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Hepatitis A outbreak among men who have sex with men (MSM) predominantly linked with the EuroPride, the Netherlands, July 2016 to February 2017

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Between July 2016 and February 2017, 48 male cases of hepatitis A were notified in the Netherlands. Of these, 17 identified as men who have sex with men (MSM). Ten of the 13 cases for whom sequencing information was available, were infected with a strain linked with the EuroPride that took place in Amsterdam in 2016. This strain is identical to a strain that has been causing a large outbreak among MSM in Taiwan.

In December 2016, the European Centre for Disease Prevention and Control (ECDC) issued a Rapid Risk Assessment reporting of two distinct hepatitis A virus (HAV) genotype IA strains circulating among men who have sex with men (MSM) in the United Kingdom (UK) and the Netherlands in 2016. Germany, Italy and Spain had also reported a recent increase in male HAV cases [1].

The outbreak is ongoing with 37 cases reported in the UK between July 2016 and January 2017 [2] and 30 cases in Berlin between mid-November 2016 and end of January 2017 [3]. Here we describe the current situation in the Netherlands including potential links to this international hepatitis A outbreak.

Case definition

A case was defined as a person who (i) met the surveillance definition of a case of hepatitis A, (ii) self-identified as MSM or had MSM contact i.e. sexual contact with another man two months before the onset of symptoms, (iii) developed symptoms after mid-2016 (3 July 2016) and (iv) was a resident in the Netherlands.

The surveillance case definition comprises (i) non-specific symptoms (e.g. fatigue, abdominal pain, loss of appetite, intermittent nausea and vomiting), (ii) fever or jaundice and (iii) laboratory confirmation or an epidemiological link with a laboratory-confirmed case i.e. either hepatitis A-specific IgM antibodies in serum or detection of HAV in serum or stool by means of PCR [4].

Surveillance of hepatitis A in the Netherlands

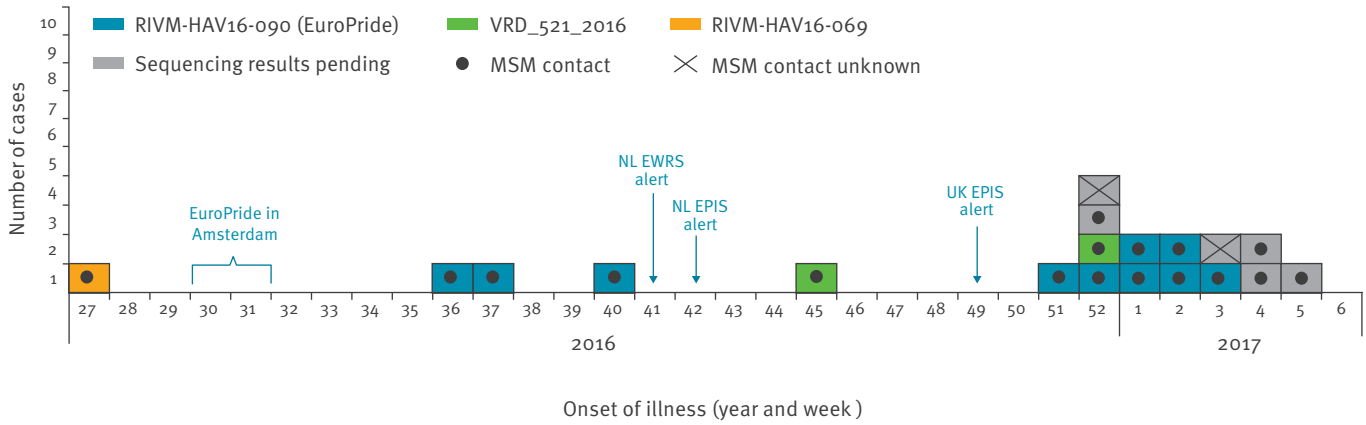
In the Netherlands, hepatitis A is a notifiable disease. Laboratories and physicians report HAV infections within one working day to the regional Public Health Services (PHS). The PHS then collect epidemiological information on demographics, occupation, symptoms, suspected source / country of infection, MSM contact (for males only) and consumption of specific food items. The PHS reports all information in the national surveillance system for notifiable diseases. In addition, serum and / or stool samples of HAV cases are routinely sent to the National Institute of Public Health and the Environment (RIVM) for molecular analysis. In case men did not explicitly report having had MSM contact two months before disease onset, MSM status was assessed by asking whether they identified themselves as MSM.

Molecular analyses

HAV IgM-positive serum samples were analysed by sequence analysis of a 460 nt PCR fragment in the VP1/P2A region according to a shared protocol available through Hepatitis A Lab-Network HAVNET [5].

Figure 1

Epidemic curve of hepatitis A cases by MSM status and week of onset of illness, July 2016–February 2017, the Netherlands (n = 19)



EPIS: Epidemic Intelligence Information System; EWRS: Early Warning and Response System; MSM: men who have sex with men; NL: the Netherlands; UK: United Kingdom.

Two cases for whom MSM status was unknown at the time of the investigation, are also included.

Outbreak description

In the first half of 2016 (including week 26), 22 sporadic hepatitis A cases were notified through the Dutch national surveillance system. Half of these were men and none reported MSM contact.

On 19 September 2016 (week 38), the outbreak investigation was triggered by the notification of two male cases of hepatitis A, in their 30s and 40s, who fell ill in mid-September. Both cases reported having had MSM contact during the EuroPride. The EuroPride, which took place in Amsterdam between 29 July and 6 August, is an international event to celebrate equality rights of the lesbian, gay, bisexual and transgender community. In 2016, this event attracted over half a million visitors [6]. Sequencing showed that strains from both cases were identical (RIVM-HAV16–090). Given the international character of the EuroPride, alerts were placed on the Early Warning and Response System (EWRS) and on ECDC's Epidemic Intelligence Information System for Food- and Waterborne diseases (EPIS-FWD) to inform other European countries.

From mid-2016 (week 27) to 7 February 2017, 48 male cases of hepatitis A were reported nationally. Of these, 17 identified as MSM. Two cases did not (yet) meet the case definition, as MSM status was unknown at the time of the investigation. For comparison, in 2013, 2014 and 2015, 56, 58 and 45 male cases of hepatitis A were reported each year, respectively. Among these, none were identified as MSM.

The Table shows the characteristics of the cases recorded in the current outbreak. The onset of illness ranged from week 27, 2016 to week 5, 2017 (Figure 1).

Of the 17 cases, 11 were born outside the Netherlands (Argentina, Brazil, Canada, France, Italy, Lebanon, Peru, Spain (n = 3), Surinam). The median age of the 17 cases was 33 years (range: 26–52). None of the cases were vaccinated and about a third was hospitalised (Table). Sequence information was available for 13 of the 17 cases, which showed co-circulation of three different hepatitis A strains (Table, Figure 2).

Ten of the 13 cases with available typing information were infected with the EuroPride strain. The majority of cases (n = 11), irrespective of sequence type, clustered in the Public Health Service region of Amsterdam, whereas other Public Health Service regions only reported incidental cases (Table, Figure 3).

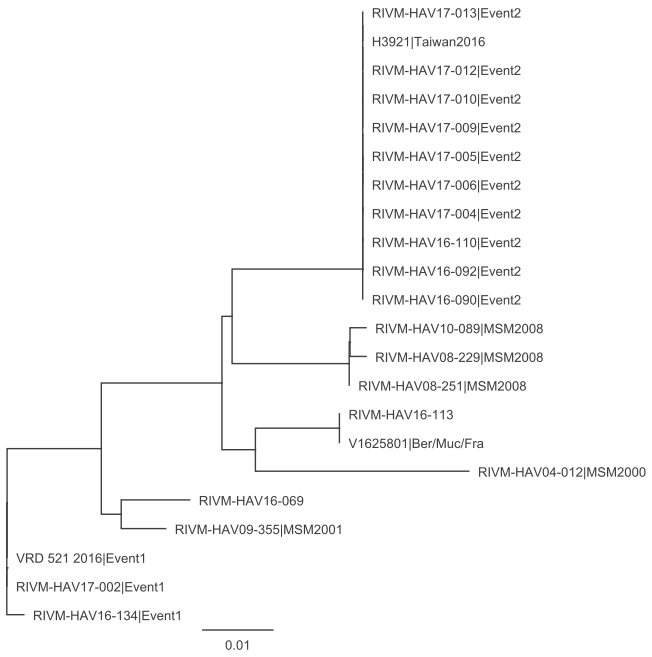
In comparison, among the 29 male cases who became ill after mid-2016 and were not MSM (median age: 20.5 years, range: 0–82) we found strains that were unrelated to the current outbreak. We detected genotype IA and IB strains from Morocco, IB strains from Egypt, Turkey, West Africa and East Africa, a IIA strain from Cameroon, a IIIA strain from Romania or no hepatitis A virus, respectively. As none of these cases was infected with a strain involved in the current outbreak, we are confident that these cases reported their MSM status truthfully.

EuroPride strain RIVM-HAV16–090

When comparing sequence information of the EuroPride strain with available sequences in the databases HAVNET [5] and GenBank, we found that the EuroPride strain was 99.57% identical to a sequence submitted by Japan (accession number: AB020565, release date: 14 August 2001). In addition, in response to a post on ProMED-mail from May 2016 that reported

Figure 2

Phylogenetic analysis of virus strains from hepatitis A cases who self-identified as men who have sex with men, the Netherlands, 2000–2017

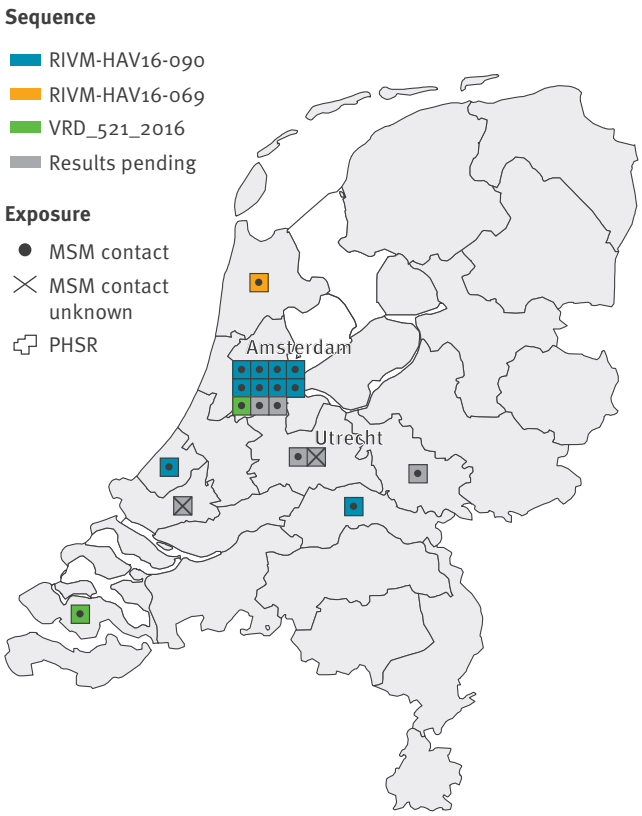


Neighbour-joining tree of sequences of 445 nt of the VP1/P2A region of hepatitis A virus strains.

The tree was constructed in PHYLIP (DNADIST) (Joe Felsenstein, Department of Genome Sciences, University of Washington, Seattle, USA). Strains are identified by sample number and cluster. Event 1 and Event 2 refer to terminology used in the Rapid Risk Assessment published by the European Centre for Disease Prevention and Control (ECDC) [1]. Event 1 refers to strains detected in the United Kingdom and Spain [2]. Event 2 refers to the EuroPride strain detected in the Netherlands and the strain circulating in Taiwan in 2016, respectively. One asymptomatic case is identical to a sequence that caused a cluster in Germany (Ber/Muc/Fra) [3]. For comparison, we also included older hepatitis A virus strains detected in men who have sex with men (MSM) in 2000, 2001 and 2008, respectively.

Figure 3

Geographic distribution of hepatitis A cases who self-identified as men who have sex with men, by available sequence information, the Netherlands, July 2016–February 2017 (n = 19)



MSM: men who have sex with men; PHSR: Public Health Service region.

Two cases for whom MSM status was unknown at the time of the investigation, are also shown. Notified cases are centred in the respective Public Health Service region. The majority of cases (n = 11) occurred in the PHSR Amsterdam.

on a hepatitis A outbreak among MSM in Taiwan with 275 notified cases [7], we investigated whether the EuroPride strain might be related to the Taiwanese outbreak strain. Direct comparison and phylogenetic analyses showed that the Taiwanese outbreak strain was identical to the EuroPride strain (Figure 2). Eight of the ten cases reported to have likely been infected in the Netherlands, and a further two cases were likely infected in Barcelona, Spain (n = 2; onset of illness for both cases: week 2, Figure 1).

Strains VRD_521_2016 and RIVM-HAV16–069

Two cases were infected with strain VRD_521_2016, first reported by the UK in December 2016 and likely imported from Spain several times [1,2]. One of the Dutch cases reported having travelled to Spain (onset of illness in week 45), whereas the other case stated to have likely been infected in the Netherlands (onset of illness week 52, Figure 1).

One case infected with strain RIVM-HAV16–069 reported having travelled to Argentina and became ill shortly before the EuroPride (week 27). The UK also reported one MSM case with the same sequence [2].

Discussion

Here we report on an ongoing hepatitis A outbreak among MSM in the Netherlands that started in 2016. Hepatitis A is an acute, self-limiting liver disease which is transmitted via the faecal-oral route. Infection occurs via contaminated food or water, or through person-to-person contact, including sexual contact. The average incubation period is 28 days (range: 15–50 days) [8]. In western Europe hepatitis A endemicity is low [9] and is primarily associated with travelling to endemic countries [10] or consumption of contaminated, imported food [11]. Outbreaks among MSM have also been described [12]. In Europe, the last outbreak of hepatitis A among MSM occurred between 2008 and 2011 [13].

Table

Characteristics of hepatitis A cases by MSM status and strain, the Netherlands, July 2016–February 2017 (n = 19)

	Men who have sex with men (MSM)					Sequence information and MSM status unknown / pending
Hepatitis A strain	RIVMHAV16090 (EuroPride)	VRD_521_2016 (UK / Spain)	RIVM-HAV16–069	Sequence information pending	Total	
Number of cases	10	2	1	4	17	2
Number per 10-year age group	20–29 (n = 3) 30–39 (n = 4) 40–49 (n = 3)	20–29 (n = 1) 40–49 (n = 1)	20–29 (n = 1)	20–29 (n = 2) 30–39 (n = 1) 50–59 (n = 1)	33 (26–52) Median (min–max)	20–29 (n = 1) 40–49 (n = 1)
Number of cases hospitalised	3	1	1	0	5	0
Number of cases vaccinated against hepatitis A	0	0	0	0	0	0
Suspected place of infection	The Netherlands (n = 8) Spain, Barcelona (n = 2)	The Netherlands (n = 1) Spain (n = 1)	Argentina (n = 1)	The Netherlands (n = 3)[a] Germany, Berlin (n = 1)	4	The Netherlands (n = 1)[b]

MSM: men who have sex with men; UK: United Kingdom.

[a] One case reported Portugal as second suspected country of infection.

[b] For one case information on suspected place of infection was not available.

Ages were estimated because only year of birth was known.

Characteristics of two male cases of hepatitis A for whom MSM status was unknown at the time of the investigation, are shown separately.

Between 2012 and mid-2016, hepatitis A infection in MSM was only notified twice in the Netherlands.

In the currently ongoing outbreak in the Netherlands, the majority of cases for whom sequence information was available, were infected with strain RIVM-HAV16–090. This strain had only been detected once before in 2010 and was absent in the Netherlands until it was detected in two MSM cases who attended the EuroPride in 2016. The strain is identical with a strain causing an ongoing outbreak among MSM in Taiwan. As at 29 September 2016, Taiwan reported 845 hepatitis A cases among MSM, of which 56% were HIV-positive or had another sexually transmitted diseases [14].

In the Netherlands, information on HIV status is not routinely collected for hepatitis A surveillance purposes. In the course of this outbreak investigation, in week 43, we detected one HAV infection in a HIV-positive MSM who was asymptomatic and therefore did not meet the case definition. Sequencing showed infection with a strain identical to the Berlin/Munich/Frankfurt HAV cluster in Germany (V16–25801) [3]. Asymptomatic individuals, even if they do not fulfil the case definition, can still be epidemiologically relevant and should therefore be included in epidemiological analyses.

In the Netherlands, besides risk groups, i.e. persons with chronic liver disease or occupational exposure to HAV, hepatitis A vaccination is recommended to individuals who travel to HAV endemic countries. Hepatitis A vaccine uptake is unknown. Because of several outbreaks among European and Dutch MSM [15,16], hepatitis A vaccination is also recommended to MSM in the Netherlands. For MSM, vaccination against HAV is available at reduced costs and is administered in combination with hepatitis B vaccine that is free of charge for this risk group. The uptake of hepatitis B vaccination among MSM in Amsterdam is high and hepatitis B incidence has dropped markedly since 2005 [17]. In contrast, financial aspects might hamper wide uptake of hepatitis A vaccination. Vaccination coverage among MSM is unknown.

In the Netherlands, hepatitis A control is based on vaccination of household- and other close contacts [4]. Tracing and vaccination of sexual contacts of MSM can be challenging due to anonymous sexual contacts. To better understand transmission chains and the epidemiology of this outbreak, we recently introduced an additional, more detailed questionnaire for hepatitis A-positive MSM to complement routinely collected epidemiological data. Given the high outbreak potential of hepatitis A in the MSM community and the high interconnectedness through global travel of this risk group [18], increasing awareness of hepatitis A among MSM as well as health professionals at sexually transmitted disease clinics and public health services should be emphasised. To increase hepatitis A vaccination uptake, the Regional Public Health Services and 'STI AIDS the Netherlands' (centre of expertise for HIV and

other sexually transmitted infections) have been engaging in activities to remind professionals and the Dutch MSM community of the availability of hepatitis A vaccination within the hepatitis B vaccination programme.

Acknowledgements

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No external funding was required to carry out this work.

Conflict of interest

None declared.

Authors' contributions

GSF analysed epidemiological data and wrote the manuscript. LPMJB, FvS, ECS and GJBS interviewed cases and coordinated outbreak investigation within the Public Health Service region GGD Amsterdam. IHMF, WLMR, GGCvR were involved in the outbreak management and coordination of the outbreak response. J-YY provided sequence information from Taiwan. HV conducted molecular analyses. All authors commented on the manuscript.

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Emergence of a novel subclade of influenza A(H3N2) virus in London, December 2016 to January 2017

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We report the molecular investigations of a large influenza A(H3N2) outbreak, in a season characterised by sharp increase in influenza admissions since December 2016. Analysis of haemagglutinin (HA) sequences demonstrated co-circulation of multiple clades (3C.3a, 3C.2a and 3C.2a1). Most variants fell into a novel subclade (proposed as 3C.2a2); they possessed four unique amino acid substitutions in the HA protein and loss of a potential glycosylation site. These changes potentially modify the H3N2 strain antigenicity.

