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Special edition:

25 years of public health impact

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Editorial team

Based at the European Centre for
Disease Prevention and Control (ECDC),
169 73 Stockholm, Sweden

Telephone number

+46 (0)8 58 60 11 38

E-mail

eurosurveillance@ecdc.europa.eu

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Eurosurveillance editorial team[1]
1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

Correspondence: Eurosurveillance editorial team (eurosurveillance@ecdc.europa.eu)

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The scientific community's interest in communicable diseases gained momentum during the last two decades of the 20th century, following the emergence of HIV and the re-emergence of (drug-resistant) tuberculosis, as well as a general increase in mortality and morbidity from infectious diseases [1]. In the mid-1990s, a number of initiatives were established in Europe that comprised, among others, the European Union (EU) dedicated surveillance networks and field epidemiology training programmes. In this context, there was an evident need for an exchange of information on results from the surveillance activities, on emerging public health threats and on the detection and response to outbreaks to support public health action and decision-making.

The monthly *Eurosurveillance* journal, created in 1995 and fully operational since 1996, was set up as a platform for outputs from the EU surveillance networks and to disseminate national experiences that other countries of the EU may find useful [2]. To complement the monthly journal, the *Eurosurveillance* weekly bulletin was created in 1996. It contained short edited news-like articles on outbreaks and important infectious disease events globally, reviewed by editors and their colleagues, with a European focus. The weekly and monthly editions were merged into today's Eurosurveillance in 2008 to capitalise on the strengths of the two journals [3]. In particular the short articles have, over time, developed into a source of sound scientific information that has helped shape public health response and policies at local, national and international level.

Being at the forefront of establishing rapid information exchange as an accepted means of peer-reviewed, scholarly communication has been an important pillar of the journal's impact. Over the years, a small but dedicated team – supported by a pan-European editorial board and working closely with many scientists and public health experts – has established the journal firmly among the prestigious journals in the field of infectious diseases. *Eurosurveillance* has ranked among the top 10 in its category for 10 years in the Journal Citation Reports impact factor and other metrics (SCImago Journal Rank, Scopus CiteScore, Google Scholar) have also been good. We take the combination of anecdotal/narrative evidence (see examples in our anniversary collection), the feedback from formal evaluations [4] and good metrics as an indication of the journal's public health impact during its 25 years of existence.

An increasing number of scholarly journals have implemented fast-tracked, peer-reviewed publishing. Together with the increased use and acceptance of preprint servers, this has facilitated important early information exchange and informed public health decision-making. The urgent need for evidence during the ongoing coronavirus disease (COVID-19) pandemic has accelerated such initiatives and led to profound changes in scholarly publishing. We continue to follow these developments closely, weighing which would be of benefit to our readers and contributors and which we would approach more conservatively.

In 2021, *Eurosurveillance* has celebrated its 25th anniversary, prompting us to look backwards as well as forwards. Looking to the future, we have identified a number of areas that will guide our operations in the coming years, while we will of course remain a fully open-access and non-profit journal. Aside from the speed of our rapid communications, quality and correctness of content and clear public health messages have been our focus. We believe and have evidence to suggest that we have gained the trust of our audience and that articles published in the journal are considered to provide sound and reliable scientific information [4,5]. Quality control (peer review, evaluation and editing), however, has come with a certain cost with regards to

the time to publication for longer articles. In the future, both speed and authoritativeness are key features of the journal that we wish to preserve and strengthen further. We also plan to work with our authors to tease out clear public health messages even more than in the past. The application of classical infectious disease epidemiological methods, in combination with public health microbiology in outbreak investigations and surveillance, has increasingly been established as a principle and has been reflected in many articles over the years. The growing complexity of our environment and the continuous development of novel methods in other disciplines that can be applied for infectious disease prevention and control, necessitates an even stronger focus on interdisciplinary aspects in the articles we select for review. Taking a gradual approach, we will address this through annual themes and subthemes that should help guide the selection of attractive and relevant articles for our audience.

