's natural resistance to most clinically available antibiotics [17-19]. Studies on clinical outcome with respect to specific therapeutic regimens are scarce and mainly focus on pulmonary disease [20,21]. Antimicrobial chemotherapy of *M. abscessus* infections is guided by in vitro drug susceptibility testing results and should include a macrolide, e.g. clarithromycin or azithromycin, and an aminoglycoside, preferably amikacin [3,13,19]. Some *M. abscessus* isolates show an inducible macrolide resistance phenotype conferred by a ribosomal methylase, Erm(41), and the clinical efficacy of macrolides against such strains remains unclear [4]. Acquired high-level resistance to macrolides (MICs>256 mg/L), however, is due to mutations in the 23S ribosomal RNA gene [22-24]. For extensive extrapulmonary disease, administration of additional compounds, e.g. moxifloxacin, linezolid and/or tigecycline is recommended [13,15,17,19]. Surgical revision of the infected tissues is often necessary to reduce the bacterial load at the site of infection.

Other ubiquitous RGM species like *M. conceptionense*, a member of the *M. fortuitum* group, have also been reported to cause infections following medical or cosmetic procedures [15,25-27]. Treatment is generally more effective than against *M. abscessus* infections due to the less pronounced innate antibiotic resistance [28]. Thus, fluoroquinolones show comparably low MICs against species in the *M. fortuitum* group and

Figure 2

Abdominal computed tomography scan of a patient following abdominoplasty at a medical centre in Ecuador showing multifocal subcutaneous abscesses of the anterior abdominal wall, Switzerland, October 2013



doxycycline, a tetracycline antibiotic, is effective in vitro against about 50% of *M. fortuitum* group isolates [13,17].

Discussion

Previous studies described serious post-surgical complications due to *M. abscessus* infections following cosmetic surgery among 20 American 'lipotourists' in the Dominican Republic between 2003 and 2004 [29-31]. Part of the infections were caused by identical strains following surgical procedures performed at the same clinic, which led to an on-site investigation by national public health authorities. However, the cause for this outbreak has not been reported. A literature search did not reveal any reports on similar infections related to medical tourism to the Dominican Republic or other Latin American countries during the following years with the exception of a large *M. abscessus* outbreak affecting 311 patients who underwent various surgical procedures including mammoplasty and liposuction in Belém (Brazil) between February 2004 and June 2005 [32]. A recent warning published by Schnabel et al. after 16 female United States residents underwent plastic surgery at eight clinics in the Dominican Republic between March 2013 and April 2014 [33] as well as our case series indicate that the problem is either unresolved or that a new source of RGM infections has emerged. Our observations highlight that cases are not restricted to the Dominican Republic and that patients residing outside the Americas are also affected.

Since RGM infections do not require compulsory reporting to public health authorities, the number of unreported cases may be considerable. We recommend that microbiological work-up of medical tourists with infections following cosmetic surgery should always include RGM. Furthermore, attending physicians should seek expert advice to timely prescribe antibiotic therapy and to prevent the emergence of drug-resistant subpopulations.

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Conflict of interest

None declared.

Authors' contributions

Microbiological investigations (FPM, GVB, ECB, AS), genotyping (CC, FPM, GVB), patient care (AVB, AW), Figures 1 and 2 (AVB, AW). The manuscript was prepared by FPM, AVB, GVB, ECB and AS.

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Burden of influenza B virus infections in Scotland in 2012/13 and epidemiological investigations between 2000 and 2012

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We describe the burden of influenza B infections in Scotland during a 13-year study period. Influenza A and B viruses cocirculated throughout the period, with numbers of influenza B cases approaching or exceeding those of influenza A during six influenza seasons. Influenza B viruses of both Victoria and Yamagata lineage were detected in two of six seasons investigated. For the 2012/13 season, influenza B accounted for 44.4% of all influenzas, with the highest incidence in those under the age of five years. Influenza B virus infections led to fewer admissions to an intensive care unit (ICU) and a lower mortality rate than influenza A (37 vs 81 ICU admissions and three vs 29 deaths) during the 2012/13 season. However, a quarter of those admitted to ICU with influenza B had not been immunised and 60% had not received specific influenza antiviral therapy. This highlights the need for consistent influenza vaccination and prompt usage of antiviral treatment for identified risk groups. Combining the newly introduced vaccination programme for children with the use of a tetravalent vaccine may provide the opportunity to improve the control of influenza B in those with the highest influenza B burden, children and young adolescents.

Introduction

Both influenza A and B viruses show antigenic variation with sequence variability in the haemagglutinin (H) and neuraminidase (N) genes, altering neutralising properties and protection from reinfection [1]. However, they differ in the dynamics of turnover of antigenic variants and in their propensity for reassortment [2]. Variants of influenza A viruses are classified into subtypes based on the H and N genes; two subtypes (H1N1 and H3N2) are currently found in humans in Europe. Although influenza B viruses are not formally classified into subtypes, two antigenically distinct lineages defined by the reference strains B/Victoria/2/87 (Victoria lineage) and B/Yamagata/16/88 (Yamagata lineage) have been circulating globally since 1983 [3]. Influenza B viruses of the Victoria and Yamagata lineages have been shown to circulate simultaneously or individually in the past and switching between lineages occurs almost every other year [2]. In contrast, antigenic shift of influenza A, characterised by changes in the circulating H and N types occurs less frequently. Because several influenza variants are present in the same season, influenza vaccines currently in use in the United Kingdom (UK) and elsewhere in Europe are trivalent, containing two influenza A subtypes (H1N1 and H3N2) and one influenza B lineage. However, it provides only limited immunity against the influenza B strains of the other lineage [4].

In order to improve our understanding of the burden and epidemiology of influenza B, we have reviewed the influenza B viruses circulating in Scotland over the period 2000 to 2013 and estimated the clinical burden of influenza B virus infections in more detail for the season 2012/13.

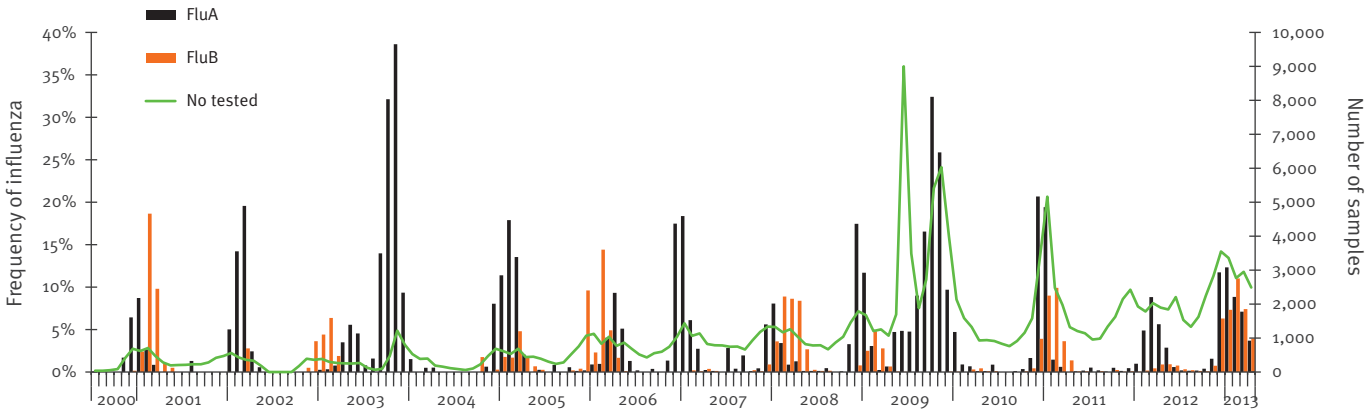
Methods

Routine laboratory testing

In Scotland, diagnostic testing by reverse transcription-polymerase chain reaction (RT-PCR) for influenza B virus forms part of multiplex real-time screening for a range of respiratory viruses. PCR-based influenza B virus screening has been carried out in National Health Service (NHS) laboratories in Glasgow since early 2000 [5], in Edinburgh since 2005 [6], in Dundee since 2007 and in Aberdeen since 2008. Thus Figure 1 from 2000 to 2005 is based on Glasgow results only. In addition to individuals presenting to hospital with presumed

Figure 1

Frequency of influenza A and B virus infections compared with total number of samples tested, Scotland, November 2012–April 2013



	2000/01	2001/02	2002/03	2003/04	2004/05	2005/06	2006/07	2007/08	2008/09	2009/10	2010/11	2011/12	2012/13
Influenza A	128	171	39	720	326	193	556	387	1,266	4,620	1,774	488	1,439
Influenza B	198	10	56	0	71	318	7	291	161	13	942	72	1,125
Influenza A and B	326	181	95	720	397	511	563	678	1,427	4,633	2,716	560	2,564
% influenza B	60.7	5.5	59.0	0	17.9	62.2	1.2	42.9	11.3	0.3	34.7	12.9	43.9
Total tested	3,803	3,694	2,648	4,538	4,744	8,490	9,940	12,008	25,447	31,360	21,869	21,148	29,612

Years when more than 30% of all influenza infections were caused by influenza B virus are shown in orange.

respiratory virus infection, a sample of those presenting in the community is tested for respiratory viruses including influenza B virus via the Scottish sentinel surveillance scheme [7].