The ongoing influenza season started early in eleven European Union countries, including England, on week 46 of 2016 [1]. The majority of reported infections have been caused by clade 3C.2a or 3C.2a1 influenza A(H3N2) viruses. The clade 3C.2a contains the current vaccine strain A/Hong Kong/4801/2014, and the first few viruses within the more recently emerged subclade 3C.2a1 were earlier shown to be antigenically matched with the vaccine component [2]. However, evidence for suboptimal vaccine effectiveness (VE) against laboratory-confirmed influenza A infection in people over 65 years-old was obtained in the first studies from Finland [3] and Sweden [4].

An outbreak of influenza A(H3N2) was first notified in our London centre on 30 December 2016. The outbreak coincided with unusually high ongoing circulation of respiratory syncytial virus (RSV) (Figure 1), and affected both patients and staff in the acute medical unit (AMU).

While infection control precautions were intensified, it resulted in multiple bay closures. We suspected that the sharp increase in the number of influenza A(H3N2) infections may have been caused by the emergence of a new genetic variant of H3N2, a hypothesis investigated through next generation sequencing (NGS) of influenza A(H3N2) strains.

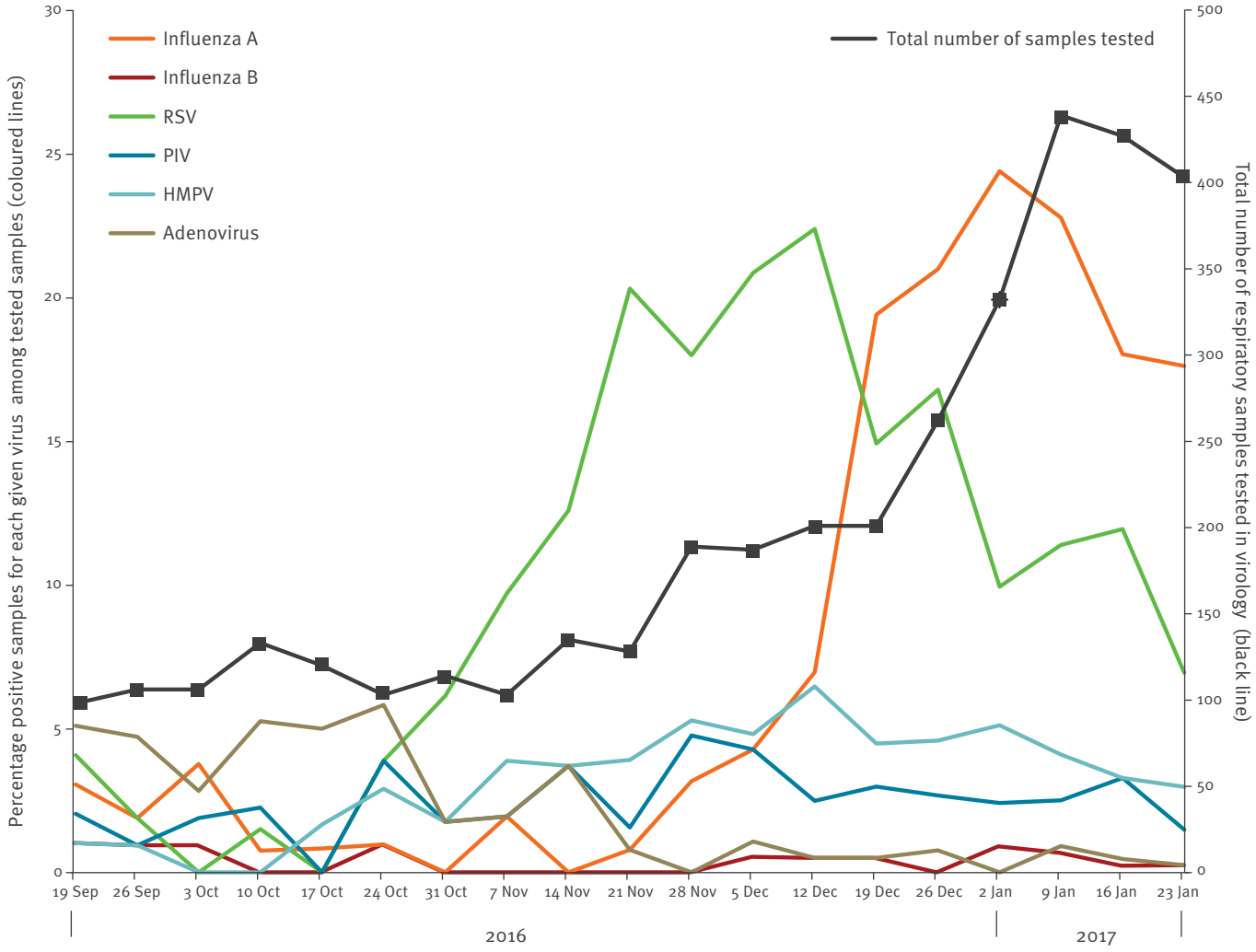
Collection and analysis of respiratory samples

The main study was based on respiratory samples (n = 1,690) analysed at the Department of Virology, University College of London Hospital (UCLH), United Kingdom between 21 December 2016 and 24 January 2017. Most samples were collected as part of routine diagnostics from inpatients and patients seen at the Accident and Emergency department, and to a lesser extent from outpatients. The basic epidemiological data including patients' age, admission and sampling dates as well as data on intensive care unit (ICU) admissions and deaths were collected. For comparison, results from influenza A and other virus testing from UCLH since 19 September 2016 were also analysed. The study was approved by the NRES Committee London, Surrey Borders HRA, (REC reference: 13/LO/1303).

All samples were initially screened for influenza A virus by reverse transcription-PCR targeting the matrix gene. A total of 67 influenza A(H3N2) virus-positive samples obtained between 8 December 2016 and 3 January 2017 were sequenced. RNA was amplified using a modified eight-segment method [5]. Library preparations were generated as previously described [5,6]. A neighbour joining phylogenetic tree was constructed using Molecular Evolutionary Genetics Analysis (MEGA) 6 software [7]. Some sequences in the phylogenetic analysis were from the Global Initiative on Sharing All Influenza Data (GISAID); the authors gratefully acknowledge the 36 originating and submitting laboratories

Figure 1

Percentage of positive respiratory samples for given viruses, and total number of respiratory samples tested per week, at the Department of Clinical Virology, University College of London Hospital, 19 September 2016–30 January 2017 (n=1,690 samples)



Adeno: adenovirus; HMPV: human metapneumovirus; PIV: parainfluenza virus; RSV: respiratory syncytial virus.

who contributed sequences to GISAID (www.gisaid.org).

Characteristics of the influenza A(H3N2) outbreak

Of the 1,690 respiratory samples obtained between 21 December 2016 and 24 January 2017, 352 samples were positive for influenza A(H3N2) virus (21%; Figure 1). Of those, 294 influenza A(H3N2)-positive samples had been obtained from 253 UCLH patients. Of patients with influenza A(H3N2) infection, over 50% (128/253) required hospital admission. An average of three inpatients (either existing inpatients or new admissions) were identified as influenza A(H3N2)-positive each day, and the highest number of hospital admissions was recorded on 10 January (n=11; Figure 2a). Over the outbreak period, six patients required ICU admission and five died. Over a third of influenza A(H3N2) infections were seen in adults over 65 years-old (99/253; 39%), most of them admitted to hospital (72/99; 73%, Figure 2b).

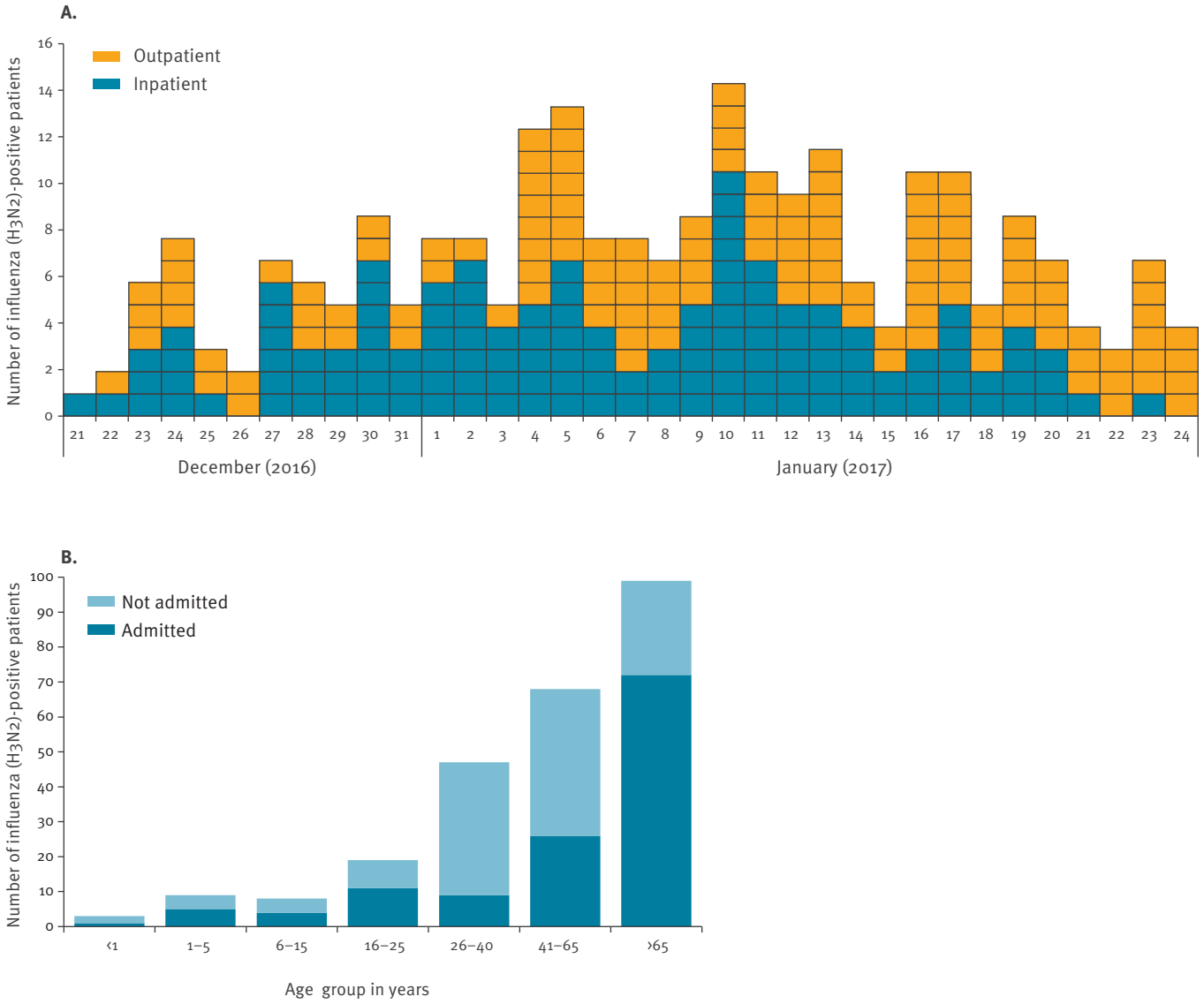
Description of influenza A(H3N2) viruses circulating in London

Phylogenetic analyses of haemagglutinin (HA) sequences indicated co-circulation of variants from subclades of 3C.3a (n=2), 3C.2a1 (n=31) and 3C.2a (n=34) (Figure 3).

Interestingly, our 3C.2a virus strains differed from the previously characterised subclade 3C.2a strains as well as from subclade 3C.2a1, and hence we have proposed them as a new subclade 3C.2a2. This subclade in turn split into two well defined but internally homogenous sub-clusters (cluster I and II; Figure 3), and also included all suspected outbreak cases admitted to AMU between 27 December 2016 and 3 January 2017 (n=15).

Figure 2

Number (A) and age distribution (B) of influenza A(H3N2)-positive patients diagnosed at the University College of London Hospitals, 16 December 2016–24 January 2017 (n=253 patients)



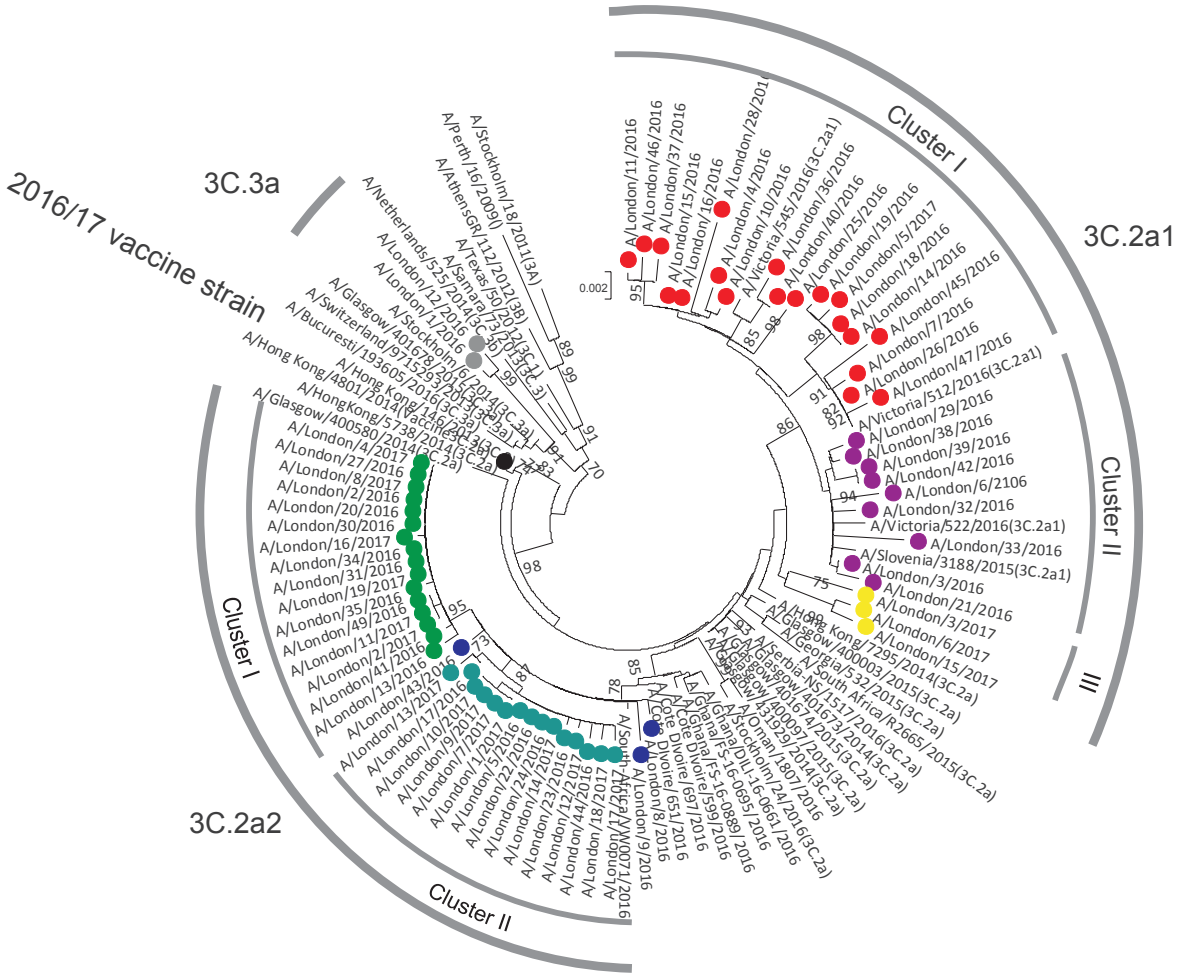
Individual clades of influenza A are typically defined by amino acid substitutions that occur as they diversify from parental strains. Such substitutions are potentially functionally relevant as they may influence the antigenicity and susceptibility to neutralising antibody induced by infection with other lineages of H3N2. Thus, investigated variants within the subclades 3C.2a and 3C.2a1 (n=65) inherited amino acid substitutions known to define their parental clades (Figure 4). All variants within the proposed subclade 3C.2a2 shared two substitutions N121K and S144K, whereby S144K is an antigenic site flanking the receptor binding site (RBS). A further two additional substitutions were observed in each 3C.2a2 cluster (I58V and S219Y in cluster I and N122D and S262N in cluster II), all in the HA1 region and based on HA1 numbering. Cluster II viruses lost the potential N-linked glycosylation site (N122D).

Discussion

In our centre in London, the early start and higher intensity of the 2016/17 influenza A(H3N2) virus epidemic mirrored that of the season 2014/15 where the subtype H3N2 also predominated. During the 2014/15 season, most influenza A(H3N2) infections in Europe were shown to be caused by antigenically drifted virus variants within the new genetic subgroup 3C.2a [8]. Our genetic analysis of London A(H3N2) viruses demonstrates ongoing co-circulation of drifted variants from multiple subclades (3C.3a, 3C.2a1 and proposed 3C.2a2). Four or more substitutions in two or more antibody binding sites are predicted to give an antigenically different virus [9] as in our case. Although we did not observe mutations in the seven positions suggested as being responsible for major transition clusters [10], position 144 is at the flank of the RBS, and additionally recognised as antigenic [11].

Figure 3

Phylogenetic tree of the haemagglutinin gene sequences of virus strains recovered in this study using reference viruses for the different phylogenetic influenza A(H3N2)clades (n = 103 sequences)



Bootstrap values obtained with 100 replicates are shown on some branches of the neighbour joining tree. The scale represents the percentage of nucleotide substitutions. Sequences are annotated in the following way: influenza type/geographical area of sample collection/sample number/year. For sequences previously characterised as belonging to the subclade 3C.2a, this is indicated after the year in parentheses. London sequences retrieved in this study are indicated with coloured circles (n = 67), and the vaccine strain with a black circle.

The authors gratefully acknowledge the 36 originating and submitting laboratories who contributed sequences used in the phylogenetic analysis to the Global Initiative on Sharing All Influenza Data (GISAID; www.gisaid.org).

Although not necessarily determining major antigenic drift, the alterations of N-linked glycosylation sites are likely to contribute to more complex conformational changes in the HA due to gain or loss of glycosylation and can thus facilitate immune escape [12]. Furthermore, any amino acid changes in the 140–146 region of HA have been shown to be characteristic for antigenically distinct viruses of epidemic significance [9,13,14]. The amino acid substitution S144K in the emerging subclade 3C.2a2 viruses together with the loss of an N-linked glycosylation site (N122D) shows potential for antigenic drift that warrants further monitoring during this ongoing season. A limitation of our study was the lack of detailed vaccination data.

Our findings in London of the rapid emergence of genetically drifted influenza A(H3N2) viruses underscore the potential for such strains to spread rapidly in hospital environments among patients and staff. Characterising emerging strains of influenza by next generation sequencing adds to the local and national monitoring of influenza trends. Further studies are needed to investigate the antigenic effects of substitutions occurring within the newly described subclade.