We remain vigilant to new developments in publishing and we are attentive to our core mission. We will carry on supporting public health practice and policymaking through sharing knowledge and authoritative evidence. Sustainability and diversity will be important guiding principles for our work. They should help us make an impact on public health also in the future and, as in the past, we count on the support of our peer-reviewers, board members and colleagues to reach this goal.

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Preliminary report of an international outbreak of Salmonella anatum infection linked to an infant formula milk

International Investigation Collaborating Units[1]

1. Public Health Laboratory Service (PHLS) Laboratory of Enteric Pathogens, PHLS Communicable Disease Surveillance Centre, PHLS Statistics Unit, Scottish Centre for Infection and Environmental Health, Glasgow, Scottish Salmonella Reference Laboratory, Glasgow, Réseau National de Santé Publique, Paris, Centre National de Référence des Salmonella et Shigella, Institut Pasteur, Paris, Direction Générale de l'Alimentation, Paris, Direction Générale de la Concurrence de la Consommation et de Répression des Fraudes, Paris, Institute for Hygiene and Epidemiology, Brussels, European Union Salm-Net surveillance network (EU concerted action funded by DG XII within the BIOMED programme)

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The number of isolates of *Salmonella anatum* from infants (aged 1 to 11 months) in England and Wales was higher than expected in November and December 1996 and early January. The Public Health Laboratory Service (PHLS) Laboratory of Enteric Pathogens (LEP) recognised the increase and an investigation began in late January 1997.

Introduction

The number of isolates of *Salmonella anatum* from infants (aged 1 to 11 months) in England and Wales was higher than expected in November and December 1996 and early January. The Public Health Laboratory Service (PHLS) Laboratory of Enteric Pathogens (LEP) recognised the increase and an investigation began in late January 1997. Initially, 12 cases of *S. anatum* infection in infants in the United Kingdom (UK) were identified by the PHLS Communicable Disease Surveillance Centre (CDSC) and the Scottish Centre for Infection and Environmental Health (SCIEH), eight in England and four in Scotland. The age of these cases directed suspicion at baby food as a possible vehicle. Salm-Net's international salmonella surveillance database was examined and national collaborators in all participating European countries were asked about recent cases in their countries.

Methods

Within 24 hours of the increase being recognised, a case control study began. It was conducted over a 48 hour period in England and Scotland to test the hypothesis that recent *S. anatum* infections in infants were associated with consumption of a particular baby food. Mothers or general practitioners of cases nominated age and neighbourhood matched control infants. None of the control households with which contact was made refused to participate. Mothers of the first 12 cases ascertained (whose ages ranged 2 to 8 months, mean 4.9 months) and 40 control infants (age range 1 to 9 months, mean 4.6 months) were interviewed by telephone by one of two interviewers from CDSC and SCIEH. A standardised questionnaire was used to record details of the foods and liquids fed to each of the case infants in the three days before they became ill and to the control infants in the three days before the interview.

Parents of all infant cases of *S. anatum* since 30 October 1996, including those in the case control study, were interviewed to establish the onset dates and duration of illness, whether their children had been admitted to hospital, and the types of food and liquids consumed before becoming ill.

Isolates of *S. anatum* from 39 recent cases in the UK, including the 12 in the case control study, were studied by plasmid profile analysis and by pulsed-field gel electrophoresis (PFGE) at LEP. Isolates of *S. anatum* from France were also studied.

Results

The case control study showed that illness was strongly associated with consumption of a particular infant formula milk. Ten of the 12 cases were reported to have been fed this product compared with three of the 40 control infants (odds ratio 62, $p<10^{-6}$). No other risk factors were associated with illness.