Prospective data collection

All reports of influenza B virus detection from NHS laboratories in Scotland are collated centrally by the national public health body Health Protection Scotland (HPS) via the Electronic Communication of Surveillance in Scotland (ECOSS) non-mandatory reporting system. The population of Scotland was estimated to be 5,295,400 in 2011 [8].

General practitioners (GP) participating in the Scottish sentinel surveillance scheme are requested to systematically submit nose and throat swabs from the first five patients per week consulting with influenza-like illness or acute respiratory tract infection (based on clinical judgement) [7]. Supplementary data on demography, clinical signs and risk group as well as vaccination status are collected for each patient. Since season 2000/01 between 18 and 89 GPs per season have participated in the scheme, submitting between 600 and 5,000 samples per season.

Furthermore, the Scottish Severe Acute Respiratory Illness (SARI) surveillance system collects individual-level data on confirmed influenza cases admitted to an intensive care unit (ICU) during the influenza season from all 30 hospitals with intensive care provision across the 14 NHS boards.

Retrospective data collection

All reports of influenza B virus detections submitted to HPS via ECOSS between 1 November 2012 and 31 April 2013 were obtained electronically and analysed in this study. Where multiple samples collected from the same patient tested positive for influenza B within an eight week period, only the first influenza B detection was included. Data were anonymised by HPS before extraction for analysis by month and year of reporting, age group and source of referral (GP/hospital). Since the ECOSS records do not include a denominator of total number of tests performed, additional data were collected on the numbers of respiratory samples referred for influenza B testing in Glasgow (January 2000–May 2013), Edinburgh (October 2005–May 2013), Aberdeen (November 2012–May 2013) and Dundee (November 2012–May 2013). Furthermore, all cases of severe influenza B admitted to ICU in Scotland between November 2012 and April 2013 were identified from the SARI surveillance system. For each case, anonymised data on age group, sex, date of onset, outcome, vaccination status, antiviral treatment and specific clinical risk group including underlying illnesses were obtained.

Estimates of the incidence of infection in different age groups were calculated for central and southern Scotland based on the most recent mid-year population

estimate of 4,252,000 including 235,200 children under the age of five years [8].

Sequencing

Nucleotide sequences of the influenza B haemagglutinin gene were obtained for each influenza season from a representative subset of archived nucleic acid samples derived from anonymised respiratory samples from patients with confirmed influenza B (collected since 2007). The haemagglutinin gene was amplified using hemi-nested primers (sense: TACTACTCATGGTAGTAACATCC; outer antisense: TTTCGTTRTGSAGTTCATCCATSGC; inner antisense: AATCATK CCTTCCCAKCCKCCT). Nucleotide sequences were aligned with reference sequences using SSE v1.1 [9] and phylogenetic analysis was performed using Mega 5.1 [10]. In addition, influenza B virus-positive samples tested in Glasgow in 2012/13 were typed using lineage-specific real-time PCR [11], and sequences from 39 previously characterised influenza B virus strains from England were obtained from the database of the Global Initiative on Sharing All Influenza Data (GISAID; http://platform.gisaid.org/epi3/frontend#48fe7c). The authors gratefully acknowledge the originating and submitting laboratories who contributed these sequences to GISAID, in particular Public Health England (formerly Health Protection Agency) and the National Institute for Medical Research in the UK (n=37 sequences) and the National Institute for Medical Research and the Centers for Disease Control and Prevention in the United States (US) (n=2 sequences).

Results

Epidemiology of influenza B virus during 2000 to 2013

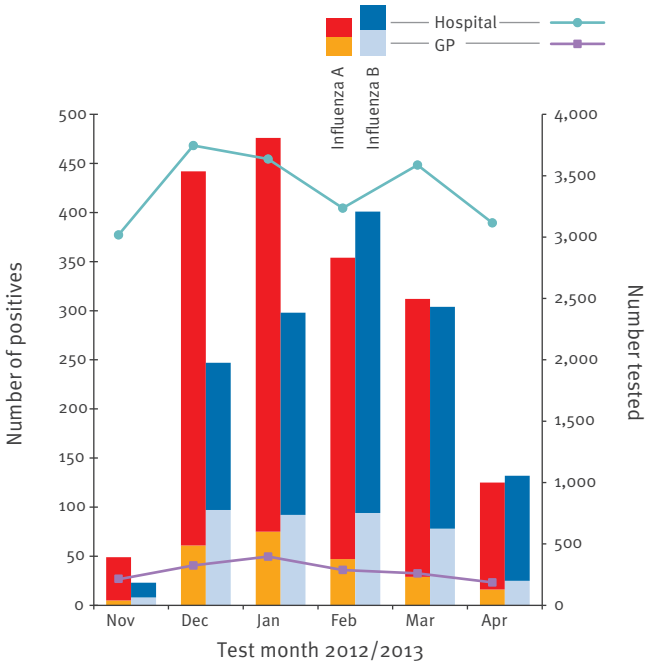
During the period January 2000 to April 2013, several distinct waves of influenza A and B viruses infections were observed in Scotland (Figure 1). Influenza A and B viruses cocirculated during most influenza seasons, with numbers of influenza B infections approaching or exceeding those of influenza A virus during six seasons (2000/01, 2002/03, 2005/06, 2007/08, 2010/11 and 2012/13). The number of samples tested for influenza viruses by PCR in Scotland increased by a factor of 7 over this period from an average of 3,381 per season in 2000 to 2003 (all Scottish samples were tested in Glasgow) to an average of 24,209 per season in 2010 to 2013 (testing done in all four centres).

Burden of influenza B infections in the 2012/13 season

More detailed information on influenza virus incidence and clinical outcomes was available for the season 2012/13. A total of 22,015 respiratory samples were tested in Scotland for influenza A and B viruses between November 2012 and April 2013. The overall detection frequency of influenza viruses was 14.4%, varying from 2.3% in November to 20.8% in February. Influenza B virus infections accounted for 44.4% of all influenza detections (1,405/3,163), exceeding the number of influenza A infections in February (Figure 2).

Figure 2

Detection frequency of influenza A and B virus infections and total number of samples submitted for testing by general practitioners and hospitals, Scotland, November 2012–April 2013 (n=22,015)



Virology testing in Scotland is performed in four NHS laboratories, located in Dundee, Aberdeen, Edinburgh and Glasgow.

Community versus hospital

Compared with 1,405 laboratory detections of influenza B virus in the 2012/13 season, 1,279 episodes of influenza B virus infection were reported to HPS during the same time period, showing the robustness of automated reporting (data not shown). The discrepancy probably reflects individuals who tested positive for influenza B more than once. Of the 1,279 reported infections, 394 related to samples collected by GPs and represented influenza B infections in the community, whereas the remaining 885 influenza B-positive samples were obtained during hospital visits (Figure 2).

The community-based influenza B infections will not be discussed further; the remaining analysis focuses on hospital-based influenza B infections only.

Age distribution

In the 2012/13 season, hospital-based influenza A and B infections were widely distributed between age groups (Table 1). The highest frequency of influenza A virus detection (over 80% typed as H3N2) was seen in individuals over 65 years of age (23.5%; 451/1,916), whereas influenza B detections were significantly underrepresented in this age group in Scotland (7.0%, 134/1,916; $p<0.0001$ by chi-square test of association). When comparing the age-specific incidence of

Table 1

Age distribution of influenza A and B virus infections in individuals tested in Edinburgh and Glasgow, November 2012–April 2013 (n=16,146)

Age group	Samples	Influenza A virus		Influenza B virus	
		Positive	Rate	Positive	Rate
‹5 years	5,555	399	7.2%	258	4.6%
5–10 years	1,637	129	7.9%	194	11.9%
11–15 years	719	53	7.4%	78	10.8%
16–20 years	551	40	7.3%	41	7.4%
21–36 years	1,494	63	4.2%	184	12.3%
37–65 years	4,274	485	11.3%	397	9.3%
›65 years	1,916	451	23.5%	134	7.0%

laboratory detections of hospital-based influenza in the Edinburgh and Glasgow area, based on the total population size in each age group, the highest incidence of influenza A(H3N2) and B infections were seen in children under the age of five years with, respectively, 170 and 110 infections per 100,000 (Figure 3). This age group was also the most frequently tested and accounted for 34% of samples tested for influenza (5,555/16,146), although this group represented only 5.6% of population (235,200/4,237,200). A similar age distribution of hospital-based influenza A(H3N2) and B infections was also observed in the previous seasons 2000 to 2012, whereas a higher incidence of influenza A(H1N1) infections was noted in adults between 21 and 65 years (data not shown).