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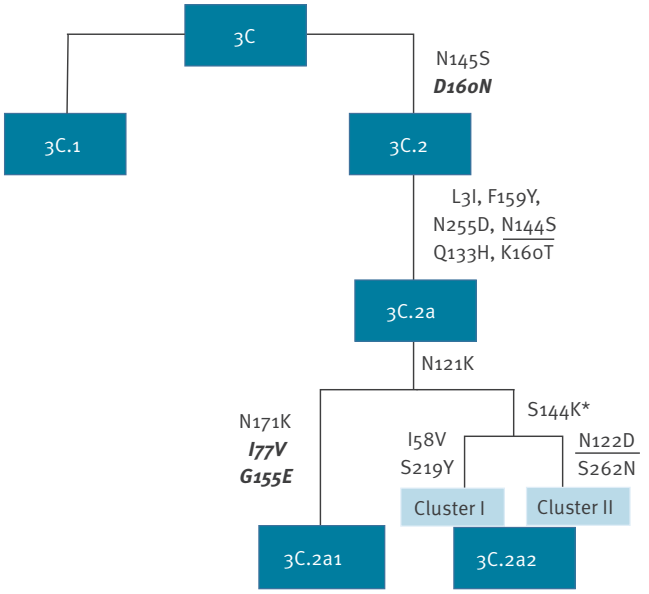
Figure 4

Schematic diagram demonstrating the shared haemagglutinin (HA) amino acid changes between clades 3c, 3C.2, 3C.2a, 3C.2a1 and 3C.2a2 based on HA1 and HA2 numbering



Changes in HA1 are indicated in normal font while changes in HA2 are in bold and italic. Changes in antigenic sites are shown with an asterisk (*) while changes resulting in the loss of a potential N-linked glycosylation site are underlined.

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Conflict of interest

None declared.

Authors' contributions

Dr Heli Harvala, wrote the manuscript and contributed to the analysis of the sequencing data. Dr Dan Frampton and Dr Paul Grant did the bioinformatics analysis. Jade Raffle and Dr Ruth Bridget Ferns performed the next generation sequencing experiments in the laboratory. Dr Zisis Kozlakidis coordinates the whole genome sequencing pipeline for ICONIC. Professor Paul Kellam, Professor Deenan Pillay and Professor Andrew Hayward analysed data and contributed to writing. Dr Eleni Nastouli designed the study, analysed data and co-wrote the manuscript. All ICONIC Consortium co-authors contributed constructively in the writing of the manuscript and offered valuable advice for the discussion part of the manuscript.

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Increase in outbreaks of gastroenteritis linked to bathing water in Finland in summer 2014

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An increased number of suspected outbreaks of gastroenteritis linked to bathing water were reported to the Finnish food- and waterborne outbreak (FWO) registry in July and August 2014. The investigation reports were assessed by a national outbreak investigation panel. Eight confirmed outbreaks were identified among the 15 suspected outbreaks linked to bathing water that had been reported to the FWO registry. According to the outbreak investigation reports, 1,453 persons fell ill during these outbreaks. Epidemiological and microbiological data revealed noroviruses as the main causative agents. During the outbreaks, exceptionally warm weather had boosted the use of beaches. Six of eight outbreaks occurred at small lakes; for those, the investigation strongly suggested that the beach users were the source of contamination. In one of those eight outbreaks, an external source of contamination was identified and elevated levels of faecal indicator bacteria (FIB) were noted in water. In the remaining outbreaks, FIB analyses were insufficient to describe the hygienic quality of the water. Restrictions against bathing proved effective in controlling the outbreaks. In spring 2015, the National Institute for Health and Welfare (THL) and the National Supervisory Authority for Welfare and Health (Valvira) published guidelines for outbreak control to prevent bathing water outbreaks.

Introduction

Since 1997, municipal authorities in Finland have been submitting notifications on suspected outbreaks of illness caused by drinking water to a national food- and waterborne outbreak (FWO) registry online, developed and maintained by the National Institute for Health and Welfare (THL) and the Finnish Food Safety Authority (Evira) [1]. Notification is mandatory and involves outbreaks with more than five cases who are not family members, have similar symptoms and have been exposed to the same water in a particular time period. The purpose of the FWO registry is to promote surveillance and outbreak investigations and to facilitate reporting at national and international level. During the period from 2009 to 2013, between one and eight waterborne outbreak notifications were sent to the FWO registry each year. Since 2012, the FWO registry has also been receiving notifications on suspected outbreaks caused by bathing water [2].

In Finland, the general provisions governing water quality at public beaches are included in the Health Protection Act (763/1994). More specific provisions concerning the monitoring and management of water quality at large and small public beaches (≥ 100 and < 100 bathers in a day, respectively) are included in the Decrees of the Ministry of the Social Affairs and Health (177/2008 and 354/2008) [3,4]. Legislation for large public beaches is based on the European Union's Bathing Water Directive (BWD) 2006/7/EC and includes a requirement for the classification of bathing waters based on frequent monitoring during the last four bathing seasons [5]. The BWD also requires beach owners to inform the public about bathing water quality and beach management, through signs at the beaches and via the Internet. Furthermore, bathing water profiles are used for public information. These profiles contain, for instance, information on the sources of pollution that may affect the quality of the bathing water and are a risk to bathers' health.

The quality of bathing waters is assessed according to the monitoring results of two faecal indicator bacteria (FIB), *Escherichia coli* and intestinal enterococci. In

Table 1

Classification criteria used for evaluating the strength of association for waterborne outbreaks, Finland, 2014

A: Same pathogen identified in patients and in the environment	B: Water quality failure or other deviation in the quality of environment
C: Association between illness and environment shown in analytical epidemiological investigation	D: Descriptive epidemiological investigation suggests that the outbreak is related to the environment and excludes other obvious exposures

Strong association: A+C or A+D or B+C. Probable association: B+D or C or A. Possible association: B or D.
Criteria modified from Tillett et al. [12].

the Finnish legislation, the microbiological values for management actions have been set separately for *E. coli* and intestinal enterococci [3,4]. For inland bathing waters, these values are 1,000 and 400 colony-forming units (CFU) or most probable number (MPN) per 100 mL, and for coastal bathing waters, 500 and 200 CFU or MPN per 100 mL for *E. coli* and intestinal enterococci, respectively. If these values are exceeded, the municipal health protection authority has to assess the impact of the deteriorated water quality on bathers' health. The authority may give instructions or impose restrictions such as advice against bathing or a temporary bathing prohibition so as to prevent health hazards. Bathing water on large public beaches is classified as excellent, good, sufficient or poor [5]. The higher the concentration of indicator bacteria and their standard deviation in bathing water, the lower is the status of the bathing water. In excellent bathing waters, the concentrations of *E. coli* and intestinal enterococci are very low, indicating no source of faecal pollution.

It has been estimated that globally ca 120 million cases of gastrointestinal disease and ca 50 million cases of respiratory diseases are caused by swimming in wastewater-polluted waters each year [6]. Thus, the health risks related to bathing water have been commonly recognised and several pathogenic microbes are known to spread via water. Viruses have caused an increasing number waterborne outbreaks associated with recreational activities [7-10] and according to a survey of 55 viral outbreaks, noroviruses were with 45% the most prevalent causative agent [11].

In July 2014, THL received primary information on several suspected outbreaks linked to bathing water via the media, while no notifications were reported to the FWO registry. This resulted in direct contacts with the health authorities, and a reminder about notifying outbreaks related to bathing water was posted in a THL Infectious Disease Bulletin sent to the municipal health authorities. The message was also distributed to municipal environmental authorities by the National Supervisory Authority for Welfare and Health (Valvira). Following these reminders, several notifications were reported to the FWO registry. We identified outbreaks caused by bathing water from the FWO registry for 2014 and reviewed the epidemiological and microbiological data in order to assess and compile guidelines for outbreak control to prevent similar outbreaks in the future.

Methods

Epidemiological investigation

We reviewed outbreak notifications and investigation reports from the FWO registry for 2014. Outbreaks with a suspected link to bathing water were included in this study. We evaluated the strength of association for waterborne outbreaks based on classification criteria (Table 1) modified from those presented by Tillett et al. [12] and on information collected from local investigation reports (i.e. time and place of swimming, number of ill persons, clinical and microbiological findings).

Microbiological investigation

Description of the laboratories and their roles

Analyses of enteric virus were carried out in four laboratories. Clinical samples were analysed at the Helsinki University Hospital (HUSLAB) and/or at the Viral Infection Unit of the National Institute for Health and Welfare (THL). Water samples were analysed either at the Water and Health Unit of the National Institute for Health and Welfare (THL) or at the Department of Food Hygiene and Environmental Health, University of Helsinki (UH). Surface samples were analysed at the UH. Pathogenic bacteria, faecal indicator bacteria (FIB) and water temperature analyses were conducted in local clinical and/or environmental laboratories.

Clinical samples

Viruses were analysed in patients' stools for seven outbreaks. At the HUSLAB laboratory, noroviruses were analysed according to Kanerva et al. [13]. For astroviruses, viral RNA was extracted from a 10% suspension of the stool using MagNa Pure LC (Roche, Germany). After RT-PCR, the amplified DNA was detected by liquid hybridisation using an astrovirus-specific probe [14]. At the THL laboratory, norovirus RNAs were extracted using the RNeasy Mini Kit (Qiagen, Germany) and the polymerase/capsid gene junction was amplified as previously described [14]. Genotyping analysis was done for several norovirus isolates at the THL laboratory. Viral RNA was amplified in polymerase region A using a one-step RT-PCR kit (Qiagen) according to Vinjé et al. [15]. Sequences were analysed using Geneious software. NoroNet online software was used for genotyping. For three outbreaks, stool specimens were tested for pathogenic bacteria (*Campylobacter, Salmonella*, *Shigella* and *Yersinia*) by routine methods [16].

Water samples

At the THL laboratory, noroviruses and adenoviruses were concentrated from 0.5–2 L water samples as

Table 2

Description of beaches with outbreaks linked to recreational water, Finland, summer 2014 (n = 13)

Outbreak	Type	Size (ha)	Category	Estimated number of bathers/day	EU BWD classification (2014)[a]	Estimated outbreak start time	Restriction against bathing
I	Lake	2,420	Small	‹100	NA	26 July	1–6 August
II	Lake	2.9	Large	150–500	Excellent	25 July	29 July–21 August
III[b]	Lake	5.5–141	2/6 small 4/6 large	‹ 100 ›100	NA Excellent	24–27 July	28 July–12 August
IV	Lake	16.6	Large	100–2,000	Excellent	24 July	31 July–31 August (until the end of the bathing season)
V	Lake	9.7	Small	‹100	NA	3 August	15–22 August
VI	Lake	71.1	Large	≤ 150	Excellent	5 August	11–21 August
VII	Sea	393,00,000	Small	‹100	NA	NK	13–15 August and 19 August–9 September
VIII	Lake/pond	0.8	Large	1,000	NA[c]	27 July	6–21 August

EU BWD: European Union's Bathing Water Directive [5]; NA: not available; NK: not known.

[a] Based on frequent monitoring during the last four bathing seasons [5].

[b] Combined results from six beaches.

[c] New beach, no classification.

previously described [17] and using glass fibre prefilters (Millipore). Viral nucleic acids were extracted and detected using RT-qPCR and qPCR methods, as previously described [18,19], with the exception of using Taqman Environmental Master Mix 2.0 (Life Technologies) in the adenovirus qPCR.

At the UH laboratory, noroviruses and adenoviruses were concentrated by using membrane disks HA and Nanoceram to filter a total volume of 4.5 L of water. When necessary, a prefilter (Waterra) was used, otherwise the protocol was as described in Maunula et al. [14]. As a modification, Taqman primer–probe sets were applied as published in ISO/TS 15216–2 [20] for norovirus GI and GII. Mengovirus was added as a process control.

MPN of *E. coli* and CFU of intestinal enterococci were analysed according to standards ISO 9308–2 and ISO 7899–2, respectively [21,22].

Surface samples

In outbreak IV, 10 environmental swabs were taken from the toilet facilities (toilets for females, toilets for males and two latrines). Swabs taken from taps, door handles and toilet seats were analysed for noroviruses according to Rönnqvist et al. using nucleic acid detection by RT-qPCR [23]. For adenovirus investigation, a primer–probe set from Jothikumar et al. was included [24].

Statistical analyses

The statistical analyses were conducted using SPSS 22 software for Windows. The related samples Wilcoxon signed-rank test was used to test the significance of temperature and FIB analyses, while comparing the outbreak samples with frequent-monitoring samples collected during the summer. Differences were considered significant if the p value was ‹ 0.05.

Results

Review of the outbreak notifications and investigation reports

In 2014, 15 outbreaks suspected to be caused by bathing water were reported to the FWO registry. We identified eight outbreaks in which an association between bathing water and the illness could be confirmed based on classification criteria (Table 1). These outbreaks occurred on public beaches in different parts of Finland in July and August, 2014 (Table 2; Table 3).

Six of eight confirmed outbreaks occurred at rather small lakes or ponds (‹ 141 ha) and eight of 13 beaches were categorised as large public beaches with more than 100 bathers per day (Table 2). According to the BWD classification criteria based on the last four bathing seasons, all these large public beaches were classified as excellent, except for one beach that was opened in 2012 and therefore did not have data for classification.

Restrictions against bathing were set for each beach (Table 2). The length of these restrictions varied from 2 days to more than 3 weeks and for one beach, the advice against bathing was set for the rest of the bathing season. Seven of eight outbreaks occurred at inland lakes where no clear source of contamination was identified according to the bathing water profiles and/or outbreak investigation reports, although for five of these outbreaks at inland lakes, non-specific quality deviations were reported (Table 3). In the one

Table 3

Strength of association for waterborne outbreaks, number of patients, virological findings and observed quality deviations, Finland, summer 2014 (n = 1,453 patients)

Outbreak	Strength of association[a]	No. of patients	Viruses found in patients	No. of virus findings per water samples tested	Viruses found in water	Observed quality deviation
I	Possible (D)	40	NA	0/1	ND	Not observed
II	Probable (A+B)	85	Norovirus GI.2	2/4	Adenovirus, norovirus GI	Untidy toilets
III[b]	Strong (B+C)	819[b] 1,093[c]	Norovirus GI.2, GI.4, GII.2	0/3	ND	Untidy toilets, defecation in water
IV	Strong (A+B+D)	185	Norovirus GII	0/1	ND	Untidy toilets
V	Probable (A)	4	Norovirus GI.2 and GII.4	1/2	Norovirus GII	Not observed
VI	Possible (B)	17	Norovirus (not typed)	0/2	ND	Untidy toilets, used nappies in water
VII	Possible (B)	2	Norovirus GI	NA	NA	Wastewater overflow
VIII	Possible (B)	27	Astrovirus	1/3	Adenovirus	Faeces on the dock

NA: not analysed; ND: not detected.

[a] Letters refer to classification criteria detailed in Table 1.

[b] Combined results from six beaches that were investigated in detail.

[c] Total number from all 32 suspected beaches from which the local health authority received notifications of illness.

coastal sea water outbreak, a wastewater overflow was identified as a potential source of contamination.

According to the outbreak investigation reports, 1,453 persons fell ill in these outbreaks (Table 3). The most common symptoms were vomiting, diarrhoea, stomach pain, and fever. Information on the incubation period was available for four outbreaks, the median incubation period ranging from 20 to 62 hours. The duration of illness was reported for five outbreaks, with a median ranging from 19 to 60 hours. None of the patients required hospital care.

Microbiological findings

Patient samples were collected in seven outbreaks and tested for gastrointestinal pathogenic viruses and bacteria. Several types of norovirus were identified, with norovirus GI.2 detected in three outbreaks (Table 3). In addition, norovirus GI.4, GII.2 and GII.4 were detected in patient samples. In one patient, astrovirus was identified. According to outbreak investigation reports, pathogenic bacteria were analysed in three investigations (outbreaks III, IV and VIII). *Campylobacter* was found in one patient (outbreak III). *Salmonella*, *Shigella* or *Yersinia* spp. were not found in any of the specimens tested.

Water samples were collected for noro- and adenovirus analyses in seven outbreaks, and noro- and/or adenoviruses were detected in the samples from three outbreaks (Table 3). In the remaining outbreak, these analyses were not requested by the municipal health protection authority. FIB were analysed from water in all outbreaks. In addition, water quality monitoring was carried out at every beach according to EU BWD and national regulations. Elevated levels of both FIB were found in two of the outbreaks (VII and VIII; Table 4), but only in outbreak VII did the number of *E. coli* exceed the limit for management actions, with maximum concentrations of 1,100 and 190 CFU/100 mL for *E. coli* and enterococci, respectively. Elevated levels of enterococci were also noted in outbreak I. In the remaining outbreaks, the levels of FIB were low. Overall, no statistical difference in the levels of *E. coli* ($p = 0.8$) or enterococci ($p = 0.086$) were noted between the outbreak samples ($n = 14$) and the frequent-monitoring samples ($n = 42$), excluding the samples from outbreak VII, where a clear contamination source was noted.

At one outbreak (IV), 10 surface samples from the toilet area were analysed, and norovirus GII was found on the tap of the women's toilet. Adenoviruses were not detected in the surface samples.

Water temperature

During the outbreak period, exceptionally warm weather raised the temperature of the bathing water by several degrees (Table 4). The average temperature of the bathing water samples collected during the outbreaks was 24.3 ± 1.3 °C ($n = 16$), while the average temperature of other frequent-monitoring samples collected at these beaches in summer 2014 (2 June to 26 August) was 19.4 ± 3.6 °C ($n = 47$; $p = 0.002$).

Table 4

Levels of faecal indicator bacteria and water temperature in outbreak samples (n = 17) and frequent-monitoring samples (n = 47), Finland, summer 2014

Outbreak	No. of analysed water samples	*Escherichia coli* MPN/100 mL	Intestinal enterococci CFU/100 mL	Temperature °C
I				
Outbreak samples	1	6	190	25.7
Monitoring samples	3	8±6	4±2	22.1±3.3
II				
Outbreak samples	2	39±26	9±8	25.0±1.4
Monitoring samples	6	72±72	6±4	20.5±4.4
III[a]				
Outbreak samples	5	14±10	3±3	25.2±0
Monitoring samples	18	19±4	15±22	19.0±3.8
IV				
Outbreak samples	1	9	7	24.0
Monitoring samples	4	3±3	1±2	19.8±4.2
V				
Outbreak samples	1	12	22	24.0
Monitoring samples	2	34±47	6±8	19.3±2.5
VI				
Outbreak samples	2	4±1	3±2	23.0±0
Monitoring samples	4	1±0	1±1	17.5±3.7
VII				
Outbreak samples	3	670±580	110±98	22.3±1[b]
Monitoring samples	5	2±4	4±4	20.2±3.5
VIII				
Outbreak samples	2	130±120	48±46	23.9±1
Monitoring samples	5	17±5	8±7	18.1±2.2

CFU: colony-forming units; MPN: most probable number.