By 10 February the investigation had identified 22 cases of *S. anatum* infection in infants from September

Table 1

Recent Salmonella anatum isolates reported to Salm-Net

Country	Period when isolates received	Total number	N° of infants isolates
Germany	Oct 1996 - Jan 1997	7	0
England	Oct 1996 - Jan 1997	21	13
Austria	Oct 1996 - Jan 1997	3	0
Belgium	Oct 1996 - Jan 1997	5	1
Denmark	1996	9	0
Scotland	Oct 1996 - Jan 1997	6	4
Spain	Oct 1996 - Jan 1997	4	0
Finland	1996	27	0
France	Sept 1996 - Jan 1997	18	4
Ireland	1996	1	0
Italy	1996	22	0
Norway	1996	7	0
Netherlands	Oct 1996 - Jan 1997	3	0
Portugal	1996	1	0
Sweden	1996	8	0
Switzerland	Oct 1996 - Jan 1997	1	0

1996 to January 1997 in four different countries, 13 in England, 4 in Scotland, 4 in France, and one in Belgium (table 1). No deaths were reported. The increase in cases reported in infants continued through December 1996 and January 1997 (figure 1).

The 17 cases in England and Scotland were aged between 1 and 7 months at the time of their illness and 15 of the cases had been fed the implicated infant milk formula. The average duration of their illness was 20 days, and four cases were admitted to hospital.

In France, the mothers of the four cases identified were interviewed using the same questionnaire as used in the UK. Three cases had gastroenteritis and one was asymptomatic. None was admitted to hospital. Dates of isolation of *S. anatum* ranged from the beginning of September 1996 to the end of January 1997 (figure 1) and the age of the cases from 4 to 7 months. The cases were not clustered geographically. Two of the cases had consumed an infant formula milk made by the same manufacturer at the same plant as the product implicated in the UK cases. The asymptomatic case, who had been screened for salmonella because of the occurrence of *S. typhimurium* infection in a 6 year old sibling, had been fed a different product made by the same manufacturer. The fourth case had not received any product from this manufacturer.

In Belgium, the mother of the case was also interviewed using the same questionnaire, but it transpired that the illness had begun in Italy and the mother and infant had returned before further details could be obtained.

Microbiological results

Molecular analysis of the UK isolates showed that nine of the 12 isolates from infants in the case control study possessed a single plasmid of about 50 megadaltons (MDa) and one had this plasmid plus two additional plasmids (40 and 45 MDa). All 10 infants had consumed the implicated product. The two isolates from infants who were not fed the implicated product were plasmid free.

Other isolates of *S. anatum* in England and Wales were also examined; 10 from infants during 1994 and 17 from adults and infants from January 1996 through January 1997. Four different plasmid profiles were identified in the isolates from 1994, none of which resembled the pattern found in the isolates associated with the implicated product. Five of the 17 isolates from 1996/97 had the single 50 MDa plasmid; three of these isolates were from infants who had been fed the implicated product, the others were from a 3 year old sibling and a mother of infants who had been fed the implicated product. Eight different plasmid profiles were identified in the remaining 12 isolates.

Five isolates of *S. anatum* from infants in the case control study that possessed the 50 MDa plasmid were studied by PFGE. All had an identical macrorestriction DNA fingerprint. The isolate with the two additional plasmids differed only in having an extra DNA fragment of about 40 kilobase pairs. Five of the 12 other isolates from 1996/97 examined by PFGE had distinct DNA fingerprints, which differed from each other and from isolates from the case control study. From these results it was concluded that the outbreak was caused by a single strain defined by DNA fingerprinting.