Severity of influenza B

In 2012–13, a total of 37 influenza B virus-infected individuals admitted to ICU were reported through the Scottish SARI surveillance system; three of them died. In comparison, 21 H1N1 and 60 H3N2 influenza A virus-infected individuals were admitted to ICU, of whom nine and 22 died, respectively. Most patients admitted to ICU with influenza B were older than 37 years (n=20), whereas a small number (n=9) of ICU admissions were observed in those younger than 20 years (Figure 4). The three mortalities occurred in the age groups 15–19, 20–24 and ›65 years.

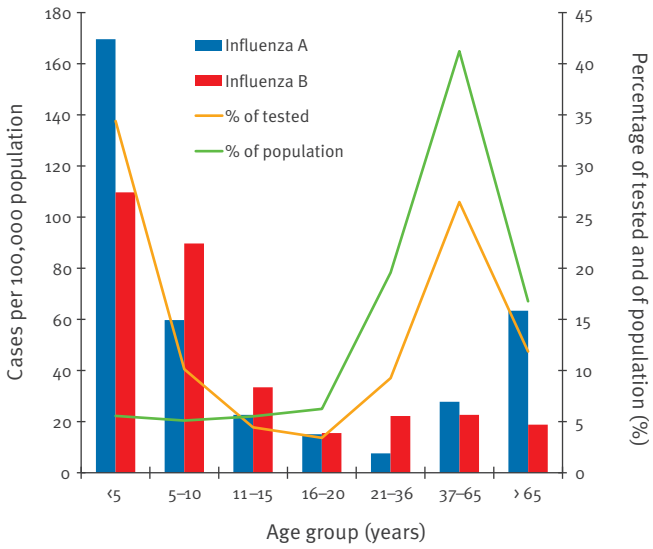
Only 16 of 27 influenza B-infected individuals were recorded to have been treated with antiviral drugs in the ICU (data not available for the remaining 10 individuals). Of the 37 influenza B patients treated in ICU, 24 belonged to groups recommended for vaccination (65 years and older or a clinical risk group), but only six had actually received the seasonal influenza vaccination.

Influenza B virus genetic lineages

Nucleotide sequence analysis of the haemagglutinin gene from viruses sampled monthly in Edinburgh (n=61) and Glasgow (n=86) between 2007/08 and 2012/13, and real-time PCR typing data in Glasgow in 2012/13 (n=488) revealed complex changes in the circulating lineages of influenza B virus (Table 2). The distribution of influenza B virus genetic lineages did not differ between Edinburgh and Glasgow. Before 2010/11, only one lineage was found per season, either B/Brisbane/60 (belonging to the Victoria lineage) or B/Florida/4 (Yamagata lineage). However, since only a limited number of influenza B virus strains were available for this study, it cannot be excluded that both genetic lineages may have been circulating simultaneously. Furthermore, in 2010/11 and 2012/13, both lineages were represented among sequences from both Edinburgh and Glasgow. Sequences collected in

Figure 3

Estimated incidence of influenza A and B virus infections in southern and central Scotland by age group, based on laboratory testing data from Edinburgh and Glasgow, November 2012–April 2013 (n=16,146)

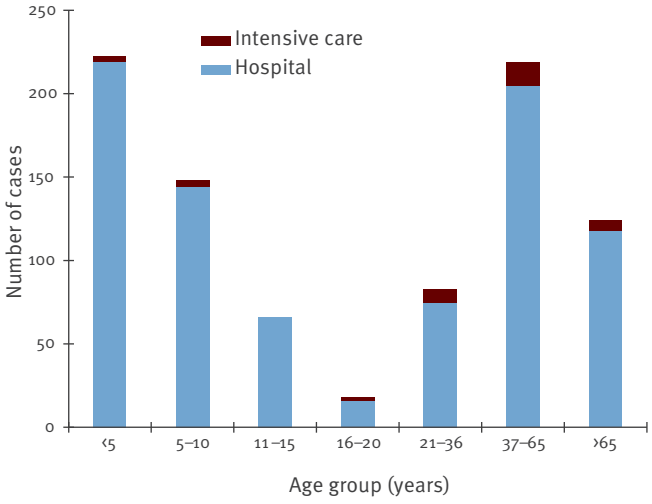


Incidences are calculated based on and census data from 2011 [8].

Line graphs refer to the right y-axis and show the percentage of samples tested per age group vs the percentage of each age group in the population of southern and central Scotland.

Figure 4

Hospital vs intensive care admissions due to influenza B infection, by age, Scotland, November 2012–April 2013 (n=880)



the same period in England (n=39), followed a similar trend. Which strains were circulating did not appear to be correlated with the vaccine strain used in a given season (Brisbane apart from 2008/09 and 2012/13) or with the intensity of influenza B infection (low in 2006/07, 2009/10 and 2011/12). In seasons with two circulating lineages there was no relationship between the distribution of virus lineages and the patients' age (data not shown).

Most influenza B viruses typed from patients treated in ICU were identified as Yamagata lineage viruses (16/19, two of which were vaccinated and thus vaccine failure was suspected); the remaining three were two Victoria lineage viruses and one untyped. This is in line with the finding that 78.5% (400/509) of influenza B viruses were identified as Yamagata lineage viruses in Scotland during the last season 2012/13.

Discussion

This epidemiological study of influenza B virus infections in Scotland is timely in view of the recent changes in the influenza vaccination programme in the UK and the possible introduction of a tetravalent influenza vaccine containing influenza B viruses of both the Victoria and Yamagata lineage. Our study reveals that influenza B virus was the predominant overall cause of influenza in four of the 13 influenza seasons from 2000/01 through 2012/13, and cocirculation of the Yamagata and Victoria lineages was observed in two of the six seasons investigated. Similar patterns of influenza B virus circulation have been described in the US, Europe and Hong Kong [12-13].

Laboratory testing

With influenza diagnostic screening now improved through faster turnaround times and the capacity for screening larger numbers of samples in all four centres (a sevenfold increase during the past 10 years), virological data currently obtained through routine testing of samples from patients presenting to hospitals can be used effectively and directly to support influenza surveillance. Interestingly, 45% of samples submitted in 2012/13 for virological investigations in Edinburgh (NHS Lothian Health Board) were obtained from children under the age of five years. The high rate of testing in this age group results from a policy that all children presenting with respiratory symptoms to the accident and emergency department at the Royal Hospital for Sick Children in Edinburgh will be sampled and tested for respiratory viruses irrespective of whether they require hospital admission or not. Almost 10% of the population under five years of age in Lothian (4,141/48,600) were tested for influenza between November 2012 and

Table 2

Number of influenza B isolates grouping with the Victoria lineage (Brisbane/06) or Yamagata lineage (Florida/04 or Wisconsin/10), by season and location, Scotland, November 2007–April 2013 (n=186)

Season	Edinburgh		Glasgow		United Kingdom		Vaccine
	Victoria	Yamagata	Victoria	Yamagata	Victoria	Yamagata	
2007/08	0	10	No data	No data	0	3	Brisbane
2008/09	9	0	24	0	1	0	Florida
2009/10	2	0	1	0	3	0	Brisbane
2010/11	16	3	33	9	8	3	Brisbane
2011/12	0	0	3	0	4	7	Brisbane
2012/13	2	19	8 (107)[a]	8 (381)[a]	4	6	Wisconsin

[a] Additional typing results based on lineage-specific real-time PCR.

The vaccine strain used in each season is indicated.

The authors acknowledge the laboratories that submitted 39 UK sequences used in this Table to GISAID: Public Health England (formerly Health Protection Agency) and the National Institute for Medical Research in the UK (n=37 sequences) and the National Institute for Medical Research and the Centers for Disease Control and Prevention in the United States (n=2 sequences).

April 2013. These screening results can therefore be an indicator for influenza A and B virus incidence and circulation in that age group irrespective of whether infections lead to hospital admission. However, this approach is not routinely used elsewhere in Scotland: Only 20% of samples submitted for virological screening in Glasgow were obtained from children under the age of five years. This is likely to reflect different screening policies adopted in hospitals (i.e. screening only those admitted vs everybody screened).

Influenza B, a paediatric infection?

Influenza is generally recognised as an important disease with high mortality rate among the elderly, although children have been shown to have an important role in the dissemination of influenza [14-15]. In contrast to influenza A(H3N2) virus, influenza B virus predominantly infects children and young adults [16-17]. In the current study, influenza B contributed to 45% of influenza-related hospital visits overall during the 2012/13 season, as much as 60% for children aged six to 15 years and 75% for young adults aged 21 to 36 years. The detection rate for influenza A dropped remarkably from those younger than five years to those aged six to 10 years, whereas only a slight drop was seen for influenza B infections (Figure 3) This is consistent with previous studies [12,18] and may be due to children accumulating natural immunity to influenza B more slowly than to influenza A [19].

Severity of influenza B infections

Data on severe influenza B virus infections and mortality are limited. A large study from the US covering the period from 1976 to 1999 reported that 25% of all influenza-related mortality could be attributed to influenza B virus. This is substantially higher than seen in this study where influenza B infections accounted for ca 9% of all influenza virus-related deaths (3/34) during the 2012/13 season. Among those admitted to ICU with influenza B infections, we saw two deaths in young adults (15 to 24 years; one without underlying health problems). Whether they and others who were admitted to ICU with influenza B virus infection were genetically predisposed for severe infection remains to be investigated further [20].