[a] Combined results from the five beaches for which indicator bacteria were analysed.

[b] Average from n = 2 samples.

Discussion

In 2014, an increased number of suspected outbreaks linked to bathing water were reported to the Finnish FWO registry. Reminders about the need to notify outbreaks borne by bathing water were sent to the municipal authorities and probably triggered the following notifications seeing as only one outbreak linked to bathing water had been reported during the period 2012 to 2013. In addition, the publicity around outbreaks in 2014 probably made the beach users' more alert so that they reported their suspicions of bathing water-related sickness to the health authorities. Generally, it could be difficult to attribute individually reported gastroenteritis cases to a particular bathing activity and therefore these outbreaks may remain undocumented.

Nearly 1,500 persons fell ill during the outbreaks linked to bathing water in 2014. Although the exact number of people visiting the beaches was not known, some municipal investigation reports estimated that hundreds to thousands of persons per day had been swimming at each beach during the outbreak period before restrictions against bathing were set. In the summer of 2014, the period of continuous hot weather in Finland, with temperatures of more than 25 °C, was exceptionally long and lasted for 38 days [25]. Because of this heatwave, it is likely that more people than usual were visiting the beaches and spent more time in the water. A previous study noted a positive correlation between the number of days with temperatures over 25 °C and the number of outbreaks per bathing season [26]. Some investigation reports also stated that the toilets at the beaches were untidy, rubbish bins were overloaded, and used nappies were floating in the water, indicating overcrowded conditions. In 2015, no outbreaks linked to bathing water were reported. This was probably due in part to the weather conditions, namely 3 days with temperatures over 25 °C in July 2015, compared with 26 such days in July 2014. In Helsinki, the average temperature and precipitation in July differed considerably between 2015 and 2014 (16.2 °C/76.1 mm vs 20 °C/12.5 mm) [27].

Most of the beaches were small, suggesting that the volume of users exceeded the self-cleaning capacity of the beach. For example, the volume of the smallest lake (outbreak VIII) is 20,800 m3. In theory, if a single infected person excreted large numbers of noroviruses (up to 1011 genomic copies/g) [28], and if these viruses were evenly diluted in the total volume of the lake, 1 g of faeces would result in a virus concentration of nearly 5,000 genomic copies/L. Considering the low infectious dose of norovirus (as few as 18 virus particles) [29] and the average ingestion of water while swimming (37 mL and 16 mL for children and adults, respectively, per

45 min swimming session [30]), it is obvious that the bathing water at this particular beach would have the potential to cause a considerable number of infections.

Norovirus was detected in ill persons in most of the outbreaks. The symptoms reported by municipal authorities fit the clinical picture of a norovirus illness [31]. In three outbreaks, norovirus GI.2 was identified. In addition, also GI.4, GII.2 and GII.4 were detected in patient samples. The prevalence of GI in these outbreaks is consistent with the observation that GI genotypes are more frequently involved in food- or waterborne outbreaks than GII, which could imply that GI is more stable in the environment [32,33]. Genotype GII.4 is the most common genotype causing infections in humans and is more likely to be associated with person-to-person transmission [34].

In two outbreaks, norovirus GI and GII were found in bathing water and in one outbreak, GII was determined in a swab taken from the tap of the toilet, but the number of particles obtained was too small to allow typing of these viruses. Therefore, an exact comparison between patient and water samples could not be carried out. In two outbreaks, adenovirus was found in water. Adenoviruses are commonly found in human wastewater and owing to their high stability in aqueous environments, they are recognised as good viral indicators of human sewage pollution [19,35,36]. Moreover, adenoviruses can spread via contaminated water and they have been linked to waterborne outbreaks [14,37,38]. Since adenoviruses most often result in subclinical disease, and symptomatic infections tend to be mild and self-resolving, most infections remain undocumented [39]. In the outbreaks of this study, no adenoviruses were identified in ill persons.

In Finland, the hygienic quality of the bathing water is evaluated according to BWD and national regulations [3-5]. According to Finnish legislation, the minimum number of bathing water samples to be taken during a bathing season is three for small public beaches and four for large public beaches. The legislation contains rules how to monitor and manage bathing waters, indicates microbiological threshold values, regulates measures to be taken when bathing water fails to meet the quality and requires the dissemination of information about bathing water quality. In Finland, the concentrations of FIB in bathing water are typically very low; 70% of the *E. coli* and 58% of the intestinal enterococci concentrations were < 10 CFU or MPN/100 mL in bathing water samples collected from all large public beaches (n = 302) during the seasons from 2013 to 2015 (data not shown). In this study, the microbiological threshold for management actions was exceeded only in one of eight outbreaks. For this outbreak, a clear external contamination source was identified as 2,000–3,000 m3 of raw wastewater had overflowed near the bathing site. In the other outbreaks, the levels of FIB were low and the bathing water quality was classified as excellent according to the BWD criteria. The sources of contamination in these outbreaks were most probably the bathers and other beach users. This suggestion is supported by the observed pollution of the beach environment.

The poor indicator value of FIB in these outbreaks raises questions about the current practices for assessing bathing water quality. This finding is consistent with a recent study showing high prevalence of adenoviruses (75%) in bathing water samples, which nevertheless complied with the regulations for recreational use [40]. Moreover, Boehm et al. reviewed the lack of correlation between FIB and human pathogen concentrations and between FIB and human health, especially in recreational areas of non-point-source contamination [41]. It is also widely known that pathogenic microbes, especially enteric viruses, survive substantially better than the currently used FIB in water environments. Therefore, new candidates, such as *Clostridium perfringens*, coliphages, *Bacteroides* and human enteric viruses as well as new genomic approaches, e.g. metagenomics, have been proposed for water quality assessment [41-43]. However, during the summer, the higher temperature of bathing water and the increased amount of ultraviolet light have a negative impact on microbe survival. In this study, noro- and adenoviruses in outbreak II were detected in the water on at least six days but fewer than 12 days. These relatively short contamination episodes may remain undetected with routine FIB sampling. In most of the outbreaks, the quality of bathing water was questioned only after people visiting the beaches fell ill, and restrictions against bathing were set for the beaches only then. The length of the restrictions was determined according to the results of water analyses and proved effective in controlling of the outbreaks.

Investigation reports of outbreaks linked to bathing water were assessed by a panel that included experts from THL, Valvira and UH. By using agreed criteria, reports can be assessed more consistently over time [12]. When the same pathogen has been identified in patients and in the beach environment, results from the analytical epidemiological study point towards a certain source and water quality failures have been detected, outbreaks are often easy to categorise. More discussion in the panel will be needed on the relation between illness and the beach environment when pollution of the beach is mentioned but no obvious other exposures are described in outbreak reports. In this study, eight outbreaks were identified among the 15 outbreaks suspected to be caused by bathing water that were reported to the FWO registry. Four outbreaks were classified as having a strong or probable association with the beach environment, and four as having a possible association. Analytical epidemiological investigations were lacking in all but one investigation, indicating that more training and practical experience in analytical epidemiology may be needed in the municipal outbreak investigation groups.

Because of an increase in the number of bathing water outbreaks in the summer of 2014, THL and Valvira published guidelines for outbreak control in spring 2015 to prevent bathing water outbreaks. If, based on the laboratory or epidemiological findings, the water is considered to be contaminated, visitors should be informed about a bathing prohibition or advice against bathing should be posted by means of the international symbols presented in the Commission Implementing Decision (2011/321/EU) [44]. To prevent outbreaks, rooms intended for washing and dressing as well as toilets at the beach should be kept clean, and soap, hand towels and toilet paper should be available. Visitors should be encouraged to wash their hands or use freshen-up towels. Nappies should not be changed and the babies' bottoms should not be washed in the bathing water, and people with gastrointestinal illness should avoid swimming. In the case of an outbreak suspicion, municipal authorities should notify the FWO registry and an outbreak investigation, including epidemiological and microbiological analyses, should be initiated.

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Conflict of interest

None declared.

Authors' contributions

Ari Kauppinen, Haider Al-Hello, Outi Zacheus, Jaana Kilponen, Leena Maunula, Sari Huusko, Ilkka Miettinen, Soile Blomqvist and Ruska Rimhanen-Finne participated in the national outbreak evaluation panel and the design of the study. Ruska Rimhanen-Finne coordinated the national panel. Ari Kauppinen was responsible for performing the data analyses and virus analyses from water performed at THL. Haider Al-Hello, Soile Blomqvist and Maija Lappalainen were responsible for analysing viruses from patient samples. Leena Maunula was responsible for analysing viruses from the water and environmental samples performed at UH. Ari Kauppinen and Ruska Rimhanen-Finne drafted the manuscript. All authors were involved in the preparation and review of the manuscript and approved the final version.

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Twenty years and counting: epidemiology of an outbreak of isoniazid-resistant tuberculosis in England and Wales, 1995 to 2014

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An outbreak of isoniazid-resistant tuberculosis first identified in London has now been ongoing for 20 years, making it the largest drug-resistant outbreak of tuberculosis documented to date worldwide. We identified culture-confirmed cases with indistinguishable molecular strain types and extracted demographic, clinical, microbiological and social risk factor data from surveillance systems. We summarised changes over time and used kernel-density estimation and k-function analysis to assess geographic clustering. From 1995 to 2014, 508 cases were reported, with a declining trend in recent years. Overall, 70% were male (n = 360), 60% born in the United Kingdom (n = 306), 39% white (n = 199), and 26% black Caribbean (n = 134). Median age increased from 25 years in the first 5 years to 42 in the last 5. Approximately two thirds of cases reported social risk factors: 45% drug use (n = 227), 37% prison link (n = 189), 25% homelessness (n = 125) and 13% alcohol dependence (n = 64). Treatment was completed at 12 months by 52% of cases (n = 206), and was significantly lower for those with social risk factors ($p < 0.05$), but increased over time for all patients ($p < 0.05$). The outbreak remained focused in north London throughout. Control of this outbreak requires continued efforts to prevent and treat further active cases through targeted screening and enhanced case management.

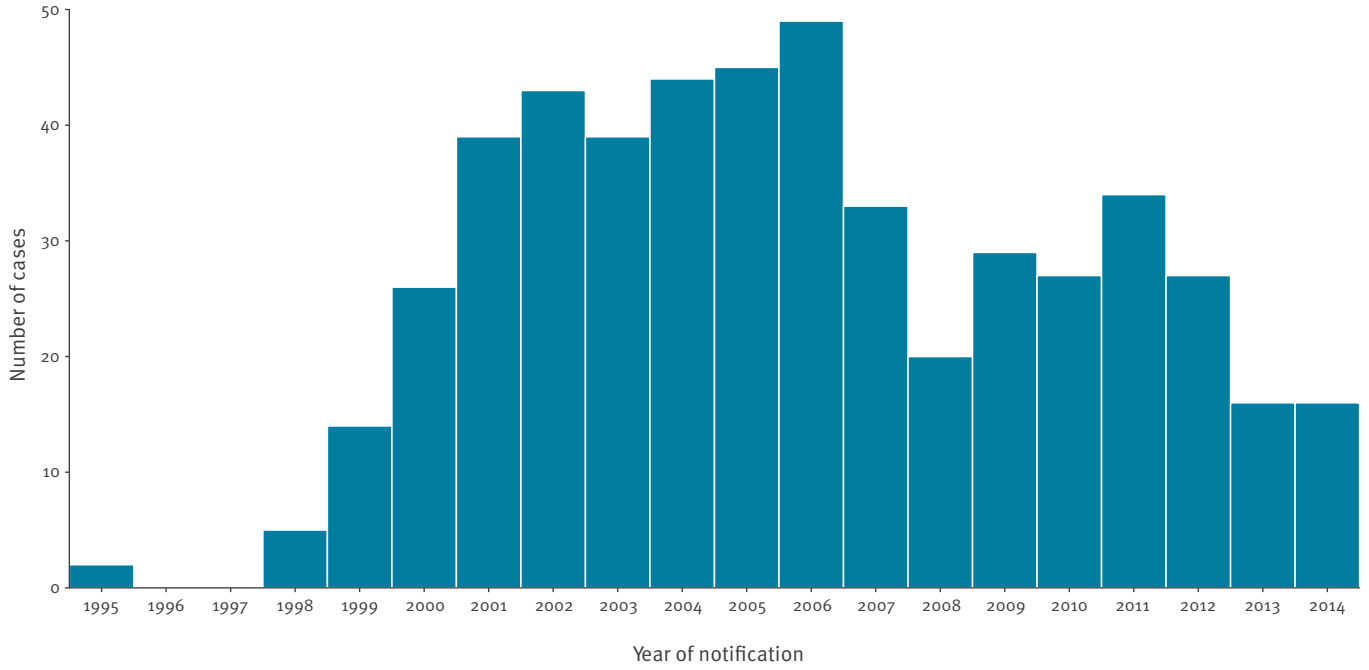
Introduction

Incidence rates of tuberculosis have fallen in many European countries in recent years, but were increasing until 2009 in England and Wales, and have since remained relatively high [1]. In 2014, 6,520 cases were reported in England (12/100,000 inhabitants) and 115 in Wales (4/100,000). The highest incidence rate (30/100,000) was reported in London, where 39% of cases in England resided [2]. Multidrug-resistant (MDR) disease poses a particular threat to tuberculosis control as it cannot be managed using standard treatment regimens. Resistance to a single first-line drug is a precursor for development of MDR-tuberculosis, and isoniazid resistance is the most commonly identified form of resistance worldwide [3].

In England and Wales, 6–7% of cases with drug-susceptibility results are resistant to isoniazid [2], and in 2013, 7% of isoniazid-resistant tuberculosis cases in England had a strain type known to be associated with an ongoing outbreak [4]. This outbreak was first identified in 2000 at a hospital in north London where three young men were diagnosed with an identical strain type of the Euro-American lineage within a week. Retrospective strain typing of isolates available at the time identified a further 15 cases, with the first case dating back to 1995 [5]. Cases have since been ascertained prospectively, and the outbreak now spans 20 years [6].

Figure 1

Number of cases in the isoniazid-resistant tuberculosis outbreak by year, England and Wales, 1995–2014 (n = 508)



Epidemiological characteristics of this ongoing outbreak were last described for cases up to 2006 [7]. These cases have previously been shown to include a high proportion of young males, particularly of white or black Caribbean ethnicity, who were born in the United Kingdom (UK) and lived in north London. Cases were also significantly more likely to present with social risk factors including imprisonment, unemployment, drug use or sex work [5,7,8]. An Outbreak Control Committee (OCC) established in 2000 recommended action on interagency working, improved identification and management of cases including use of directly observed therapy (DOT) and contact tracing, and improved control in prisons [9].

As cases continue to be reported 20 years since the first identified case, this cluster now represents one of the largest documented outbreaks worldwide of drug-resistant tuberculosis. In this study, we aimed to describe the evolution of the outbreak in time and space and discuss implications for future tuberculosis control.

Methods

Outbreak case definition and data sources

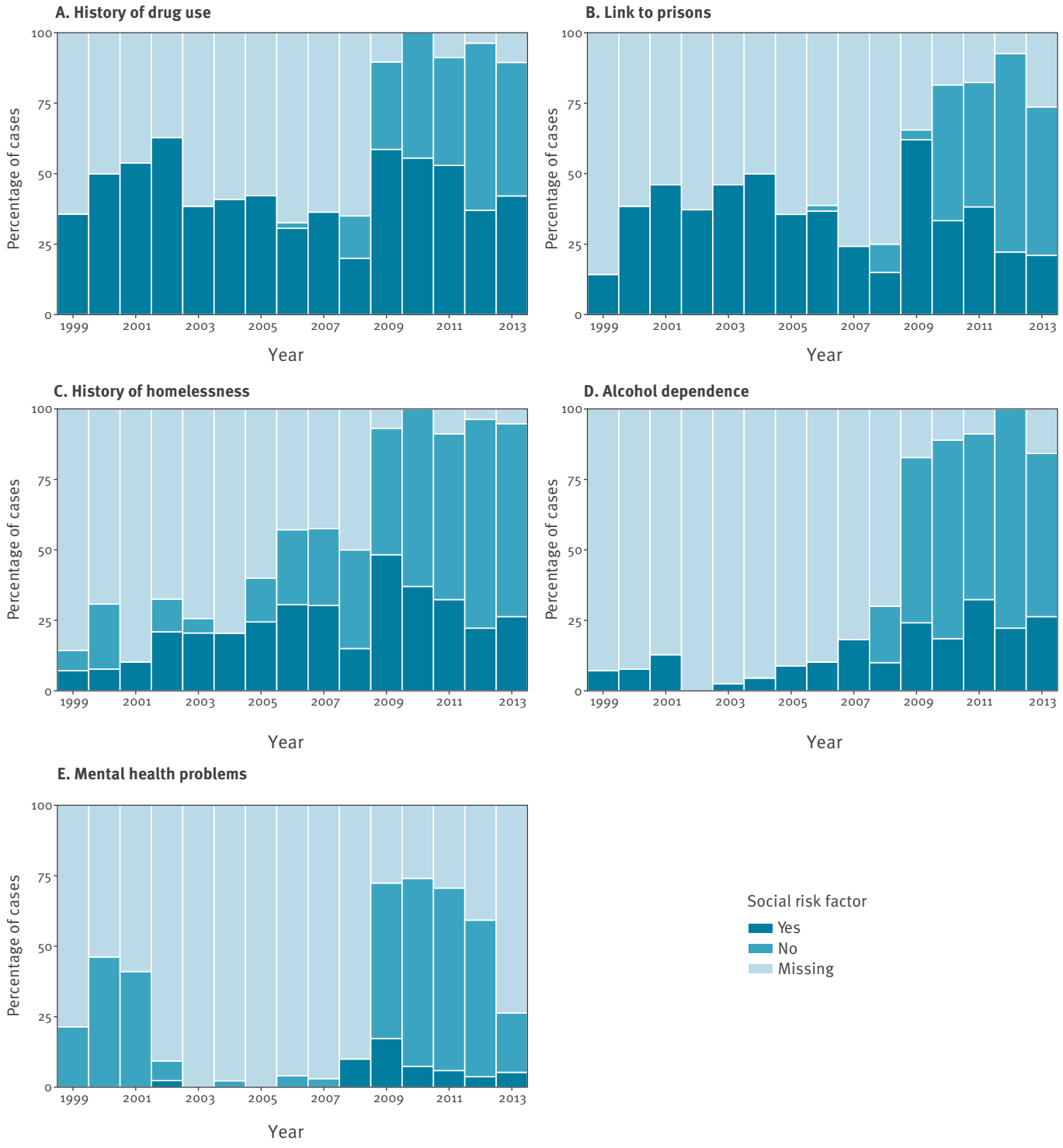
Cases were defined as individuals diagnosed from 1995 to 2014 in England and Wales with a *Mycobacterium tuberculosis* isolate that was indistinguishable from the outbreak strain. Following identification of the outbreak in January 2000, cases were ascertained prospectively by strain typing of all isoniazid-resistant isolates from patients resident in, or with known epidemiological links to, London. Prior to 2000, cases were ascertained retrospectively through review of microbiological databases and strain typing of identified isolates [7]. From 2010 onwards, strain typing was conducted on all tuberculosis isolates in England and Wales, regardless of links to London.