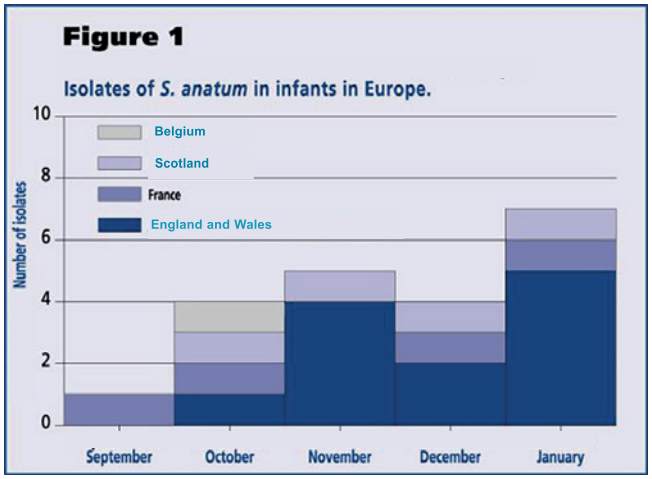
Four isolates of *S. anatum* from infants in France were sent to LEP for molecular analysis, of which results are available for three isolates. One was plasmid-free and two possessed a plasmid of about 50 MDa; one of these isolates also possessed an additional plasmid of 70 MDa coding for resistance to ampicillin and sulphonamides. When studied by PFGE the two plasmid-carrying isolates had pulsed-field profiles corresponding to that of the UK epidemic strain whereas the plasmid-free strain had a completely different pulsed-field profile. The two plasmid-carrying isolates were from cases identified in September and October who had consumed infant formula milk from the same plant as the UK cases.

Control measures

The infant formula milk implicated was produced in a factory in France using dried milk that may have originated from either of two spray drying plants, one in France and the other in the Netherlands. This factory produces a range of baby foods for export to different countries in addition to the implicated formula milk that is specially formulated for the UK market.

Figure 1

Isolates of *S. anatum* in infants in Europe.



On 24 January 1997 the implicated product was withdrawn from sale in the UK, a public warning was issued, and agencies in other European Union countries were informed through the " rapid exchange of information " system.

In France, *S. anatum* has not been detected by inspection of routine product samples or samples taken by inspection authorities in the past 15 months of production and from the factory›s environment. Nevertheless, the factory has been closed for cleaning. It was established that the implicated formula milk in France and the UK had been produced from the same batch of raw materials. On 7 February 1997 the infant formula milk produced from this batch in the French factory was withdrawn from distribution in France.

Conclusion

The results of the molecular fingerprinting of the *S. anatum* isolates, the case control study, and the food consumption histories of other cases together provide overwhelming evidence that a particular infant formula milk, manufactured in France, was the source of an outbreak of *S. anatum* infection in infants in late 1996 and early 1997. Active surveillance of cases of *S. anatum* infection in infants in the European Union is continuing. The company and food safety authorities are conducting investigations that aim to reveal the primary cause of the contamination.

Last minute note

S. anatum of the same plasmid profil as the epidemic strain has been isolated from an unopened sachet of the formula milk retrieved from the home of a recent case. The result of PFGE are awaited.

Travel associated legionellosis among European tourists in Spain

A Galmés Truyols[1] , J. F. Martinez Navarro[2]
1. Field Epidemiology Training Programme (FETP) 1995-97
2. National Centre for Epidemiology, FETP, Madrid, Spain

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Introduction

Travel associated Legionnaires' disease has caused concern among European countries since the second half of the 1980s because of the morbidity among citizens of the European Union and because of the threat posed to the economies of the Mediterranean countries by the occurrence of the disease among tourists. As a result, the European Working Group for Legionella Infections (EWGLI) (1) was set up in 1986 coordinated by the National Bacteriology Laboratory in Stockholm until 1993 when this role was transferred to the Public Health Laboratory Service Communicable Disease Surveillance Centre in London. Case reports are sent from patients' countries of residence to countries they have visited.

EWGLI has developed a surveillance scheme based on a computer software program, the European Legionellosis Surveillance Scheme (ELSS). Monthly updates are sent to all collaborators of all available data from throughout Europe since 1987 (2). The aim of this study was to analyse data covering cases of legionellosis associated with travel to Spain, including the Balearic and Canary islands.

Method

An Epi Info analysis was run on the ELSS program database, updated as of 31 December 1995. The numbers of travellers who had arrived from other European countries and stayed in tourist accommodation in Spain were obtained from the Spanish National Statistics Office (INE) (3,4) and used as denominators to calculate rates.