Prevention and treatment

Influenza immunisation has been recommended in the UK since the late 1960s and has been targeted to those predisposed towards greater morbidity following influenza. Although influenza vaccine uptake in Scotland during the 2012/13 season was 77% for those older than 65 years and 56% for those 65 years and younger in a risk group [21], only a quarter of those treated in ICU who should have been vaccinated actually had received the seasonal influenza vaccine. Most individuals (16/18) in ICU who had missed their vaccination were younger than 65 years but should have been vaccinated due to an underlying chronic medical condition. Whether the mechanism to target these individuals for influenza vaccinations could be improved remains to be studied.

The neuraminidase inhibitors zanamivir and oseltamivir have been shown to be effective in the treatment of influenza [22-23], and are recommended for those hospitalised with influenza. Only 60% of individuals admitted to ICU with influenza B were treated with antiviral drugs; none of those who died were reported to have received antiviral treatment. However, the effectiveness of neuraminidase inhibitors against influenza B virus has been questioned and needs to be investigated further because inhibitory concentrations in vivo differ by up to 10-fold [24].

As the disease burden of influenza B is higher in children and young adolescents than in older age groups and because of recent cocirculation of influenza B viruses of both lineages, tetravalent vaccines may need to be combined with the newly introduced vaccination programme for children to improve the control of influenza B. The national programme aims to offer annual influenza vaccination to all school children across Scotland by autumn 2015, but there is no defined time frame for the introduction of the tetravalent vaccine in this group. Our study demonstrates that influenza B virus infections are associated with substantial morbidity and that influenza surveillance and interventions including vaccination and treatment are still suboptimal.

Acknowledgements

We acknowledge the authors, originating and submitting laboratories of the sequences from GISAID's EpiFlu Database from which 39 UK influenza B virus sequences were obtained for comparison.

Conflict of interest

None declared.

Authors' contributions

Heli Harvala analysed the data and wrote the manuscript. Donald Smith, Peter Simmonds and Beatrix von Wissman also wrote part of the manuscript. The study was planned together with Heli Harvala, Rory Gunson, Kate Templeton, Arlene Reynolds, Beatrix von Wissmann and Jim McMenamin. Peter Simmonds designed the influenza B virus sequencing primers, whereas Donald Smith and Karina Salvatierra did the virus sequencing, and Alastair MacLean virus typing based on real-time PCR. Catherine Frew, Alison Hunt and David Yirrell provided the laboratory data, whereas Beatrix von Wissmann and Arlene Reynolds provided the data from Health Protection Scotland.

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Hepatitis A outbreak in Italy, 2013: a matched case–control study

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Between January and May 2013 a hepatitis A (HA) incidence increase was detected in Italy, signalling an outbreak. A retrospective matched case–control study was conducted to identify the source of infection. A case was defined as a resident of any of five regions (Apulia, autonomous province of Bolzano, Emilia-Romagna, Friuli–Venezia-Giulia and autonomous province of Trento), who had symptom onset between 1 January and 31 May2013 as well a positive test for anti-HA virus IgM. We compared each case with four age-and neighbourhood-matched controls. Overall 119 cases and 419 controls were enrolled. Berries were found as the main risk factor for HA (adjusted odds ratio (ORadj): 4.2; 95% confidence interval (CI): 2.5–7.0) followed by raw seafood (ORadj: 3.8; 95% CI: 2.2–6.8; PAF: 26%). Sequencing the virion protein (VP)1-2a region from 24 cases yielded a common sequence (GenBank number: KF182323). The same sequence was amplified from frozen mixed berries consumed by some cases as well as from isolates from Dutch and German HA patients, who had visited some of the affected Italian provinces during the outbreak. These findings suggested berries as the main source of the Italian outbreak. Control measures included voluntary recall of the confirmed frozen mixed berry batches and a trace-back investigation was initiated. The Ministry of Health website recommends frozen berries to be cooked for two minutes before eating.

Introduction

Hepatitis A virus (HAV) is highly transmissible and one of the most frequent causes of foodborne infections. It occurs worldwide, both sporadically and in the form of epidemics, with a tendency for cyclic recurrences in time. Every year an estimated 1.4 million cases of hepatitis A occur worldwide [1].

The incubation period of hepatitis A (HA) is usually between 28 and 30 days (maximum range 15–50 days) [2], and while the disease is often asymptomatic in children under five years-old, its severity increases with age in adults over 18 years-old [2]. HAV, which can be stable in the environment for several months is able to survive freezing [3], and can be transmitted through contaminated water and food as well as from person to person, by the faecal–oral route [1,4]. Other relevant risk factors associated with HAV are travel to highly endemic areas [5] as well as individual high-risk behaviours (such as for people who inject drugs or use other illicit drugs, for men who have sex with men and for people using contaminated blood products [2]).

In Italy, HA is mandatorily reported to the Italian National Surveillance System (Ministerial Decree (DM) of 15/12/90) [6]. A sentinel surveillance system for acute viral hepatitis (SEIEVA - Sistema Epidemiologico Integrato Epatiti Virali Acute), which was implemented in 1984 [7] is also in place to strengthen the surveillance and to promote the investigation and control of acute viral hepatitis.

HA in Italy arises both in sporadic and endemic-epidemic forms, with some regions in the south (such as Apulia and in Campania) permanently affected by the disease [8-10]. Since the early 1990s, in line with a global trend and as the result of improved sanitation

and socio-economic conditions, the epidemiology of HAV has greatly changed in the country, with a clear decline in the numbers of cases over the years, whereby the incidence of HAV dropped from 4 per 100,000 in 1991 to 1.4 per 100,000 in 2006 [11]. Over the last decade, the disease incidence further declined and was below 1 per 100,000 in the last two years before the outbreak reported here.

The outbreak

A new outbreak has been ongoing in Italy since 2013. Epidemiological data showed a clear increase in the incidence of HA from 1 January to 31 May 2013 compared to the same period of the previous three years [8]. The highest increase in numbers of patients affected by HA was observed in northern Italy, in particular in the regions of Emilia-Romagna, Friuli–Venezia-Giulia, Lombardy, Piedmont, Trento and Bolzano, and Veneto. Apulia (south Italy) also showed an increase in the number of cases in 2013. In May 2013, some European Union Member States (Germany, Netherlands, Poland) reported cases of HA linked to a ski holiday in northern Italy (autonomous provinces of Trento and Bolzano). After this notification, a retrospective epidemiological investigation started in the provinces of Trento and Bolzano, whereby cases notified to the local health units were contacted. Sequencing of the virion protein (VP)1-2a region of the virus derived from five cases residing in these provinces, one Dutch, and two German cases revealed 100% sequence similarity between all isolates. Thus the outbreak strain was characterised and submitted to GenBank (GenBank accession number: KF182323). The preliminary epidemiological investigation in the autonomous provinces (AP) of Trento and Bolzano showed that the only common food consumed by different cases had been mixed berries. Frozen mixed berries eaten by cases in another region, Veneto, were found positive for HAV and the VP1-2a region of the viral sequence derived from the berries showed 100% sequence similarity with that of the cases' isolates, so more samples of frozen mixed berries were collected throughout the country and to date a total of 15 frozen mixed berry samples in Italy have been found positive for HAV [8,12].

As soon as the outbreak was detected. a series of actions and control measures were undertaken by the Italian health authorities including enhanced of surveillance and awareness of HAV, the collection of additional epidemiological information on associated risk factors, and the characterisation of the virus RNA by genotyping and sequencing of the VP1-2a region from all new cases [13,14], since normally in Italy genotyping and sequencing are not performed on a routine basis.

Moreover, the voluntary recall of the confirmed mixed frozen berries batches was performed and advice to the population regarding the use of frozen mixed berries was given (i.e. advice in supermarket and shops to cook frozen berries for two minutes). In addition risk communication concerning the consumption of frozen berries (i.e. cook frozen berries for two minutes) was provided to the general public through Ministry of Health (MoH) and Istituto Superiore di Sanità (ISS) websites.

Through a Ministerial Directive on 23 May 2013, the Italian MoH designated a task force of experts from different professions to participate in the management of the HAV outbreak in Italy in 2013. In this framework, an analytic epidemiological study was planned in some of the Italian regions (Apulia, AP of Bolzano, Emilia-Romagna, Friuli–Venezia-Giulia and AP of Trento) that experienced the highest increase of HAV cases.

Methods

Study design and objectives

A retrospective matched case–control study was performed to identify risk factors for HAV infection acquired among the population, from 1 January to 31 May 2013, in some of the regions where the largest increase in the number of cases was observed (Apulia, AP of Bolzano, Emilia-Romagna, Friuli–Venezia-Giulia and AP of Trento). The potential risk factors explored were the consumption of berries, other food items described as potential sources of HAV infection [2,5] and history of travel [15,16].

Case definition and control selection

The study population consisted of all residents in the four selected regions of northern Italy (AP of Bolzano, Emilia-Romagna, Friuli–Venezia-Giulia and AP of Trento) and from one of the southern regions of Italy (Apulia Region).