The outbreak strain was initially characterised using restriction fragment length polymorphism (RFLP) analysis and before 2000, the isolates selected for typing were those of isoniazid monoresistant organisms cultured at four laboratories serving the area where first cases were reported. After 2000 all such strains across London were RFLP-typed, and from 2006 onwards, due to a change in routine practice, 24-locus mycobacterial interspersed repetitive sequence variable-number tandem repeat (MIRU-VNTR) typing was used to identify the corresponding strain [7]. Strain typing was conducted at the Health Protection Agency National Mycobacterium Reference Laboratory.

We extracted information on outbreak-related cases from multiple data sources. Demographic, clinical, microbiological and treatment outcome data were provided by a bespoke outbreak database, and from the electronic surveillance systems for London (the London Tuberculosis Register, LTBR) and the rest of England and Wales (the Enhanced Tuberculosis Surveillance System, ETS). Information on social risk factors (drug use, link to prisons, including patients who were in prison at time of diagnosis, homelessness, alcohol dependence and mental health concerns) was collected initially in the bespoke outbreak database, and from 2009 in surveillance systems.

Figure 2

Percentage of cases in the isoniazid-resistant tuberculosis outbreak by year and social risk factor, England and Wales, 1999–2014



We also extracted information on outbreak cases from data collected by Find and Treat, a pan-London tuberculosis outreach service [10]. This service aims to identify cases of tuberculosis in hard-to-reach populations, typically those with social risk factors, and support them to complete treatment. We identified outbreak-related cases who had been screened and managed by the service, and used data to supplement information on social risk factors.

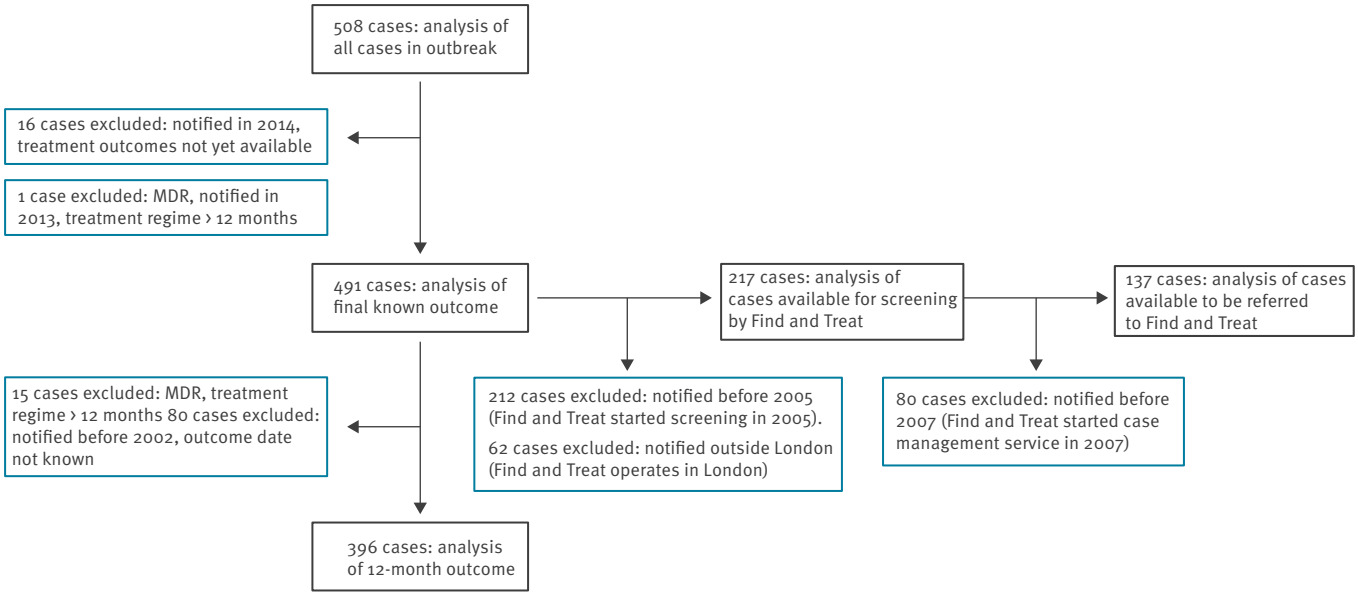
Databases were combined on the basis of unique identifiers, patient names and dates of birth. Patients with multiple episodes of tuberculosis with the outbreak strain were identified, but only the first period was included in analyses.

Epidemiological analysis

We plotted annual numbers of outbreak cases as an epidemic curve. We described demographic,

Figure 3

Eligibility criteria for cases included in analyses of treatment outcomes and Find and Treat data, isoniazid-resistant tuberculosis outbreak, England and Wales, 1995–2014



MDR: multidrug-resistant.

Black boxes show analyses, blue boxes show exclusions.

clinical and microbiological characteristics of all cases in counts and proportions, and used the chi-squared test for trend to identify changes over time. For social risk factors, we calculated overall proportions and identified changes over time by plotting proportions of cases reporting risk factors by year.

We identified treatment outcomes at 12 months for non-MDR cases who were notified between 2002 and 2013. MDR cases were excluded from this analysis because their planned treatment regime exceeds 12 months; cases notified before 2002 were excluded as they did not have a date of treatment outcome recorded, and outcomes had not yet been collected for cases after 2013. We tested for changes in proportions of cases completing treatment over time and used the chi-squared test to compare proportions of cases with and without social risk factors who completed treatment. Final known outcomes were also defined, and included cases notified before 2002, MDR cases notified before 2013, and incorporated 12- or 24-month treatment follow-up where appropriate.

We used Find and Treat data to identify the proportion of cases notified in London who had been screened by the service (cases from 2005 onwards), and referred for case management (cases from 2007 onwards). We calculated the proportions of patients referred to Find and Treat who had social risk factors, and used chi-squared tests to compare the rates of treatment completion in patients referred to Find and Treat with those who were not.

Spatial analysis

We determined case locations using geocoded residential postcodes where available. Prison or clinic postcodes were used where relevant for patients diagnosed while in prison or with no fixed abode. We plotted numbers of cases nationally by region and calculated incidence rates by London borough using population data from the 2001 census. We visualised the spatio-temporal progression of the outbreak within London through a series of smoothed-incidence maps. Each map displays the spatial intensity of case locations in a 5-year period during the outbreak, generated through kernel-density estimation [11].

We further explored the spatial point pattern of cases in London through k-function analysis. The k-function is a method for detecting spatial clustering and is defined as the expected number of cases within a given distance from an arbitrary case location [12]. First, we tested the hypothesis that the points were completely spatially random by comparing the k-function of the observed point locations with the function generated by 99 simulated point patterns. We then tested the hypothesis that the locations of cases in the first 10 years of the outbreak were part of the same spatial distribution as those in the second decade by calculating their cross k-function. This is the number of points from one distribution within a range of distances of a typical point from the other distribution [11]. The observed cross k-function was compared with the functions defined by 99 simulations based on random re-labelling of the joint spatial distribution of

Figure 4

Numbers of cases in the isoniazid-resistant outbreak in England (by Public Health England Region) and Wales, 1995–2014 (n=505)

Total number of cases is 505 because three cases had no geographical information. Contains Ordnance Survey data, Crown copyright and database right 2014.

points to the two time periods. If the observed function lay within the upper and lower bounds of these limits, this would be consistent with the null hypothesis that the points were part of a common spatial distribution.

Data management, validation and analysis were performed using R 3.1 and Stata 13.0. The R package *spatstat* was used for kernel-density and k-function analyses [13].

Results

From 1995 to 2014, 508 cases with the isoniazid-resistant tuberculosis outbreak strain were identified. The epidemic curve (Figure 1) shows that, after initial ascertainment of the outbreak in 2000, the number of cases rose steeply, reaching a peak of 49 in 2006. After a subsequent decrease in numbers, there appears to have been a second peak in 2011, followed by another decline in cases.

Characteristics of cases

Table 1 presents the demographic characteristics of cases in this outbreak by 5-year period. The majority of cases (71%) were male; of white (39%), black Caribbean (26%) or black African (13%) ethnicity; and born in the UK (60%). There were no significant changes in proportions of these characteristics over time (chi-squared trend p=0.97, 0.39, and 0.28 for sex, ethnicity and place of birth respectively). The median age of cases increased from 25 years (range 6–71, interquartile range (IQR) 21–28) in 1995–1999 to 42 in 2010–2014 (range 12–79, IQR 31–49); and there was a significant increase in the proportion of cases aged 45–64 years over the outbreak (chi-squared trend: p<0.001).

Most cases (85%) had pulmonary tuberculosis, and this proportion did not change over time (chi-squared trend: p=0.83). All cases had isoniazid-resistant disease; there were 14 cases of MDR-tuberculosis (3%), of which nine were MDR at their initial drug resistance test, and five were initially isoniazid-resistant but subsequently acquired resistance to rifampicin. One MDR case additionally developed pyrazinamide resistance. Twenty-four patients were diagnosed with this strain on more than one occasion, half of whom had initially completed treatment. The longest interval between diagnoses was 14 years; median 3.5 years.

One or more social risk factors were reported for 308 (61%) cases. History of drug use (227, 45%), links to prisons (189, 38%), and homelessness (125, 25%) were most frequently reported. Alcohol dependence (64, 13%) and mental health concerns (13, 3%) were reported less often. For 108 cases, two risk factors were reported (21%); for 66 cases, three risk factors (13%) and for 23 cases, four risk factors (5%). Figure 2 displays the proportion of cases with each social risk factor by year. This demonstrates the continued importance of prisons and drug use over the duration of the outbreak; as well as the change in data-collection methods in 2009, which resulted in an increased proportion of cases reported with presence or absence of risk factors and decreased proportion with missing data. Prior to this, reports were commonly made only if a risk factor was present and left missing if absent.

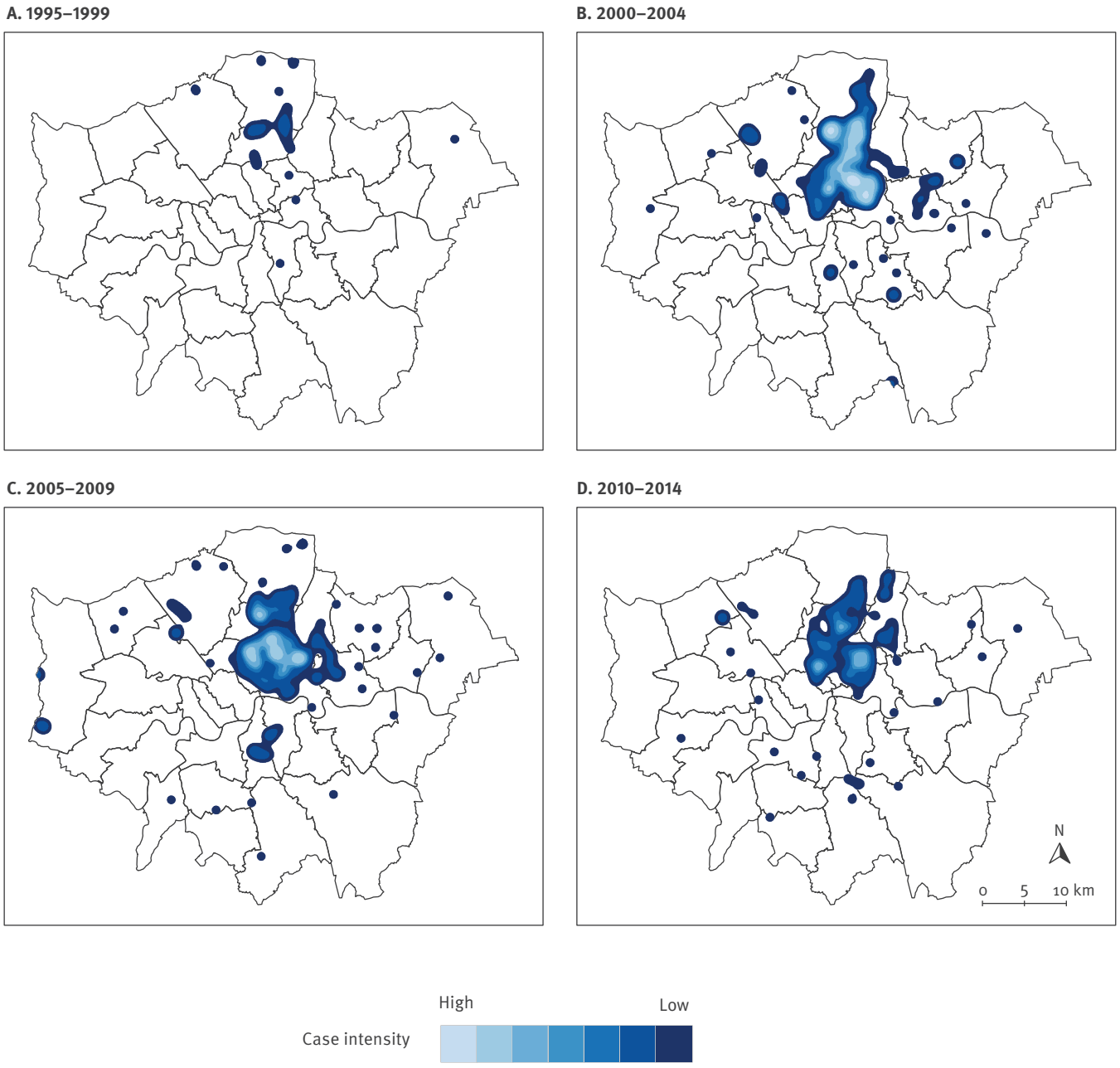
Treatment outcomes and Find and Treat

Figure 3 defines the eligibility criteria for including cases in these analyses.

Treatment was completed by 206 (52%) of 396 eligible patients at 12 months, with a significant increase in this proportion during the outbreak (p<0.05). Cases with at least one social risk factor had a significantly lower percentage of treatment completion at 12 months than those with none or missing information on social risk factors (42% and 67% respectively, chi-squared test: p<0·05). Treatment completion was lowest for those with a history of homelessness (n=42/125, 39%), links to prisons (n=58/189, 39%), or a history of drug use (n=72/227, 52%). At final known outcome, 372 (76%) of 491 eligible cases completed treatment, and those with at least one social risk factor also had a lower percentage of treatment completion (71% vs 82% for those with none or missing information on social risk factors, chi-squared test: p=0·006). Twenty cases were reported to have died (4%). Tuberculosis is known to have contributed to the deaths of three patients at final known outcome, was incidental for seven, and

Figure 5

Smoothed incidence maps of cases in the isoniazid-resistant tuberculosis outbreak in London, by 5-year time period, 1995–2014



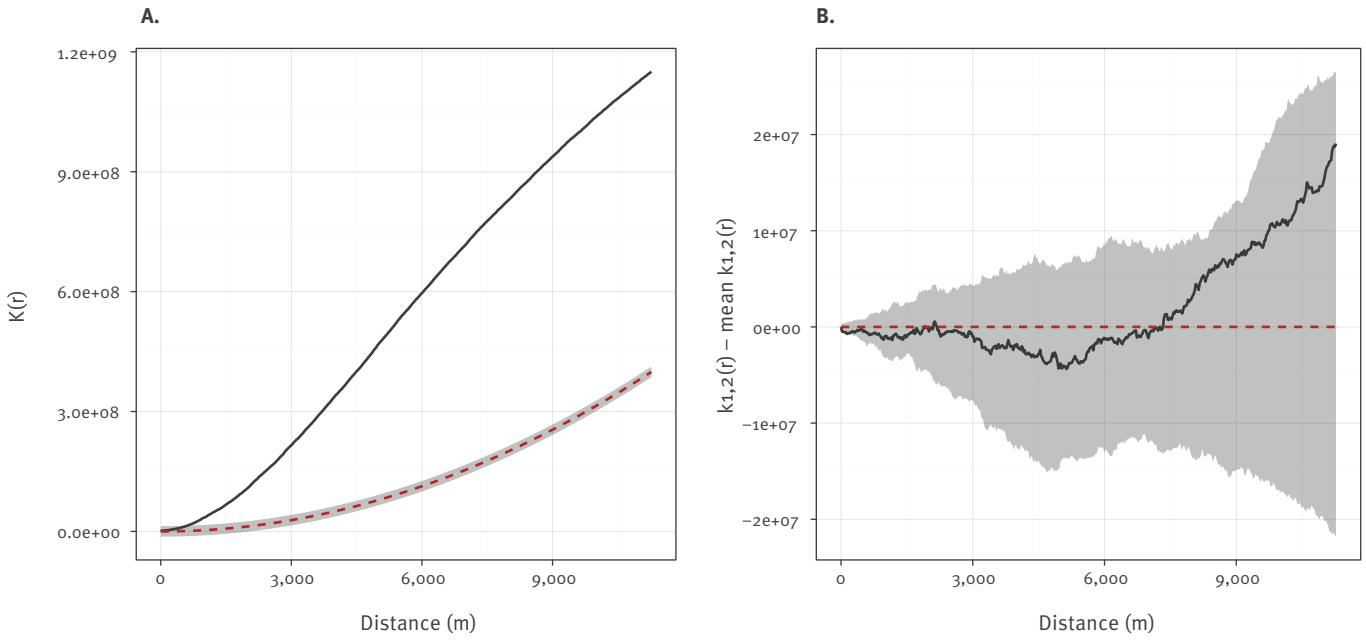
Spatial intensity determined using kernel-density estimation, bandwidth 597 m. Contains Ordnance Survey data, Crown copyright and database right 2014.

has an unknown link to the deaths of the remaining 10 patients. Reasons for failing to complete treatment are shown in Table 2; 8.6% and 8.8% of cases had been lost to follow-up at 12 months and final known outcome respectively.

The Find and Treat screening programme aims to identify cases of tuberculosis in 'hard to reach' populations and has been operating in London since 2005. During this period (2005–2013), it screened 11.5% (25/217) of the individuals which were subsequently found to be part of the outbreak. Since 2007, Find and Treat has also operated a case management service, and one quarter (35/137) of outbreak patients notified in London in this time period have been referred to the service. The majority of these patients had a history of homelessness (n = 30/35); drug use (n = 29/35) and links to prisons (n = 21/35). These patients were significantly less likely to have completed treatment at 12 months (15/35) compared with those who were not managed within the service (72/102, chi-squared p = 0.006). However, treatment completion at final known outcome

Figure 6

K-function analysis of spatial clustering in isoniazid-resistant tuberculosis outbreak in London, 1995–2014; A: K-function test of complete spatial randomness; B: Cross k-function comparing first and second 10 years of outbreak



Simulation envelopes shaded in grey.

was not significantly different between the two groups (26/35 vs 87/102; chi-squared test: p = 0.22).