The ELSS program contains two interrelated databases: each record on one database consists of a single accommodation address for a given patient during the incubation period, so that for any one case there are as many records as there are accommodation addresses during a specific trip; the second database contains information about individual patients, with one record per case. These two databases can be linked and cross-referenced using a case-ID field. For the purposes of analysis, Dbase III Plus and Epi Info 6.01 software packages were used. Since all cases reported in Europe are pooled in the same databases, the first task was to separate patients who had travelled to Spain from those who had been to other European destinations. The second step was to code regions (using the designated Spanish Autonomous Region codes) and accommodation addresses.

Hotels and holiday apartments in Spain have a similar structure and management and so we studied both together.

The duration of stay in Spain was calculated for all patients, as were the periods between arrival in Spain and the onset of symptoms and between return to country of permanent residence and the onset of symptoms. These periods were calculated for all hotel stays in the case of travellers who had stayed in more than one hotel.

The incubation period for legionellosis was taken as two to 10 days (5). The disease was said to be confirmed if any legionella was cultured or if a fourfold rise in the titre of antibodies against *Legionella pneumophila* sg1 titre was observed. A presumptive diagnosis

Table 1

Legionellosis among European tourists in Spain, 1987-1995. Distribution by age and sex.

	Sex			
Age group	Male	Female	Unknown	Total number (%)
0 - 24	0	3	0	3 (1)
25 - 44	29	16	0	45 (16)
45 - 64	104	37	1	142 (51)
› 64	58	24	0	82 (29)
Unknown	3	1	5	9 (3)
TOTAL	194 (69)	81 (29)	6 (2)	281 (100)

Figure 1

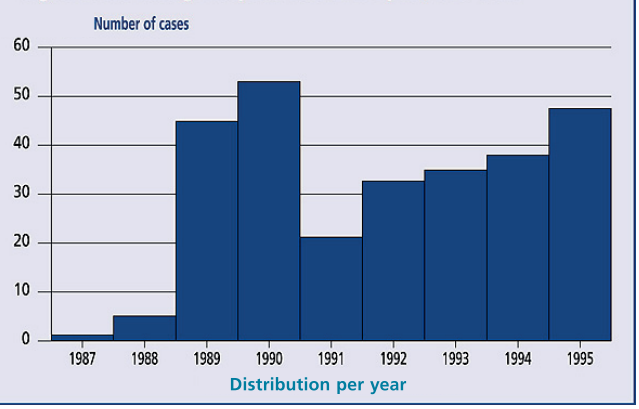


Figure 2

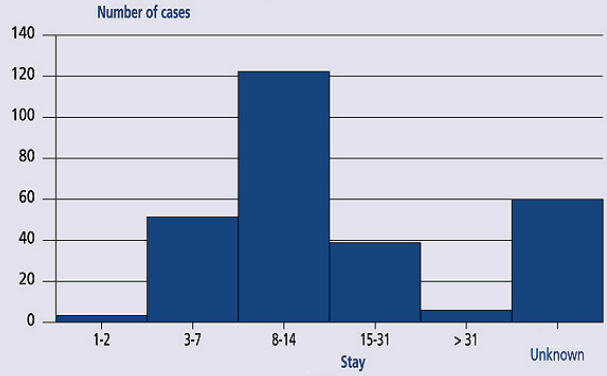
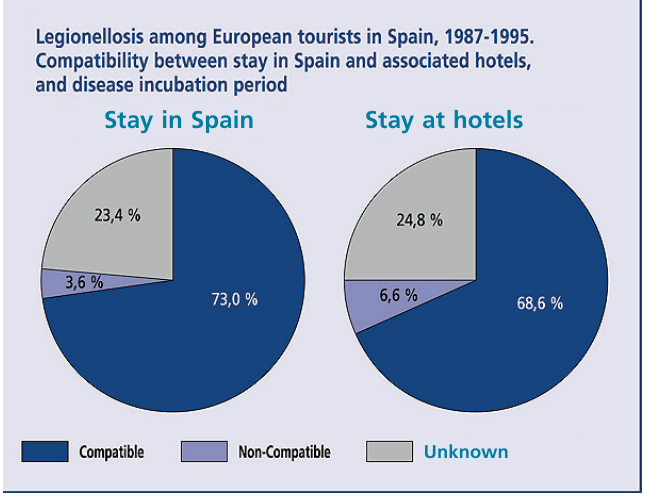