A case was defined as a symptomatic person, positive for HAV IgM with onset of symptoms (or date of testing if onset date not available) between 1 January 2013 and 31 May 2013. For Apulia, where HAV is endemic and where molecular typing of the viruses isolated from cases is a standard procedure [17], only cases presenting a sequence identical to the outbreak strain (GenBank number: KF182323) and of sub-genotype 1A were included in the study.

Potential controls, that had not presented with hepatitis A symptoms during the period from 1 January to 31 May 2013, were selected from the general population residing in the five Italian regions and matched with each case by age (±3 years) and place of residence (individual matching). The exclusion criteria for controls were: had been diagnosed with hepatitis A in the past, had previously presented symptoms consistent with a diagnosis of hepatitis A (i.e. jaundice + dark urine) or had been vaccinated against hepatitis A.

Three analyses were conducted. The first analysis included all cases and controls as defined above, while a second analysis was restricted to a group of cases, for which the virus had been sequenced and shown to harbour the 'outbreak' sequence. As in the first

analysis, the respective controls for the second analysis were individually matched by age (± 3 years) and place of residence. Because all cases from the Apulia region reported having consumed raw seafood, a third analysis based on cases with the outbreak sequence but excluding the Apulia cases was undertaken. For this, controls were also individually matched by age and place of residence.

Sample size

We selected up to four matched controls for each case (assuming 5% exposure among controls, 80% power to detect a minimum odds ratio (OR) of 3, alpha error of 5%). The minimum sample size necessary was estimated to be of 595 people (476 controls and 119 cases) using STATA 13.

Data collection

The data were collected via telephone interviews using an ad hoc questionnaire. Cases and controls were contacted by health professionals and epidemiologists specifically instructed and involved in the study, and gave verbal informed consent before enrolment. The data were anonymous and used only by the investigation team for the purpose of the study.

Data included socio-demographic features, contact with a household member or sex partner who has HA, food history and travel history that occurred in the 15 to 50 days before symptom onset.

Laboratory investigations

Regions were requested to provide leftover sera and/or faecal samples from IgM-positive cases included in the case–control study. Sera and/or faecal samples were analysed by the Institute Experimental Zooprophylactic of Lombardy and Emilia Romagna (IZSLER) and the national reference laboratory at ISS for further characterisation by genotyping and sequencing. The VP1/2a region of the HAV genome was amplified [18].

Moreover, retail frozen berry samples were analysed directly from food business operators. A reverse transcription-polymerase chain reaction (RT-PCR) was used to detect HAV. In order to further characterise the genotype of detected HAV strains, a nested PCR was performed with degenerate primers targeting the VP1-2a genomic region. Following purification, the VP1-2A region amplicons were subjected to double strand sequencing and the sequence was checked by basic local alignment search tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) in Genbank.

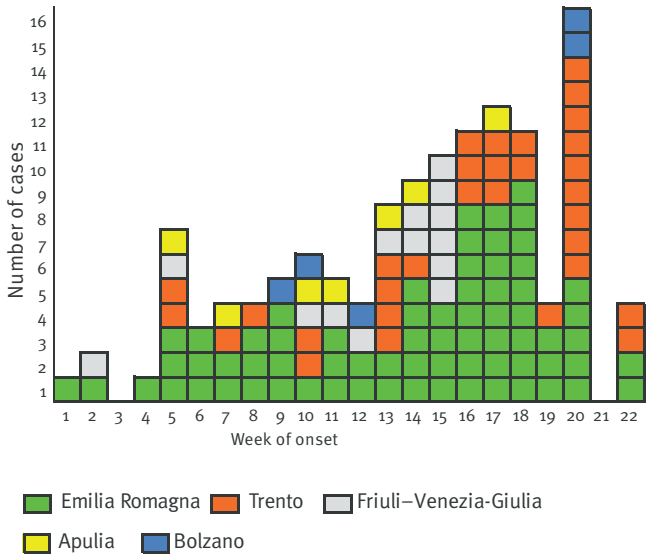
Statistical analysis

A descriptive analysis was made by time, place and person. A univariate analysis was conducted to estimate the association between various potential risk factors and HAV infection, crude ORs were calculated with 95% confidence intervals (CI) and p-values using conditional logistic regression. A multivariate analysis was done by using a conditional logistic regression model to test for possible confounding factors and interaction. All factors identified as disease-associated with p-value ‹0.15 and with OR›1 in the univariate analysis were included in the model. In order to quantify the contribution of risk factors to the disease, the population attributable fraction (PAF) was calculated for those factors with the biggest OR. P-values‹0.05 were considered as statistically significant. The same statistical analysis was conducted on the restricted samples containing only the cases with an outbreak sequence (second and third analyses described earlier). Moreover, the PAF was estimated in order to define the contribution of the risk factors to HA.

EpiInfo version 5.3.4 was used to enter questionnaire responses and to perform the whole statistical analysis.

Figure 1

Number of cases of hepatitis A by week of symptom onset in five regions, Italy, 1 January–31 May 2013 (n=127)



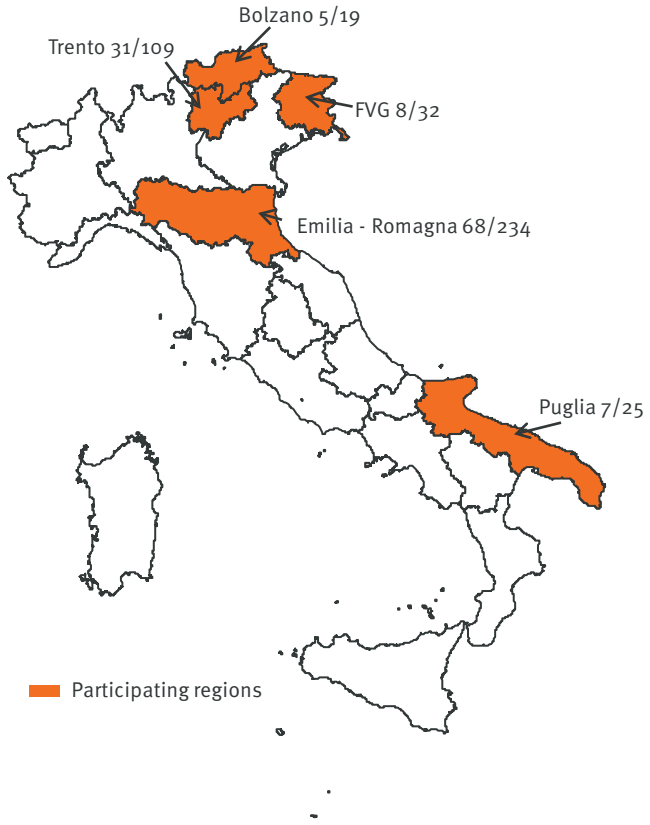
Cases are presented in different colours according to the region where they were notified.

Results

The matched case–control study included 119 sets of cases and controls. A total of 588 subjects were interviewed (127 cases and 461 controls). In Figure 1 the epidemic curve is reported. The number of cases increased progressively, peaking at week 20. As of June 2013 the outbreak was still ongoing. Two cases were excluded due to lack of appropriately matched controls within the specified time frame; another six cases that were part of a family cluster due to a common source of infection were also excluded. An additional, 42 controls were excluded due to at least one of the exclusion criteria being present.

Figure 2

Regions selected for the case–control study in an outbreak of hepatitis A, Italy, 1 January–31 May 2013 (n=5 regions)



The name of each participating region is indicated, followed by the numbers of respective cases and controls selected.

Thus, the study included a total of 538 subjects, 119 cases (22%) and 419 controls (78%). The median age was 37.0 years (range: 3–70) for HAV cases and 38.0 years (range: 1–72) for controls (p=0.6384). The majority of study participants in both groups were male. No significant difference in the sex of cases and controls was observed. As a result a median of 3.5 controls for each case were included in the study.

Most of the HAV cases involved in the study, 68/119 (57%), were from the region of Emilia Romagna (Figure 2).

Univariate and multivariate analysis

In the univariate analysis, compared with the 419 controls, cases were more likely to have eaten berries (OR: 4.42; 95% CI: 2.70–7.27), raw seafood (OR: 4.65; 95% CI: 2.70–8.00), or travelled (OR: 2.34; 95% CI: 1.45–3.77) (Table 1). In the multivariate analysis, the highest association with illness was for people who had consumed berries (adjusted odds ratio (ORadj): 4.2; 95% CI: 2.54–7.02).

Among those who reported berry consumption, the majority had eaten berries at home (70%, 169/240) or at a restaurant (15%, 36/240). The food types consumed that included berries were: yogurt (36%, 86/240), cakes (28%, 67/240), ice cream (21%, 51/240), panna cotta (8%, 19/240), cheesecake (5%, 13/240), cornflakes (5%, 11/240) and fruit juices (1%, 2/240). Other subjects (5%, 12/240) consumed only berries. The types of berries consumed were: blueberries (7%, 16/240), strawberries (3%, 7/240), raspberries (3%, 6/240), blackberries (3%, 6/240), red currants (2%, 4/240) and mixed berries (82%, 197/240).