Spatial analysis

All cases were successfully geocoded to locations in England and Wales, with the exception of which three had no location data. The majority of these cases (416, 82%) lived in London; while the Midlands and East of England (44, 9%) reported the most cases of other regions (Figure 4). Within London, most cases were reported in north-east and north central areas, with the highest rates in the boroughs of Hackney and Haringey (45 and 34 cases per 100,000 population respectively), compared with 6 per 100,000 for the whole of London.

The smoothed incidence maps (Figure 5) show that the outbreak has remained largely concentrated in north London, with the highest spatial intensity of cases located in this region in all four time periods.

The k-function of the observed point locations (Figure 6A) lies clearly outside the simulation envelope representing randomly generated point patterns. This demonstrates that the data show spatial clustering above what would be expected by complete spatial randomness. The cross k-function (Figure 6B) compares the spatial distribution of the cases in the first and second 10 years of the outbreak. The k-function of the observed data lies within the simulation envelope generated through random labelling of cases to different time periods. There was therefore no evidence that the spatial distribution of cases in London changed significantly during the outbreak.

Discussion

This isoniazid-resistant tuberculosis outbreak has now been ongoing for 20 years, despite a recent decline in incidence. It has had a consistent focus in north London, particularly among socially marginalised populations. Links to prisons, drug use, and a history of homelessness are important risk factors, and failure of these groups to complete treatment is likely to have perpetuated the outbreak.

Major impacts of this outbreak have included tuberculosis disease in over 500 individuals, at least three linked deaths, and reinfection or relapse in 24 cases. Multidrug-resistance has emerged in this strain, and appears to have been transmitted between cases, as nine patients presented with an initial drug resistance test that was MDR. There are also potentially thousands of further individuals who have undetected infections, given that the lifetime risk of developing active disease following infection is estimated at 10% [14]. Furthermore, this outbreak has contributed considerable economic costs to health and social care services: Management of an uncomplicated case of tuberculosis is estimated to cost GBP 5,000 (approximately EUR 5,790), while drug-resistant cases can cost more than 10 times this amount, before taking into

Table 1

Demographic characteristics of cases in the isoniazid-resistant tuberculosis outbreak, England and Wales, 1995–2014

	1995–1999 n (%)	2000–2004 n (%)	2005–2009 n (%)	2010–2014 n (%)	All n (% of total)
All cases	21	191	176	120	508
Sex					
Male	13 (61.9)	139 (72.8)	122 (69.3)	86 (71.7)	360 (70.9)
Female	8 (38.1)	52 (27.2)	54 (30.7)	34 (28.3)	148 (29.1)
Age (years)					
Median	25	35	36	42	36
‹15	1 (4.8)	1 (0.5)	5 (2.8)	2 (1.7)	9 (1.8)
15–24	9 (42.9)	31 (16.2)	22 (12.5)	8 (6.7)	70 (13.8)
25–34	8 (38.1)	60 (31.4)	53 (30.1)	29 (24.2)	150 (29.5)
35–44	1 (4.8)	58 (30.4)	55 (31.3)	31 (25.8)	145 (28.5)
45–64	1 (4.8)	34 (17.8)	35 (19.9)	46 (38.3)	116 (22.8)
›65	1 (4.8)	7 (3.7)	6 (3.4)	4 (3.3)	18 (3.5)
Ethnic group					
White	8 (38.1)	59 (30.9)	73 (41.5)	59 (49.2)	199 (39.2)
Black Caribbean	4 (19.0)	59 (30.9)	49 (27.8)	22 (18.3)	134 (26.4)
Black African	3 (14.3)	34 (17.8)	20 (11.4)	10 (8.3)	67 (13.2)
Indian	2 (9.5)	7 (3.7)	6 (3.4)	7 (5.8)	22 (4.3)
Black other	1 (4.8)	4 (2.1)	8 (4.5)	5 (4.2)	18 (3.5)
Other	1 (4.8)	18 (9.4)	14 (8.0)	15 (12.5)	43 (8.4)
Unknown	2 (9.5)	10 (5.2)	6 (3.4)	2 (1.7)	20 (3.9)
UK-born					
Yes	15 (71.4)	95 (49.7)	114 (64.8)	82 (68.3)	306 (60.2)
No	4 (19.0)	82 (42.9)	51 (29.0)	35 (29.2)	172 (33.9)
Unknown	2 (9.5)	14 (7.3)	11 (6.3)	3 (2.5)	30 (5.9)
Country/area of birth if not UK-born					
Sub-Saharan Africa[a]	1 (4.8)	24 (12.6)	20 (11.4)	8 (6.7)	53 (10.4)
Jamaica	0 (0)	17 (8.9)	10 (5.7)	5 (4.2)	32 (6.3)
Ireland	1 (4.8)	14 (7.3)	2 (1.1)	6 (5.0)	23 (4.5)
Indian Subcontinent[b]	1 (4.8)	4 (2.1)	3 (1.7)	6 (5.0)	14 (2.8)
Other	0 (0)	20 (10.5)	15 (8.5)	10 (8.3)	45 (8.9)
Unknown	3 (14.3)	17 (8.9)	12 (6.8)	3 (2.5)	35 (6.9)

UK: United Kingdom.

[a] Includes cases born in Angola, Congo, Eritrea, Ethiopia, Gambia, Ghana, Kenya, Liberia, Mauritania, Nigeria, Sierra Leone, Somalia, Tanzania, Uganda, Zambia and Zimbabwe.

[b] Includes cases born in India, Bangladesh, Pakistan and Sri Lanka.

account use of additional resources associated with outbreak investigations such as contact tracing and outbreak control team meetings [15].

The outbreak has proved particularly challenging to control despite great efforts overseen by the dedicated OCC established in 2000. It has consistently been associated with 'hard to reach' populations including those with a history of drug use, homelessness or imprisonment. Recommendations implemented by the OCC which attempted to target these groups included extension of contact tracing beyond household contacts to close social contacts, promulgation of advice relating to specific drug regimens for treatment, and expanded use of DOT [9]. The OCC met regularly and reviewed progress and implementation, with improvements seen in numbers of contacts traced per case and treatment completion, but the decline in cases was slow to occur. In more recent years, the Find and Treat mobile screening unit has contributed to control of the outbreak. Approximately one in 10 outbreak cases was screened by this service since it started operating, and it was also an effective service for managing complex patients: In this outbreak, patients managed by Find and Treat had a higher prevalence of social risk factors, but there was no significant difference in final treatment completion rates in these patients compared with others in the outbreak.

Table 2

Treatment outcomes of cases in the isoniazid-resistant tuberculosis outbreak, England and Wales, 2002–2013 (12-month outcome) and 1995–2013 (final known outcome)

Outcome	12-month outcome (cases 2002–2013)[a] n (%)	Final known outcome (cases 1995–2013)[b] n (%)
Completed	206 (52.0)	372 (75.8)
Still on treatment	66 (16.7)	11 (2.2)
Lost to follow-up	34 (8.6)	43 (8.8)
Died	12 (3.0)	20 (4.1)
Transferred out	10 (2.5)	17 (3.5)
Unknown / Not complete – unknown reason	68 (17.2)	28 (5.7)
Total	**396**	**491**

[a] Excluding all multidrug-resistant cases.

[b] Excluding multidrug-resistant case notified in 2013.

Our analysis provides some insights into the natural history of this outbreak and how it has progressed. In spite of its long duration, the outbreak has remained fairly circumscribed in north London, and characteristics of populations affected have remained relatively stable, although the age of the patients at notification did increase over time. The smoothed incidence maps and k-function analyses demonstrate distinct spatial clustering which persisted in the same region of north London throughout. These observations are consistent with intensive transmission among a social cohort approximately 20 years ago, whose infections have gradually progressed to active disease. If a great deal of ongoing transmission had been occurring outwith these groups, it would be expected that cases would have become more widely disseminated with smaller clusters arising in dispersed geographic areas. Detailed genetic analyses of a selection of outbreak isolates found little or no associated fitness cost and the presence of specific deletions that could be a peculiar feature of the strains and help explain their persistence over the very many years [16].The epidemic curve had a two-wave pattern, with an initial peak which may represent cases whose infections rapidly progressed to disease, and a later peak potentially driven by those presenting with symptoms following a longer period of latency. Alternatively, the second wave of cases may have resulted from a second period of intensive transmission. Whole genome sequencing of isolates could be used to investigate these hypotheses by constructing a phylogenetic tree that identifies likely chains of transmission [17].

This analysis represents the largest documented outbreak of drug-resistant tuberculosis to date. Previous outbreaks of comparable size have been reported in New York City [18-20] and South Africa [21,22]. Both incidents were linked to nosocomial transmission among HIV-positive patients, which have not been important factors in this outbreak [7]. There are more commonalities between this outbreak and one that occurred in Stockholm, Sweden between 1996 and 2005, comprising 96 cases [23,24]. The Stockholm outbreak was also characterised by confinement of cases to a distinct demographic group in a small geographic area, resistance to isoniazid, and had an epidemic curve with a two-wave pattern. This indicates the importance of community transmission of tuberculosis within European cities, and the need to focus control measures on affected groups.

Our results therefore have implications for future control of this outbreak and for control of tuberculosis more widely. We recommend targeted screening of high-risk individuals (for example in prisons) to prevent further active cases, and enhanced case management to support patients to complete treatment. Continued support of the Find and Treat tuberculosis outreach service, which provides a cost-effective approach to case finding [10] and has successfully identified and managed complex patients in this outbreak, should help to achieve this. We additionally recommend following National Institute for Health and Care Excellence guidance for tackling tuberculosis among hard-to-reach groups [15]. This includes standardised risk assessment for all tuberculosis patients; better recording and monitoring of contact tracing, and expanded use of DOT, which is used infrequently in London compared with other parts of the world [25]. In the wider context of tuberculosis outbreak control, we recommend regular reviews of the epidemiology and spatial distribution of tuberculosis clusters linked by molecular strain typing. This will enable better understanding of the important factors associated with transmission, tracking the extent of spatial dispersion of outbreak strains, and improved targeting of control measures.

A strength of this study is that we combined data from multiple sources including two surveillance systems, a bespoke outbreak database, and data from the tuberculosis outreach service, Find and Treat. This enabled us to describe all reported cases that have

been associated with the outbreak, and ensured best possible completeness of variables. However, there were also limitations with these data: We are unlikely to have ascertained all cases affected in the outbreak because we used only culture-confirmed cases, excluding those without appropriate strain-typing information regardless of epidemiological links. This also means that we are likely to have overestimated the prevalence of pulmonary disease in this outbreak. The number of cases that occurred before 2000 is likely to have been underestimated as cases were ascertained retrospectively, and before 2010 cases notified outside London were not typed unless there were known epidemiological links to the outbreak. Owing to the change in routine microbiological testing procedures from RFLP to MIRU-VNTR typing, the case definition for this outbreak also changed. It is therefore possible that these cases are not part of the same outbreak. However, all cases were isoniazid-resistant and epidemiological characteristics of cases did not change significantly following the change in strain-typing methodology. This suggests that the updated case definition was appropriate, but this is being confirmed through whole genome sequencing of isolates defined through the two different methods.

Another limitation of this analysis is that we were unable to assess the importance of risk factors which are not included in routine surveillance. These include commercial sex work and unemployment, which have previously been found to be linked to this outbreak, and HIV status, which is not thought to be an important factor [7]. Estimates for prevalence of social risk factors also represent minimum likely values, owing to non-ascertainment, non-disclosure and inconsistent definitions, particularly before 2009 when these fields were introduced to routine surveillance systems. We therefore did not assess the relative importance of risk factors in this outbreak through a formal case control study. However, surveillance data shows that the proportion of all London tuberculosis cases reported between 2009 and 2014 who had one or more social risk factor was substantially lower than for cases in this outbreak reported over the same period (ca 10% and 40% respectively) [6]. The shift towards older age groups observed here has also not been observed in London cases more widely, and, contrary to the pattern in this outbreak, highest overall rates within London have been in north-west areas [6]. It is therefore likely that the characteristics identified here are specific to this outbreak and do not merely reflect the epidemiology of tuberculosis patients as a whole.

Finally, there were limitations to the statistical and spatial methods used in this analysis. We used the chi-squared test for trend to identify changes in patient characteristics over time. An assumption of this test is that the proportions did not, for example, increase and then decrease as the outbreak progressed. We checked for this possibility by plotting these characteristics as a function of time. Spatial analyses were based on point locations of cases stratified by 5- and 10-year time periods. This provides an incomplete picture of the true spatial distribution of the outbreak and could have masked intra-period changes in distributions. However, the smoothed incidence maps demonstrate a clear and persistent focal point the north of the city, so it is unlikely that these factors have had a substantial impact on the conclusions.

Tuberculosis in Europe is increasingly a problem that is concentrated in large cities [26]. This study demonstrates that outbreaks in cities, even in low incidence countries, can persist for many years through community transmission. Resolving this outbreak of drug-resistant disease, and prevention of future outbreaks, will therefore be a key factor in strengthening tuberculosis control in Europe. As recognised by the recent Collaborative Tuberculosis Strategy for England, this will require that best practice in clinical care, social support and public health are brought together [27].

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Conflict of interest

None declared.

Authors' contributions

ACH, HM, CA, CMS and SCMT conceived the study. CMS and SCMT conducted analyses and wrote the first draft. CA, MKL, TB and AS oversaw collection of data. HF assisted with analyses. All authors revised and edited the final manuscript.

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Research article

Mid-season real-time estimates of seasonal influenza vaccine effectiveness in persons 65 years and older in register-based surveillance, Stockholm County, Sweden, and Finland, January 2017

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Systems for register-based monitoring of vaccine effectiveness (VE) against laboratory-confirmed influenza (LCI) in real time were set up in Stockholm County, Sweden, and Finland, before start of the 2016/17 influenza season, using population-based cohort studies. Both in Stockholm and Finland, an early epidemic of influenza A(H3N2) peaked in week 52, 2016. Already during weeks 48 to 50, analyses of influenza VE in persons 65 years and above showed moderately good estimates of around 50%, then rapidly declined by week 2, 2017 to 28% and 32% in Stockholm and Finland, respectively. The sensitivity analyses, where time since vaccination was taken into account, could not demonstrate a clear decline, neither by calendar week nor by time since vaccination. Most (68%) of the samples collected from vaccinated patients belonged to the 3C.2a1 subclade with the additional amino acid substitution T135K in haemagglutinin (64%) or to subclade 3C.2a with the additional haemagglutinin substitutions T131K and R142K (36%). The proportion of samples containing these alterations increased during the studied period. These substitutions may be responsible for viral antigenic change and part of the observed VE drop. Another possible cause is poor vaccine immunogenicity in older persons. Improved influenza vaccines are needed, especially for the elderly.

Introduction

Systems for register-based monitoring of vaccine effectiveness (VE) against laboratory-confirmed influenza (LCI) in real time were set up in Stockholm County, Sweden, and in Finland, before the start of the 2016/17 influenza season, using population-based cohort studies [1,2]. In both locations, after an initial moderately high VE of about 50%, a rapid and sharp 20% decline in VE was observed. In addition, reports from hospitals and outpatient clinics indicated that a majority of patients with influenza-like illness (ILI) and severe acute respiratory infection (SARI) were elderly people, i.e. those 65 years and above, and that many of them had been vaccinated with the seasonal influenza vaccine (SIV). We therefore wanted to calculate early and mid-season estimates of influenza VE and compare the results between the two populations. The aim was to evaluate VE for LCI in persons 65 years and above, an age group eligible for free SIV.

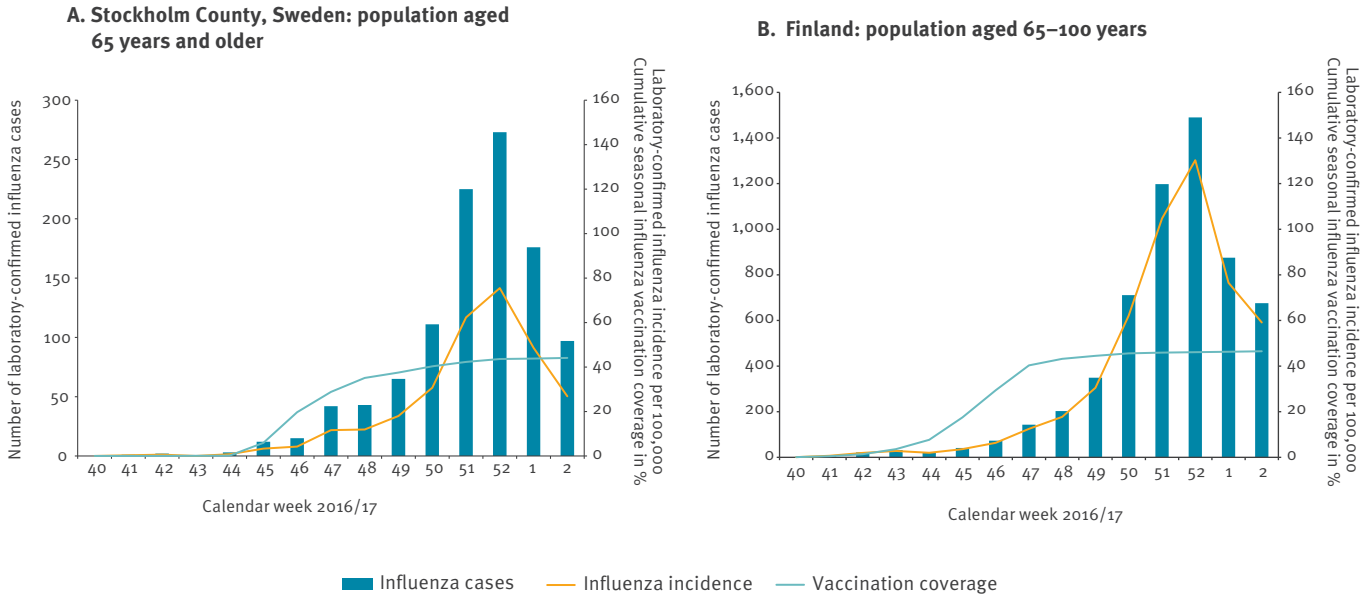
Methods

In both Stockholm County, Sweden, with 2 million inhabitants, and Finland with 5.5 million inhabitants, permanent residents have a unique personal identification number (PIN) based on which various national registers can be linked.