Raw seafood was found to have the second highest significant association with HAV (ORadj: 3.83; 95% CI: 2.54–7.02) with the third highest factor being history of travel (ORadj: 1.98; 95% CI: 1.15–3.41). The majority of the cases (60%) referring to having travelled during the incubation period indicated Italy as the destination (distributed as 61% in the north of Italy, 21% in the centre of Italy and 17% in the south). The 30% of the subjects who travelled abroad indicated a country outside of Europe. The remaining 10% indicated a travel history in European countries other than Italy.

Table 1

Factors positively associated with hepatitis A in univariate and multivariate analysis, Italy, 1 January–31 May 2013

Factor	Univariate analysis	Multivariate analysis	P-value
	Crude OR (95%CI)	Adjusted OR (95%CI)	
Fennel	1.03 (0.66–1.62)	NA[a]	–
Fresh salad	1.02 (0.57–1.73)	NA[a]	–
Bag salad	0.93 (0.59–1.45)	NA[a]	–
Radishes	0.77 (0.44–1.34)	NA[a]	–
Carrots	0.70 (0.43–1.12)	NA[a]	–
Celery	1.29 (0.80–2.07)	NA[a]	–
Raw seafood	4.65 (2.70–8.00)	3.83 (2.16–6.79)	<0.001
Milk products (non-packaged)	0.62 (0.37–1.02)	NA[a]	–
Untreated water	0.77 (0.39–1.50)	NA[a]	–
Berries	4.42 (2.70–7.27)	4.22 (2.54–7.02)	<0.001
Travel	2.34 (1.45–3.77)	1.98 (1.15–3.40)	0.014
Age	1.02 (0.93–1.13)	NA[a]	–
Sex	0.83 (0.55–1.26)	NA[a]	–

CI: confidence interval; NA: not applicable; OR: odds ratio.

[a] All factors identified as disease-associated with p-value <0.15 and with OR>1 in the univariate analysis were included in the multivariate analysis.

PAF was the same (26%) for berries and raw seafood, meaning that the impact of both risk factors was similar, while PAF for travel was lower (16%).

Of the 119 cases enrolled in the case–control study, 24 had respective viral sequence information within the time of the study. The sequenced viruses were identical or highly similar (nucleotide identity between 99.8% and 100%) to the outbreak strain (HAV genotype 1A), in an RNA sequence of 440 nucleotides overlapping the VP1/P2a region. The outbreak sequence had already been submitted to GenBank prior to this study [8].

A restricted statistical analysis was conducted on the 24 cases and respective 82 controls in order to confirm the above described results. Twelve of the 24 cases were from the AP of Trento, seven from Apulia, four from Emilia Romagna and one from the AP of Bolzano. Seventeen of the 24 cases (71%) ate berries and 10 (42%) ate raw seafood. All the 24 cases and 82 controls were included in the restricted analysis with no significant difference in the sex of cases and controls observed.

The statistical analysis (Table 2) identified berries as the highest associated risk factor for developing the disease (ORadj: 4.99; 95% CI: 1.32–18.92) followed by raw seafood (ORadj: 4.46; 95% CI: 1.10–18.04).

As all cases from the Apulia region reported to have eaten raw seafood, an analysis excluding cases from this region was undertaken resulting in berries being the unique risk factor for the disease with an OR of 7.29 (95% CI: 1.56–34.02).

Table 2

Factors positively associated with hepatitis A illness in univariate and multivariate analysis, for 24 cases having sequence information confirming infection with the outbreak strain, Italy, 1 January–31 May 2013

Factor	Univariate analysis	Multivariate analysis	P-value
	Crude OR (95%CI)	Adjusted OR (95%CI)	
Fennel	0.95 (0.32–2.89)	NA[a]	–
Fresh salad	0.31 (0.0–1.34)	NA[a]	–
Bag salad	1.78 (0.58–5.83)	NA[a]	–
Radishes	0.71 (0.17–2.92)	NA[a]	–
Carrots	0.41 (0.14–1.21)	NA[a]	–
Celery	0.58 (0.16–2.06)	NA[a]	–
Raw seafood	5.05 (1.38–18.49)	4.46 (1.10–18.04)	0.027
Milk products (non-packaged)	0.51 (0.10–2.69)	NA[a]	–
Untreated water	4.74 (1.49–15.15)	NA[a]	–
Berries	3.03 (1.00–9.17)	4.99 (1.32–18.92)	0.018
Travel	3.03 (1.00–10.81)	NA[a]	–
Age	–	–	–
Sex	0.61 (0.21–1.81)	NA[a]	–

CI: confidence interval; NA: not applicable; OR: odds ratio.

[a] All factors identified as disease-associated with p-value ‹0.15 and with OR›1 in the univariate analysis were included in the multivariate analysis.

Discussion

The analytical investigation conducted in Italy indicated that the consumption raw seafood or berries constituted risk factors for HA. The highest significant association with illness was however found for berries, which were the most likely source of the outbreak. Berries, as the most implicated risk factor in a HAV foodborne outbreak, is a new finding in Italy.

During the outbreak we were also able to demonstrate that the sequences derived from cases were 100% similar to the sequences isolated in frozen berry food samples [8]. The same sequence has also been isolated 2008 and 2009 in the Czech Republic and Slovakia as well as in Ireland among men who have sex with men (MSM) [19,20]. Sequence analysis of HAV RNA-positive samples, of some of the enrolled cases in the study, showed that all the cases tested had an identical IA genotype, supporting the hypothesis of a widespread, common source of infection.

Since HAV genotyping and sequencing is not performed on a routine basis in Italy we cannot compare the current outbreak genotype with previously identified Italian genotypes. The exception is for the Campania and Apulia regions, where HAV is endemic. The phylogenetic analysis conducted in Apulia between 2008 and 2009 revealed the co-circulation of subtypes IA (74%) and IB (26%), clustering with strains from Germany and France, and with those previously circulating in the region [17]. The sequence from the outbreak reported here was different from sequences of genotype IA found in Apulia in 2008 and 2009. In Campania, laboratory investigations conducted during an outbreak in 2004 showed the 1B HAV genotype as the most common circulating strain (90% of sera) [10].

Consumption of fresh or frozen produce is known to be associated with outbreaks of foodborne enteric viruses, particularly norovirus and HAV [21]. Produce (fruits and vegetables) can become contaminated during cultivation prior to harvest due to contact with inadequately treated sewage or sewage polluted water, or fomites. Contamination may also occur by infected food handlers, during harvesting, processing, storage, distribution or final preparation, with the virus likely to be found on the surface of the food. Fruits and vegetable are more prone to being the vehicle of

foodborne infections as they are more likely to be left uncooked before consumption [22]. Berries as a vehicle for transmission of HAV, have been described in several outbreaks with most of the infections connected with consumption of minimally processed frozen berries [22].

Furthermore, it has been shown that among berries (frozen raspberries in particular) and vegetables (i.e. parsley) those with uneven shapes are more likely to retain viruses on their surface. Raspberries, for example, have crevices and hair-like projections which may prevent the virus from being removed by rinsing [21].

Recently outbreaks connected to berry consumption have been reported in some Nordic European countries (Denmark, Finland, Norway and Sweden) [23] and in the United States [24]. The outbreak in Nordic countries was associated to frozen strawberries [25,26] while the outbreak in the United States was associated to Townsend Farms Organic Antioxidant Blend, a frozen blend containing a pomegranate seed mix [24]. However, the virus genotype 1A isolated to the Italian outbreak is different from the virus genotypes implicated in the above mentioned recent outbreaks (genotype 1B), which also have frozen strawberries and berries as the suspected vehicles [25,26].

The main limitation of this study is the recall bias: as the incubation period of hepatitis A occurs over a long period of time (range 15–50 days), the food consumption history in the weeks before symptom onset may be vague and better remembered by cases than by controls. Another possible limitation is due to some controls being not susceptible to HAV (having forgotten if they had been vaccinated or had asymptomatic infection); this underestimated the association between outcome and exposure of interest. Another limitation was that no other known hepatitis A exposures (e.g. persons who inject drugs, male same-sex sexual contact) were taken into consideration.

An additional limitation is that we were unable to compare cases and controls by exposure to different types of berries consumed, due to both cases and controls inability to discriminate between fresh or frozen berries and because most of them reported consuming a mix of berries.

Compared with international findings on viral foodborne outbreaks, what is new in this Italian outbreak associated to berries is that the epidemic involved a large number of people over a wide area. Because the batches of frozen berries consumed by some of the cases were composed of a mixture of berries respectively originating from different lots, it has been difficult to trace-back the origins of each type of berry.

Rapid and effective monitoring is critical for detecting outbreaks and new risk factors for infectious diseases. For this reason, as soon as the outbreak was confirmed [8], the MoH enhanced the national surveillance system and undertook appropriate control measures that are still in place: voluntary recall of the confirmed frozen mixed berries batches and advice to the population regarding the use of the frozen mixed berries already purchased or still available in retail (i.e. advice in supermarket and shops to cook frozen berries for 2 minutes). Moreover, risk communication to the general population through MoH and Istituto Superiore di Sanità (ISS) websites concerning the consumption of frozen berries (i.e. cook frozen berries for 2 minutes) was done. Frozen berries were also included as another potential risk factor in the epidemiological investigation and sequencing of the virus affecting HA cases was requested.

The Italian national reference laboratories (for human and food investigation) and the public health authorities are conducting epidemiological and laboratory investigations as well as trace-back and trace-forward investigations in parallel. The collaboration between the surveillance system for foodborne diseases and the molecular epidemiology has been essential to understand the risk factors associated with this outbreak.

In the future, attention should be paid to the prevention of contamination before or during food processing by implementation of good agricultural, hygienic and manufacturing practices, as well as hazard analysis critical control points (HACCP) systems. Continuous health education would also be helpful in the effective control of hepatitis A.

Central Task Force on Hepatitis A

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Conflict of interest

None declared.

Author contributions

CR contributed to the design the study, coordinated the activities, done the interviews, introduced and analysed the data and drafted the manuscript as the lead writer. LR and VA contributed to the design the study, done the interviews, introduced and analysed the data and drafted the manuscript. AB

contributed to the data analysis and interpreted the results. MET contributed with data on Italian cases. AC, RB, ST, ME and MNL contributed to the laboratory sequencing.VC and SF contributed with data on Trento province cases. BN, MA and FA contributed with data on Bolzano province cases.CG, ACF, EM and BMB contributed with data on Emilia-Romagna region cases. TL contributed with data on Friuli–Venezia-Giulia region cases. VC, MC and RP contributed with data on Puglia region cases. CR contributed as supervisor of the study and the manuscript.

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Letter to the editor: Preventing nosocomial MRSA infections by screening

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To the editor:

The systematic literature analysis and review by R Köck et al. [1] on targeted preventive measures to limit infections by meticillin-resistant *Staphylococcus aureus* (MRSA) is well done but surprisingly did not include the single most extensive intervention published to date, i.e. the Veterans Affairs (VA) initiative to combat MRSA in their acute care hospitals in the United States [2]. The introduction in the summer of 2007 of active screening, followed by contact isolation of positive patients, led to an impressive 62% decrease in MRSA infection in their intensive care units (ICUs) and a 45% reduction in non-ICU wards by the end of 2010. These reductions in MRSA infection rates were sustained and even reached 72% for ICU's and 65% for non-ICU's by June 2012 [3]. Nation-wide, virtually all (150/153) VA hospitals participated in this effort and millions of patients were screened, far surpassing the combined experiences of all studies that were included in the Köck review. Of note, such rapid positive effects of active MRSA surveillance and contact isolation are concordant with the predictions previously made by Bootsma et al. from their complex modelling of the MRSA epidemiology in acute care hospitals [4]. Interestingly, the same intervention was applied to all the 133 VA long term care facilities in 2009 and this led to a 36% reduction in MRSA infections in these facilities by the end of 2012 [5]. In that latter intervention, 12.9 million resident days were monitored.

The first evaluation of the VA initiative was mentioned by Köck et al., but only briefly in the discussion section of their review (reference 99 in their paper), but it remains to be explained why the VA initiative's experience was not included in the body of the review and presented in the Tables. The fact that the VA initiative was not primarily designed as a study but as a real-life intervention does not make it irrelevant for this review. The intervention was well executed (with very high compliance rates), closely monitored and did have pre- and post-intervention phases. As stated by Köck et al., very few randomised controlled trials have been done on MRSA control, and most data included in their Tables were derived from similar quasi-experimental observational studies [1]. As the Review is now, the readership may fail to learn about the highly instructive VA MRSA prevention initiative.

Conflict of interest

None declared.

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Authors' reply: Systematic literature analysis and review of targeted preventive measures to limit healthcare-associated infections by meticillin-resistant *Staphylococcus aureus*

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To the editor:

We thank Prof. Verbrugh for the letter in regard to our article *Systematic literature analysis and review of targeted preventive measures to limit healthcare-associated infections by meticillin-resistant Staphylococcus aureus (MRSA)* [1]. As mentioned by Prof. Verbrugh, the article describing the United States Veteran Affairs (VA) initiative to combat MRSA [2] was discussed in our review, but not included in the body of the text and not reviewed in detail. It should clearly be stated that we highly acknowledge the work of the VA initiative. This is why the results of this 'bundle approach' for MRSA prevention were highlighted in the discussion. However, in the literature review, we focused on assessing specifically the impact of three defined single measures (i.e. screening, isolation in single rooms and decolonisation) on the occurrence of MRSA. The question of interest with respect to MRSA screening was: '*Does screening of patients before or on admission reduce the incidence of MRSA infection or transmission? How do PCR-based rapid tests for the direct detection of MRSA from screening specimens influence the incidence of MRSA colonisation or infection compared with culture-based methods?*'

In the VA initiative, screening followed by contact precautions was among the measures implemented which, indeed, made the study relevant for consideration in the review. However, the article was excluded from formal review. The main reason was that different microbiological techniques (culture-based and PCR rapid test) were used for MRSA screening in the participating 153 hospitals and results were not stratified between these different methods. This prevented basic categorisation of the article according to the question of interest mentioned above (PCR vs culture). We agree that this formal aspect could have been better explained in the section defining exclusion criteria.

However, we also agree that both the fact that the VA initiative was performed as a real-life intervention (rather than a (randomised) controlled study) and that screening was implemented in a bundle together with 'institutional culture change' and hand hygiene programmes, did not make the initiative irrelevant for learning about MRSA prevention. On the contrary, as we were aware that most evidence evaluating the effects of screening is from observational studies, we explicitly did not restrict data analysis to randomised controlled trials. In addition, we think that the evaluation of care bundles is an important (and feasible) method to assess preventive effects in defined healthcare systems or clinical settings.

Members of the group

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Conflict of interest

None declared.

Authors' contributions

RK, AWF, KB, BC, JEvGP, SH, JK, MM, GP, RLS, MJS, ET and WW replied to the letter.

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Letter to the editor: Early transmission dynamics of Ebola virus disease (EVD), West Africa, March to August 2014 – Eurosurveillance 17 September 2014

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To the editor:

In their article *Early transmission dynamics of Ebola virus disease (EVD), West Africa, March to August 2014*, published on 11 September, Nishiura and Chowell estimated the effective reproductive number Rt for the mainly affected countries, Guinea, Liberia and Sierra Leone, to be consistently above 1 since June 2014, indicating that the outbreak is not yet under control [1]. Such studies are welcome and useful to understand and quantify the ongoing epidemic and to plan the response activities.

However, we would like to add a cautionary note to the interpretation of the surveillance data. Important detailed information may be missed by such general modelling approach. The study of the epidemic curves based on data retrieved by week and district of reporting, from the situational reports of the Ministry of Health of Liberia [2] shows very different patterns contributing to the overall observed dynamic at national level. Figure 1 shows the number of suspected, probable and confirmed cases reported by week in Liberia, from calendar week 21 (starting on 19 May) to week 37 (starting on 8 September) 2014. Following the steep increase in the number of cases up to week 34, which was described by Nishiura and Chowell, a levelling off in the number of newly reported cases occurred between weeks 34 and 36 resulting in the flattening of the curve, followed by a new increase in week 37.

Figure 1

Distribution of suspected, probable and confirmed cases of Ebola virus disease by week of reporting, Liberia, week 21 (starting on 19 May) to 37 (starting on 8 September) 2014 (n=2,663)