In Stockholm County, we used the central database (VAL) for healthcare utilisation, consultations and diagnoses, the vaccination register (Vaccinera) and for the outcome, the national electronic surveillance system (SmiNet) for the reporting of communicable diseases. Data from VAL, Vaccinera and SmiNet were linked using the same PIN (for details on data sources see [1,3,4]). VAL was used for obtaining data on in- and outpatient diagnoses, comorbidities, age and sex as well as the Stockholm Mosaic system. The latter is a proxy for

Figure 1

Coverage of seasonal influenza vaccination and number and incidence of laboratory-confirmed influenza cases, by calendar week, Stockholm and Finland, 1 October 2016–15 January 2017 (n = 358,583 and 1,144,894, respectively)



socioeconomic status based on 11 mutually exclusive categories, e.g. living in a low-income urban apartment block, multicultural suburb, affluent inner city, countryside, by which the County (including Stockholm city) can be divided into 120 smaller urban agglomerations [5]. Vaccinera contains all data, starting from 2009, on influenza and pneumococcal vaccination of persons aged 65 years and older or belonging to medical risk groups. Since the SIV programme in Stockholm offers persons 65 years and older vaccination free of charge and registration is mandatory and required for reimbursements to the healthcare provider, it can be assumed that all vaccinated persons in that age group are included in this database. SmiNet includes all diagnoses of influenza A and B starting from 1 December 2015 when they became notifiable diseases.

In Finland, the Population Information System (PIS) [5], the National Vaccination Register (NVR) [6] and the National Infectious Diseases Register (NIDR) [7] are also linked through a unique PIN. The PIS provides information on every person's date of birth, sex, date of death, and residential history. Also the NVR contains individual-level data, e.g. vaccine type and lot number as well as date of vaccination, for all vaccinations given within public primary healthcare (the system responsible for delivering the national immunisation programme), including free SIV for certain age and risk groups. The coverage of the NVR is assumed to reach 100% when excluding the population (‹5% of the elderly) that is affected by identified regional and temporal gaps in the NVR [6] or was temporarily living abroad during the study period. As part of the National Notification System of Communicable Diseases mandated by the Communicable Disease Act [8], all laboratories must send to the NIDR individual-level data on respiratory specimens that test positive for influenza, e.g. influenza type, date and place of sampling. The samples are taken on clinical grounds by judgement of the treating physician both in inpatient and outpatient settings.

The study populations were formed by the elderly, i.e. all individuals aged 65 years and older registered in Stockholm County on 1 October 2016 and all individuals aged 65 to 100 years permanently living in Finland on 1 October 2016.

The vaccines used for adult SIV during the current season in Stockholm were Vaxigrip (Sanofi Pasteur MSD, Lyon, France) (94.7%) and Fluarix (GSK, Brentford, United Kingdom) (5.2%), County. In Finland, it was Influvac (Abbot, Illinois, United States) in public healthcare and Vaxigrip in private healthcare. An individual was defined as vaccinated (exposed) starting from the day after (first) SIV during the ongoing season, and as previously vaccinated if they had at least one SIV record in the respective vaccination register for the previous 2015/16 season.

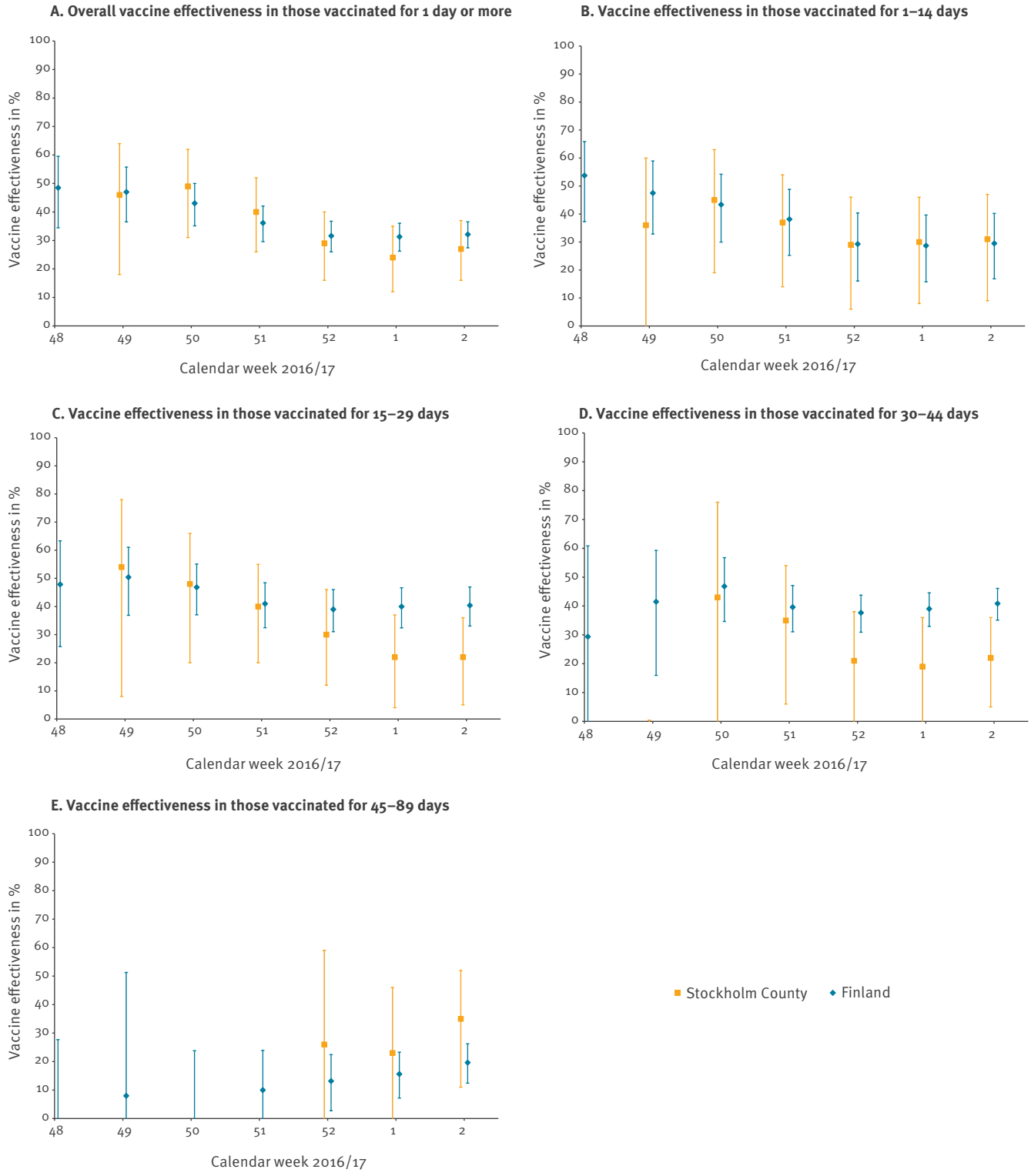
The outcome was defined as any LCI, irrespective of the influenza (sub)type, in patients sampled as in- or outpatients anywhere in the healthcare system.

Statistical analyses

Hazard rate ratios (HRR) comparing the hazard rates of LCI among vaccinated and unvaccinated individuals were calculated using Cox regression analyses. Vaccination status was modelled as a time-varying exposure, so individuals could contribute both vaccinated and unvaccinated risk time. The follow-up time, that the individuals of the two study populations

Figure 2

Weekly estimates of influenza vaccine effectiveness in the population aged 65 years and older in Stockholm County[a], Sweden and 65–100 years in Finland[b], 1 October 2016–15 January 2017 (n = 358,583 and 1,144,894, respectively)*



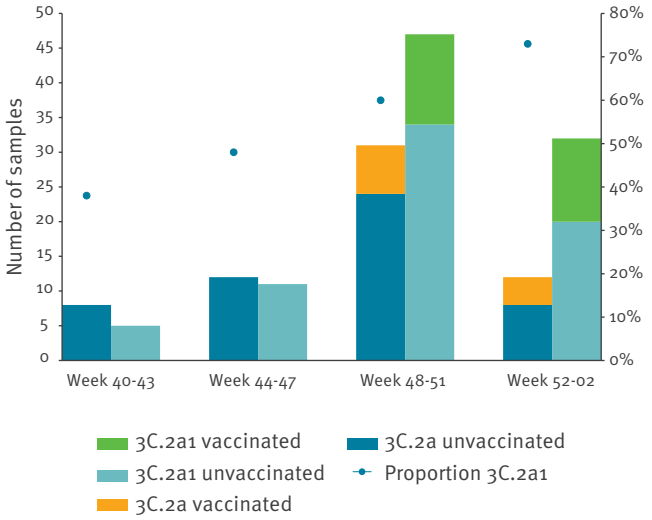
Panel A: whole population, unstratified. Panels B–E: whole population, stratified according to time being vaccinated.

[a] Models were adjusted for age, sex, comorbidity status, socioeconomic status, previous seasonal vaccination and pneumococcal vaccination. As complete case analysis was used, the number of cases decreased due to missing data on socioeconomic status.

[b] Models were adjusted for age, sex and previous seasonal vaccination.

Figure 3

Subclade distribution of influenza A(H3N2) viruses from unvaccinated and vaccinated patients, Stockholm and Finland, 1 October 2016–15 January 2017 (n = 158)



Dots: proportion of influenza subclade 3C.2a1 among total characterised samples, irrespective of vaccination status.

contributed to started on 1 October 2016 and ended with the occurrence of LCI, death (Finland only), or on 15 January 2017 (end of week 2), whatever occurred first. The cut-off in the data on 15 January reflects the time point when this publication was prepared. VE was calculated as (1 – adjusted HRR) × 100% and reported with 95% confidence intervals (CI).

The Cox models were adjusted for age in years (65–69, 70–74, 75–79, 80–84, ≥ 85 or 85–100), sex, previous influenza vaccination, and in Stockholm County also for comorbidity status, socioeconomic status and pneumococcal vaccination. The potential of previous influenza vaccination being an effect modifier was evaluated by stratifying the analysis and comparing the hazard rates among people vaccinated neither in 2015/16 nor in 2016/17 and among people vaccinated in both seasons, people vaccinated only in 2015/16 and people vaccinated only in 2016/17.

In sensitivity analyses, time since vaccination was taken into account and the time-dependent exposure variable was modified. Instead of only two levels ('not vaccinated', 'vaccinated for 1 day or more'), three levels ('not vaccinated', 'vaccinated for 1 to 14 days', and 'vaccinated for 15 days or more') and seven levels ('not vaccinated', 'vaccinated for 1 to 7 days', 'vaccinated for 8 to 14 days', 'vaccinated for 15 to 29 days', 'vaccinated for 30 to 44 days', 'vaccinated for 45 to 89 days', and 'vaccinated for 90 days or more') were considered and the respective VE estimates calculated.

In addition, the analyses were stratified by age calculating separate VE for the study population younger than 75 years and the study population aged 75 years and older.

Data management and analyses on the Swedish side were carried out using SAS Enterprise software (SAS Institute Inc., Cary, NC) and R 3.3.2 on the Finnish side.

Virus characterisation

A subset of influenza-positive specimens from clinical laboratories and sentinel surveillance systems, including patients treated in intensive care units (ICU), was chosen and characterised by sequencing of the haemagglutinin gene.

The chosen Finnish and Swedish strains represented different geographic origins and were timely distributed between weeks 40/2016 and 2/2017. Of the 158 sequenced samples, 43 of 75 (57%) and 34 of 83 (41%) were from the Finnish and Swedish sentinel systems, respectively. The remaining sequenced samples were from several clinical laboratories in both countries during the studied period.

Ethical consideration

The analysis in Stockholm was part of an ongoing evaluation of vaccine programmes required by the Department of Communicable Disease Control and Prevention, Stockholm County Council, Stockholm, Sweden, and falls outside the mandate for the Regional Ethics committee. PINs were anonymised in the linking of Vaccinera to VAL and SmiNet, and no data making individual identification possible was retained.

The National Institute for Health and Welfare, Finland (THL) carries out IVE evaluations as its statutory duty mandated by the Communicable Disease Act [6]. The umbrella protocol for influenza studies in context of the national immunisation programme, including the analyses presented here, have been reviewed by the THL Ethical committee and by the data ombudsman of Finland (THL/607/6.02.00/2016).

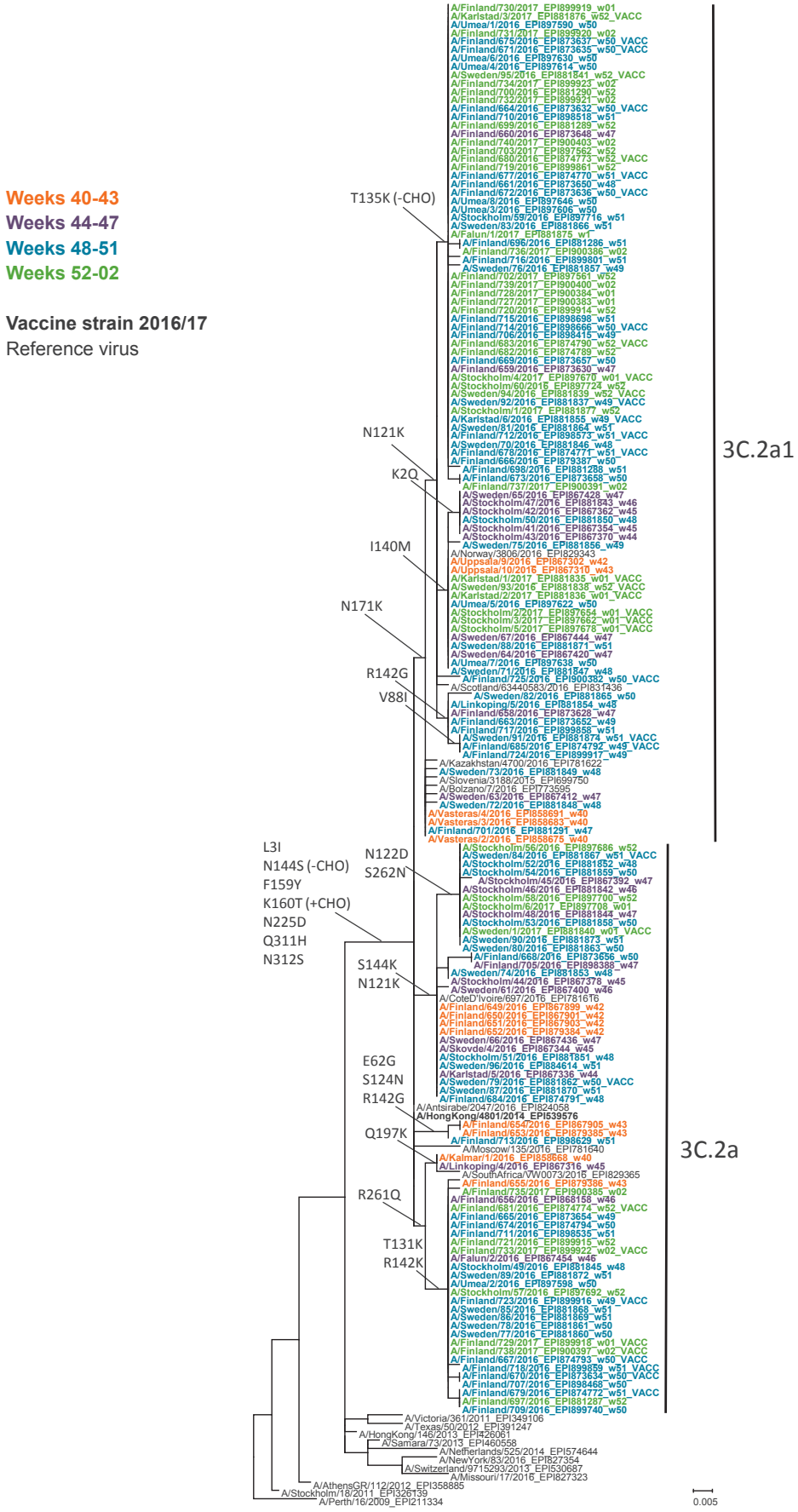
Results

The 2016/17 influenza epidemic started earlier than usual both in Sweden and Finland (Figure 1). The first cases were seen already in early November and the epidemic peaked in week 52. In both countries influenza A dominated (› 99%). Nearly all samples were influenza A(H3N2); only 10 of more than 1,300 typed samples in Sweden were influenza A(H1N1). In Finland, almost 17,000 laboratory-confirmed influenza A findings were reported to NIDR during the follow-up period. The National Influenza Centre in Finland subtyped a total 122 samples, and all were influenza A(H3N2).

In total, 1,034 and 5,845 LCI cases aged 65 years or above were reported during the study period in Stockholm County and Finland, respectively. The baseline characteristics of the population are presented in Table 1.

Figure 4

Phylogenetic analysis of amino acid sequences of the haemagglutinin HA1 subunit in influenza viruses from patients in Sweden and Finland, 1 October 2016–15 January 2017



The strains are coloured according to collection period, and samples from vaccinated patients have VACC as a suffix after the week number. The tree was constructed using the maximum likelihood method with Mega software version 5.1. The reference sequences (black colour) were downloaded from the Global Initiative on Sharing Avian Influenza Data (GISAID) and listed in Table 4.

Table 1

Comparison of baseline characteristics in the study population of Stockholm and Finland, 1 October 2016–15 January 2017 (n = 358,583 and 1,144,894, respectively)

	Not vaccinated		Vaccinated	
	n	%	n	%
Stockholm County, Sweden	**n = 201,106**		**n = 157,477**	
Age group				
65–69 years	71,999	36	35,128	22
70–74 years	54,972	27	46,418	29
75–79 years	30,787	15	32,158	20
80–84 years	19,817	10	21,572	14
≥ 85 years	23,531	12	22,201	14
Sex				
Male	91,184	45	69,482	44
Female	109,922	55	87,995	56
Previous influenza vaccination				
Not vaccinated in 2015/16	155,831	77	38,790	25
Vaccinated in 2015/16	45,275	23	118,687	75
Finland[a]	**n = 612,818**		**n = 532,076**	
Age group				
65–69 years	219,447	36	157,586	30
70–74 years	136,560	22	134,782	25
75–79 years	99,974	16	108,800	20
80–84 years	72,647	12	72,593	14
85–100 years	84,190	14	58,315	11
Sex				
Male	263,972	43	234,226	44
Female	348,846	57	297,850	56
Previous influenza vaccination				
Not vaccinated in 2015/16	535,248	87	125,622	24
Vaccinated in 2015/16	77,570	13	406,454	76

[a] By vaccination status as of 15 January 2017, i.e. follow-up was not restricted after a person was diagnosed with laboratory-confirmed influenza.

In Stockholm, 97% of the individuals with LCI had been sampled in the hospital setting, either in the emergency room or on a ward. Of the 1,034 patients with LCI, 755 (73%) were treated as inpatients. In Finland, no less than 3,787 (65%) of patients with LCI had been sampled in the hospital setting (when considering all places of sampling not unambiguously identifiable as outpatient), but no information about the setting of further treatment, i.e. whether the patient was transferred to a ward or sent home, was available for the present analysis.

The SIV campaign in Stockholm County started on 9 November 2016 (week 45). By 30 November, 100,442 persons 65 years and older were vaccinated, which corresponded to 28% of this age group, and by 31 December, the corresponding figure was 152,583 (43%) (Figure 1). In Finland, the SIV campaign started gradually, and most of the vaccinations were given in weeks 45–47. By the end of week 47, 461,323 (40%) of the 1,144,894 elderly people included in the study were vaccinated. The SIV coverage further increased to 46% by the end of 2016 (Figure 1).

A stratified analysis demonstrated (data not shown) that previous SIV (Table 1) was not an effect modifier, neither in the Stockholm nor in the Finnish data.

In Stockholm, the first two estimates of VE, in weeks 49 and 50, were 56% (95% CI: 11–78) and 49% (95% CI: 38–70) (Figure 2). After that, VE declined rapidly to the current estimate of 28% (95% CI: 16–37) in week 2, 2017 (Figure 2, Table 2). In Finland, the VE in weeks 48 and 49 was estimated at 49% (95% CI: 34–60%) and 47% (95% CI: 36–56%) (Figure 2). In the following weeks, VE dropped to the current (week 2) estimate of 32% (95% CI: 27–37) (Figure 2, Table 2). There was no significant difference when comparing VE of individuals considered vaccinated from day 1 after vaccination or considered vaccinated from day 15 after vaccination, with the unvaccinated as a reference (Table 2).

A sensitivity analysis revealed that VE for 'being vaccinated for 1 to 7 days' was‹0% (95% CI: ‹0–15%) in Stockholm and 17% (95% CI: −5 to 35%) in Finland, while VE for 'being vaccinated for 8 to 14 days' was 30% (95% CI: 4–49) and 37% (95% CI: 22–49) in Stockholm and Finland, respectively. VE for a later time after vaccination seemed more or less stable during the study period (Table 3, Figure 2 panels C and D). In Finland, VE estimates started to decrease when the exposure to SIV was 45 days or more in the past (Table 3). However, an exact evaluation of the onset of declining VE was not done. The Stockholm VE estimates were generally lower and characterised by broad confidence intervals because of small case numbers. In Stockholm, but not in Finland, the VE in persons older than 75 years were much lower than those aged 65–74 years (data not shown).

Genetic analyses

Characterisation of influenza A(H3N2) samples from Sweden and Finland showed that all viruses belonged to subclades 3C.2a or 3C.2a1, which are both considered to be antigenically similar to the vaccine strain A/Hong Kong/4801/2014 [7]. In total 158 influenza A(H3N2) viruses were sequenced, 121 from unvaccinated and 37 from vaccinated patients. The proportion of viruses belonging to subclade 3C.2a1 (n=95) increased during the study period from 38% to 73% (Figures 3 and 4). In addition, the amino acid substitutions T135K and G479E in the HA1 and HA2 part of the haemagglutinin were determined in 58 of the 95 subclade 3C.2a1 viruses (Figure 4). Twenty-five of the 95 3C.2a1 viruses and 16 of the 58 viruses with the T135K and G479E substitutions were from vaccinated patients. Among the 63 viruses in subclade 3C.2a, 12 were samples from vaccinated persons. Nine of these 12 samples had the additional amino acid substitutions T131K, R142K and R261Q in HA1. All sequences have been uploaded to the Global Initiative on Sharing All Influenza Data (GISAID) EpiFlu database (Figure 4).

Table 2

Vaccine effectiveness estimates for seasonal influenza vaccination on laboratory-confirmed influenza in persons 65 years and older, Stockholm and Finland, 1 October 2016–15 January 2017 (n =358,583 and 1,144,894, respectively)

	Cases	Person-years	Population[a]	Crude hazard rate ratio (95% CI)	Adjusted hazard rate ratio (95% CI)	Vaccine effectiveness % (95% CI)
Stockholm County, Sweden[b]						
Unvaccinated	654	83,263	201,113	Ref	Ref	Ref
Vaccinated for 1 day or more[c]	380	20,736	157,470	0.90 (0.79–1.03)	0.72 (0.63–0.89)	28 (16–37)
Vaccinated for 15 days or more	322	14,345	153,762	0.94 (0.82–1.08)	0.76 (0.65–0.89)	24 (11–35)
Finland[d]						
Unvaccinated	3,674[e]	247,456	613,202	Ref	Ref	Ref
Vaccinated for 1 day or more[c]	2,171	85,674	531,692	0.73 (070–0.77)	0.68 (0.64–0.73)	32 (27–37)
Vaccinated for 15 days or more	2,006	65,357	527,664	0.73 (0.70–0.78)	0.67 (0.63–0.72)	33 (28–38)

CI: confidence interval.

[a] By vaccination status at the end of each individual's follow-up.

[b] Models were adjusted for age, sex, comorbidity status, socioeconomic status, previous seasonal vaccination and pneumococcal vaccination. As complete case analysis was used, the number of cases decreased due to missing data on socioeconomic status.

[c] The sensitivity analysis showed that there was no protection during the first week after vaccination, but that a significant vaccine effectiveness could be observed already during days 8 to 14 (see text).

[d] Models were adjusted for age, sex and previous seasonal vaccination.

[e] The number of vaccinated/unvaccinated differs from Table 1 because 384 people were vaccinated after having a laboratory-confirmed influenza. For Table 2, they are counted as unvaccinated and then their follow-up was stopped because they turned out to be a case.

Table 4 lists all reference sequences retrieved from GISAID for the phylogenetic analysis. The authors gratefully acknowledge the originating and submitting laboratories who contributed sequences were used in this study.

Discussion

Annual vaccination against circulating influenza viruses remains the best strategy for preventing influenza illness. However, VE varies widely and in some seasons the protection of especially older persons and other medical risk groups may be very low or even non-existing, particularly in seasons dominated by influenza A(H3N2) [1,8]. In addition, VE estimates in a given season may differ depending on whether the analysis is performed early/mid-season or at the end of the season, in most cases resulting in lower end-of-season estimates [8,9]. A decrease in VE observed within a season may be due to a change in the circulating virus such as the introduction of a new clade of influenza A(H3N2) during the 2014/15 season [10], to the egg-adaptation of the vaccine influenza A(H3N2) strain [11], or to waning immunity over time [12,13]. Rapid feedback on the impact of SIV is therefore important, as it may help guide the outbreak response.

In our current study, both Stockholm and Finland noted moderately high VE of ca 50% against LCI (predominantly influenza A(H3N2)) in persons 65 years and older, 4 to 5 weeks after the start of the epidemic at the beginning of November which coincided with the start of the SIV campaigns in both countries. However, during the following four weeks, VE declined steeply and was only around 30% by week 2, 2017, in both Stockholm and Finland. Since then and up to week 6, VE has remained stable in both places (data not shown). While the reasons for this observation remain unknown, it seems unlikely that the early decline occurred because the vaccination campaigns were started too late, since the highest VEs were observed early in the season. The sensitivity analysis showed that although there was no protection during the first week after vaccination, a significant VE was observed already during days 8 to 14. Thus, VE was similar irrespective of whether we considered events and person-time accumulating during the first 14 days after vaccination as vaccinated, or whether we excluded them from the analysis. A majority of the study population had been vaccinated also in the previous year and they may therefore have had a rapid immune response to SIV and have been protected earlier than 14 days after vaccination. We believe that it is more correct to either consider persons vaccinated from day 1 or 8 after vaccination or exclude them from the analysis, than to include them in the non-vaccinated group, which results in misclassification biasing the VE estimates towards zero.

During the study period, the proportion of samples in subclade 3C.2a1 increased and in total 60% (95/158) of the viruses were in this subclade. The majority, 25/37 (68%), of the genetically characterised samples from vaccinated patients belonged to subclade 3C.2a1 and 16 of those had the additional amino acid substitution T135K. This mutation is located in a conserved element

Table 3

Vaccine effectiveness estimates for seasonal influenza vaccination on laboratory-confirmed influenza in persons 65 years and older, by time since vaccination, Stockholm and Finland, 1 October 2016–15 January 2017 (n = 358,583 and 1,144,894, respectively)

	Cases	Person-years	Crude hazard rate ratio (95% CI)	Adjusted hazard rate ratio (95% CI)	Vaccine effectiveness % (95% CI)
Stockholm County, Sweden[a]					
Unvaccinated	654	83,263	Ref	Ref	Ref
Vaccinated for 1–14 days[b]	58	5,960	0.84 (0.65–1.17)	0.69 (0.53–0.91)	31 (9–47)
Vaccinated for 15–29 days	132	6,167	0.96 (0.79–1.23)	0.78 (0.64–0.95)	22 (5–36)
Vaccinated for 30–44 days	130	5,149	0.97 (0.80–1.17)	0.78 (0.64–0.95)	22 (5–36)
Vaccinated for 45–89 days	59	3,027	0.79 (0.58–1.07)	0.65 (0.48–0.89)	35 (11–52)
Vaccinated for 90 days or more	1	1	NA	NA	NA
Finland[c]					
Unvaccinated	3,674	247,456	Ref	Ref	Ref
Vaccinated for 1–14 days[b]	165	20,317	0.74 (0.63–0.87)	0.71 (0.60–0.83)	30 (17–40)
Vaccinated for 15–29 days	369	21,529	0.63 (0.56–0.70)	0.60 (0.53–0.67)	40 (33–47)
Vaccinated for 30–44 days	675	20,641	0.63 (0.58–0.69)	0.59 (0.54–0.65)	41 (35–46)
Vaccinated for 45–89 days	957	23,107	0.89 (0.83–0.96)	0.80 (0.74–0.88)	20 (12–26)
Vaccinated for 90 days or more	5	80	2.11 (0.87–5.08)	1.69 (0.70–4.08)	−69 (−308 to 30)

CI: confidence interval; NA: not applicable.

[a] Models were adjusted for age, sex, comorbidity status, socioeconomic status, previous seasonal vaccination and pneumococcal vaccination. As complete case analysis was used, the number of cases decreased due to missing data on socioeconomic status.

[b] The sensitivity analysis showed that there was no protection during the first week after vaccination, but that a significant vaccine effectiveness could be observed already during days 8 to 14 (see text).

[c] Models were adjusted for age, sex and previous seasonal vaccination.

of the receptor-binding site in the antigenic epitope A and causes a loss of the glycosylation motif [14]. Amino acid 135 is conserved in 62% of all human H1, H2 and H3 viruses [15]. In total 12/63 (19%) of the viruses in subclade 3C.2a were samples from vaccinated persons and nine of these had the additional amino acid substitutions T131K, R142K and R261Q. Both the T131K and the R142K substitution are located in the antigenic epitope A and T131 is conserved in 45% of all human H1, H2 and H3 viruses [15]. In a study from Canada which included all age groups and reported a higher adjusted interim VE of 42% for 2016/17, 80% of the characterised influenza A(H3N2) samples belonged to subclade 3C2a1 and 19% to 3C.2a [16]. Only 19% of the 3C.2a1 samples had the T135K mutation and 74% of the 3C.2a samples had the T131K mutation. In total 29% of the characterised samples in Canada had T135K or T131K, while in our study, one of these two alterations was detected in 54% of all samples, and in 68% of the samples collected from vaccinated patients. The characterised samples were not randomly selected to be representative for vaccinated and unvaccinated persons or different time periods, and it remains to be investigated whether these specific substitutions alter the antigen similarity to the influenza A(H3N2) vaccine strain A/Hong Kong/4801/2014.

A study by Kissling et al. [8] about pooled-season VE against influenza A(H3N2) in persons 60 years and older during the seasons 2011/12 to 2014/15, showed that VE reached a peak of 44.6% at day 45 after vaccination and then gradually declined to 0% at day 140. In contrast, we found a very early VE peak of around 50% and then a rapid decline to a fairly stable low level of around 30% during the remaining study period, which for most individuals was less than 60 days after vaccination, and also during the four weeks after the end of the study period.

The sensitivity analyses, where time since vaccination was taken into account, could not demonstrate a clear gradual decline, neither by calendar week nor by time since vaccination. We do not have a satisfactory explanation for these observations; the antigenic change, discussed above, or a chance finding are two of several possibilities. As more LCI cases are observed, the power of the study increases and the estimates will become more accurate. Since the confidence intervals of the weekly overall VE estimates are now overlapping, the observed decline is not statistically significant. We will revisit this issue with more cases and follow-up time in the end-season analysis.

However, the generally low VE is probably at least partly a result of the poor immunogenicity of the present SIV in older persons, especially for influenza A(H3N2) [17]. Increased use of adjuvanted vaccines and the introduction of high-dose vaccines in Europe should therefore be considered [18]. In a large randomised placebo-controlled study of persons 65 years of age and older,

Table 4

Details of the influenza A(H3N2) reference sequences retrieved from the Global Initiative on Sharing Avian Influenza Data (GISAID)'s EpiFlu database for phylogenetic analysis of HA1 in this study

Isolate name	Segment ID	Country	Originating laboratory	Submitting laboratory
A/Perth/16/2009	EPI211334	Australia	WHO Collaborating Centre for Reference and Research on Influenza	Centers for Disease Control and Prevention
A/Stockholm/18/2011	EPI326139	Sweden	Swedish Institute for Infectious Disease Control	National Institute for Medical Research
A/AthensGR/112/2012	EPI358885	Greece	Hellenic Pasteur Institute	National Institute for Medical Research
A/Missouri/17/2016	EPI827323	United States	Missouri Department. of Health & Senior Services	Centers for Disease Control and Prevention
A/Switzerland/9715293/2013	EPI530687	Switzerland	Hopital Cantonal Universitaire de Geneves	National Institute for Medical Research
A/New York/83/2016	EPI827354	United States	New York State Department of Health	Centers for Disease Control and Prevention
A/Netherlands/525/2014	EPI574644	The Netherlands	National Institute for Public Health and the Environment (RIVM)	National Institute for Medical Research
A/Samara/73/2013	EPI460558	Russian Federation	WHO National Influenza Centre Russian Federation	National Institute for Medical Research
A/HongKong/146/2013	EPI426061	Hong Kong (SAR)	Government Virus Unit	National Institute for Medical Research
A/Texas/50/2012	EPI391247	United States	Texas Department of State Health Services-Laboratory Services	Centers for Disease Control and Prevention
A/Victoria/361/2011	EPI349106	Australia	Melbourne Pathology	WHO Collaborating Centre for Reference and Research on Influenza
A/South Africa/VW0073/2016	EPI829365	South Africa	Sandringham, National Institute for Communicable D	Crick Worldwide Influenza Centre
A/Moscow/135/2016	EPI781640	Russian Federation	Ivanovsky Research Institute of Virology RAMS	Crick Worldwide Influenza Centre
A/HongKong/4801/2014	EPI539576	Hong Kong (SAR)	Government Virus Unit	National Institute for Medical Research
A/Antsirabe/2047/2016	EPI824058	Madagascar	Institut Pasteur de Madagascar	Crick Worldwide Influenza Centre
A/CoteD'Ivoire/697/2016	EPI781616	Cote d'Ivoire	Pasteur Institut of Côte d'Ivoire	Crick Worldwide Influenza Centre
A/Bolzano/7/2016	EPI773595	Italy	Istituto Superiore di Sanità	Crick Worldwide Influenza Centre
A/Slovenia/3188/2015	EPI699750	Slovenia	Laboratory for Virology, National Institute of Public Health	Crick Worldwide Influenza Centre
A/Kazakhstan/4700/2016	EPI781622	Kazakhstan	National Reference Laboratory	Crick Worldwide Influenza Centre
A/Scotland/63440583/2016	EPI831436	United Kingdom	Gart Naval General Hospital	Microbiology Services Colindale, Public Health England
A/Norway/3806/2016	EPI829343	Norway	WHO National Influenza Centre	Crick Worldwide Influenza Centre

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a high-dose influenza vaccine was 24% more efficacious in the prevention of influenza compared with a standard trivalent vaccine (TIV) [19]. Similarly, in a randomised study in persons 65 years and older, influenza vaccine adjuvanted with MF-59 induced significantly higher antibody response, especially against influenza A(H3N2), than ordinary TIV [20].

A major limitation of this study was the inability to control for healthcare-seeking behaviour and sampling biases. If vaccinated persons with ILI seek healthcare more often and are more likely to be swabbed by doctors than unvaccinated persons, this would underestimate VE, and vice versa. However, we believe this risk to be low in this older age group of patients, most of whom had signs of severe influenza, because nearly all patients in Stockholm and a large part of the patients in Finland were sampled in the hospital setting and treated as inpatients. Elderly persons who are ill enough to seek hospital care because of an

infection will do that irrespective of their vaccination status. In the hospital setting, sampling for influenza is performed not only in order to establish a diagnosis and determine the correct treatment, but also because an LCI will mean that the patient can be treated in a cohort with other influenza patients which can prevent the spread of influenza in the hospital. In order to fully understand the dynamics of VE during an influenza season, a more detailed cohort study addressing the potential sources of bias is warranted. Also, a small amount of vaccine exposure misclassification cannot be fully excluded. The Finnish NVR does not cover vaccinations given in the private sector and the number of SIV doses missed by the NVR remains unknown, although it is expected to be a negligible number compared with the ones registered.

The strength of our study is that by using the same PIN to link vaccination, laboratory and diagnostic registers, we could study VE in real time for all inhabitants in two large geographic areas, and that the large number of LCI cases made it possible to perform and communicate an early estimate of VE. The fact that the two sites detected the same signal at the same time and that the evolution of the VE over time followed the same pattern at both sites lends further credibility to our results.

Irrespective of the cause, the low VE in older persons in this interim estimate has had implications for healthcare in Stockholm County and Finland: early antiviral therapy was recommended for ILI and SARI in risk groups, irrespective of vaccine status. Also, to keep the momentum for SIV compliance and provide a better fit, the World Health Organization (WHO) vaccine strain selection committee needs timely evidence for their decision on the composition of the vaccine for the following influenza season. Finally, our study indicates that it is possible to deliver real-time VE estimates by population-based register linkage, although further methodological analyses are needed to understand the potential confounders.

*Author's correction:

The x-axes in Figure 2 were mistakenly marked 2015/16 instead of 2016/17. This mistake was corrected on 1 March 2017 on request of the authors.

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Conflict of interest

None declared.

Authors' contributions

Maria-Pia Hergens conducted the data management and statistical analysis on the Swedish side, participated in the study's design and in writing and revising the paper. Ulrike Baum conducted the data management and statistical analysis on the Finnish side, participated in the study's design and in writing and revising the paper. Mia Brytting conducted genetic analysis on the Swedish side, participated in writing and revising the paper. Niina Ikonen conducted genetic analysis on the Finnish side, participated in writing and revising the paper. Anu Haveri conducted genetic analysis on the Finnish side, participated in writing and revising the paper. Åsa Wiman conducted genetic analysis on the Swedish side, participated in writing and revising the paper. Hanna Nohynek conceptualised the paper, participated in the analysis of the data and writing and reviewing of the paper. Åke Örtqvist conceptualised the paper, participated in the analysis of the data and writing and reviewing of the paper.

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