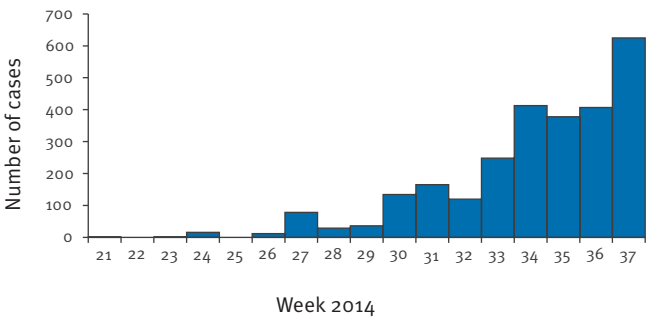


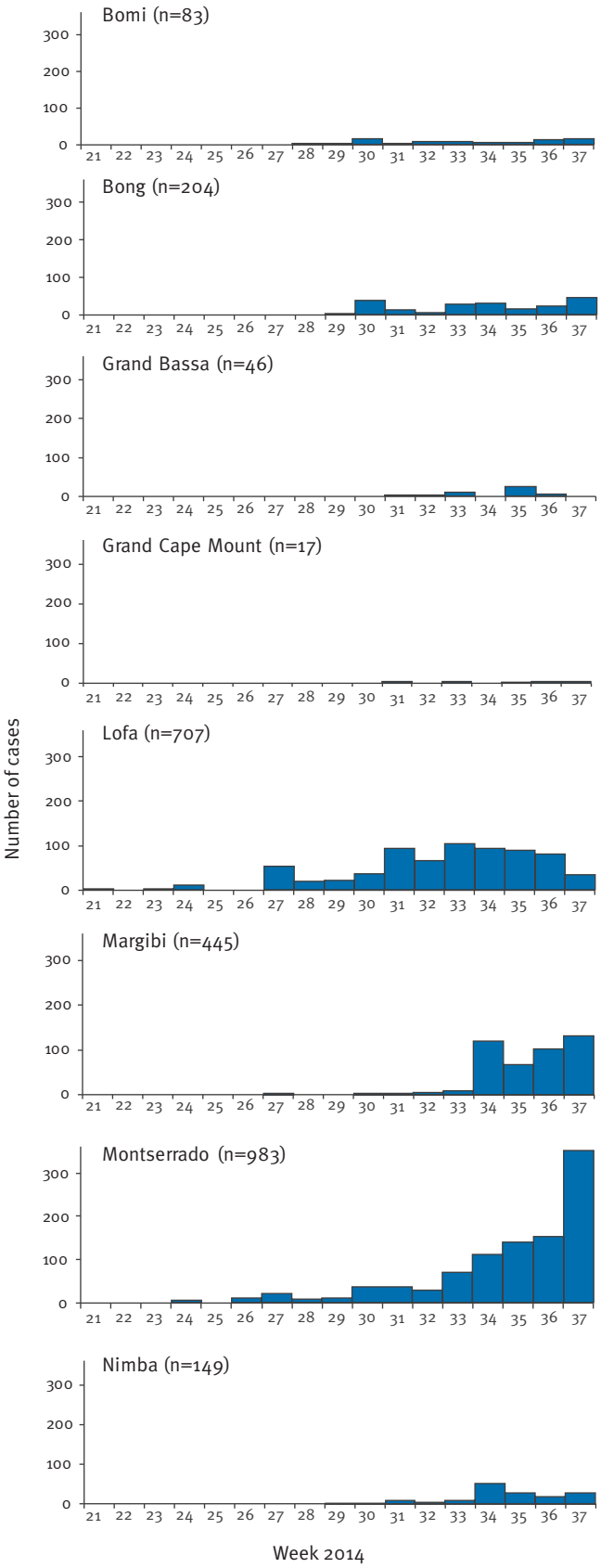
Figure 2 shows the distribution of newly reported cases by week for selected districts of Liberia. Only districts that reported more than five cases since the start of the epidemic are shown. The epidemic curves show markedly different patterns. The districts of Bomi, Bong, Grand Cape Mount, Margibi and Nimba experienced a relatively stable number of weekly cases, while the districts of Lofa and Grand Bassa reported an increase in the number of cases up to weeks 33 and 35, respectively, followed by a decrease in subsequent weeks. The district of Montserrado shows a continuous increasing pattern from week 29 up to week 37.

The presentation of aggregated data for Liberia at national level which shows a transient overall stabilising and even slightly decreasing trend in the number of newly reported cases between weeks 34 and 36 can therefore be misleading. The alarming trend in the district of Montserrado is compensated by a decreasing trend observed in the recent weeks in districts reporting fewer cases.

Furthermore, the observed dynamic based on available surveillance data can only be interpreted in the light of the performance of the surveillance system having generated them. There are reports from areas in the affected countries where hospitals have closed, health centres are overwhelmed, patients are treated at home and contact tracing and monitoring is inadequate. Caution is therefore necessary when interpreting the data, as a decrease in the number of newly reported cases could signify either a positive effect of the interventions to control the epidemic or a decrease in the

Figure 2

Distribution of newly reported cases of Ebola virus disease by week and selected districts, Liberia, week 21 (starting on 19 May) to 37 (starting on 8 September) 2014



performance of the surveillance system. Similarly, an increase in the number of cases could result not only from improved surveillance but also from increased transmission.

The use of surveillance data for setting priority intervention areas, for measuring their effectiveness and for planning resources on the basis of forecasting, needs to consider the performance of the surveillance system through which the data are generated. Simple surveillance quality indicators should be collected along with epidemiological data, such as the number of contacts identified and monitored. Moreover, studies assessing performance are a useful addition to allow better understanding of the limitation of surveillance data, e.g. capture-recapture studies, review of healthcare facilities records or household visits in affected areas. In conclusion, ensuring efficient surveillance is essential for the effective response to this devastating outbreak.

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Authors' reply: Feedback from modelling to surveillance of Ebola virus disease

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To the editor:

We appreciate the comments from Plachouras et al. on our article published in *Eurosurveillance* a week ago [1,2]. Overall we fully agree with them on both points, i.e., (i) in that there is a need to account for the geographic heterogeneity of the ongoing Ebola epidemic to better understand the transmission dynamics and guide intervention strategies and (ii) in that caution must be exercised to interpret time-dependent changes in the reported coverage of cases captured by the surveillance systems. Here we further highlight these issues by providing feedback from a mathematical modelling point of view.

First, the most recent data points comprising the last three weeks of reported case counts (weeks 35-37) presented by Plachouras et al. were not incorporated in our analysis as these data were not available at the time of preparing our study. Indeed, these additional data points might have changed our interpretation of the most recent trends of the effective reproduction number. Second, our analysis was based on an approximate strategy in line with the available aggregated data. Consequently, we were not able to consider heterogeneous patterns of transmission within each country. With detailed spatial data, we could have detected an apparent slowdown in the incidence influenced by actual decline in incidence at several regions along with a steady increase in Montserrado. With such analysis of spatial data, we would have interpreted the most recent estimate of R_t for Liberia as the result of spatial dilution of differential growth rates by different regions, possible reflection of large local clusters of cases, or the presence of significant reporting delays in the most recent data. Real-time analysis of the ongoing public health crisis in West Africa deserves the consideration of the most detailed, accessible and accurate epidemiological data in order to capture the above-mentioned aspects and explicitly identify regional variations in transmission, which could be key to guide intervention efforts.

We take this opportunity to address two critically important issues in conducting modelling studies using surveillance data subject to limited reporting coverage. First, as discussed in light of our original findings [2], the reported case data are always accompanied by reporting delays. Suppose that the unbiased number of cases and the actual reported number of cases at calendar time t are given by c_t and r_t, respectively. Then we have the relationship,

$$c_t = r_t \frac{1}{H_{T-t}}$$

where H_{T-t} is the cumulative distribution function of the reporting delay (of length $T-t$) and T represents the most recent time of observation. This indicates that most recent incidence data might be underestimated (and should be adjusted by H_{T-t}). Nevertheless, this might not be a significant issue as long as H_{T-t} is independent of calendar time.

There is a second (and perhaps more serious) issue to consider, i.e., the potential for time-dependent changes in the reporting rate. This is highly relevant to the ongoing Ebola virus disease (EVD) epidemic as the number of new cases has been exponentially growing, which generates pressure on healthcare facilities to assist an extraordinary large number of cases beyond their expected capacity. Let the reporting fraction be s_t at calendar time t which could be estimated by carefully looking into the time-dependent change in the proportion of severe (or fatal) cases among all reported cases [3]. For instance, if the fraction of critically ill cases among total cases increases at a rate b per day, reflecting a decreasing ascertainment rate, we have

$$s_t = \frac{1}{b} s_{t-1}$$

and the unbiased number of cases at t, c_t, is calculated by dividing the reported number of cases n_t by s_t, i.e., $c_t = n_t/s_t$. For instance, a modelling study made a similar

adjustment to analyse data of the influenza A(H1N1) pdm2009 pandemic. In this study, the proportion of hospitalised cases among total reported cases was used as the input data to calculate s_t [3].

It is worth noting that several efforts have already been made to estimate the reproduction number of the ongoing EVD epidemic [2,4,5,6] based on the same publicly available country-wide data of reported cases as in our study.

Potential feedback from modelling studies to surveillance can be summarised as follows: (i) The geographic differences in the evolution of the Ebola epidemic highlighted by Plachouras et al. underscore the need to access high-resolution spatiotemporal data to detect heterogeneous levels in the spatiotemporal dynamics of the epidemic. At the same time, it is critical to exercise caution in the analysis of aggregated time-series data in the presence of significant levels of spatiotemporal heterogeneity. (ii) As a possible indicator of variations in the reporting fraction, monitoring well-defined severe cases would be useful, e.g., hospitalised cases, cases in the state of disseminated intravascular coagulopathy or shock, and deceased cases in order to calculate time-dependent changes in the fraction of the severe cases among the total number of reported cases. It might be also feasible to further account for the time delay from symptoms onset to developing severe manifestations in order to adjust the reporting delay. Surveillance and mathematical modelling are two complementary instruments in the toolbox of epidemiologists. Combining their strengths would be highly beneficial to understand epidemic dynamics and take public health actions. We are keen to contribute further by analysing more detailed epidemiological data of the Ebola epidemic.

Conflict of interest

None declared.

Authors' contributions

HN and GC drafted and revised the manuscript.

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