Figure 3



of *L. pneumophila* sg1 infection was made if the case was diagnosed on the basis of a single high antibody titre or if another method was used, that is the word "other" appeared in the report of the case. Infections with all other *Legionella* species or serotypes were regarded as presumptive unless diagnosed by culture. The criterion chosen in this study to define case-clustering in any one hotel was the appearance of more than one case in the same calendar year or the appearance of a single case in two or more successive years.

Results

A total of 281 cases were reported from 1987 to 1995. In 1995 two duplicate cases (repetition of case ID codes) were eliminated. Men accounted for 69% of cases overall, 54% of whom were aged 45 to 64 years. Women accounted for 29% of cases, 46% of whom were aged 45 to 64 years, the most numerous group (table 1). The sex of 2% of cases was unknown.

The largest number of cases (53) was reported in 1990 (figure 1). The average of 40 cases were reported each year from 1989 to 1995. Only six cases had been registered before 1989.

The diagnosis of 154 cases (55%) was confirmed by culture or fourfold rise in antibody titre and presumed in 121 cases (43%). The method of diagnosis was unknown in 6 cases (2.1%).

When reported, 21 cases were ill (7%), 123 (44%) had recovered, 28 (10%) had died, and no data were available on the health status of the remaining 109 (39%). Date of onset of symptoms was known for 265 (94%) cases. Length of stay in Spain was unknown in 60 cases (21%) and less than three days in three cases (figure 2). In 66 cases (23%), analysis of the dates of symptom onset and dates of stay in Spain failed to show whether the patient had been in Spain during the incubation period of the disease. Data on a further 10 yielded periods of time incompatible with having acquired infection in Spain: five having become ill too soon after arrival in Spain and five too long after returning home or reaching another destination.

The 281 cases had stayed at a total of 303 hotels. Two hundred and eight (69%) such stays occurred within the likely incubation periods, 20 stays occurred outside

Table 2

Legionellosis among European tourists in Spain, 1987-1995. Associated hotels broken down by Autonomous Region.

Autonomous Region	Cases	Hotels		
		Total	Associated with more than one case	Clustering criteria met (1)
Balearic Isles	103	60	19	17
Catalonia	55	33	9	9
Valencian Region	39	26	9	6
Canary Islands	33	27	4	3
Andalousie	34	32	6	6
Rest of Spain	4	9	2	2
Other (2)	10	-	-	-
Unknown	3	-	-	-
TOTAL	281	186	49	43

(1) More than one case in any one year or a single case in two or more successive years.
(2) Visited more than one region, without it being possible to ascertain in which infection took place.

Table 3

Legionellosis among European tourists in Spain. Cases and annual mean rates by Autonomous Region, 1989-1995

		Annual mean rate	
	Cas / Cases	(cases per 100 000 European tourists)	
Autonomous Region		Rates	95% C.I.
Valencian Region	37	0.85	(0-2.86)
Balearic Isles	101	0.50	(0.03-1.12)
Catalonia	55	0.35	(0-1.00)
Canary Islands	32	0.32	(0-1.16)
Andalousie	33	0.31	(0-1.11)
Rest of Spain	4	0.03	(0-0.51)
Other (1)	10		
Unknown	3		
TOTAL	275	0.38	(0.16-0.65)

(1) Visited more than one region.

Table 4

Legionellosis among European tourists in Spain. Cases and annual mean rates by country of origin, 1989-1995

		Annual mean rate	
	Number of cases	(cases per 100 000 European tourists)	
Country of origin		Rates	I.C. 95% / 95% C.I.
Sweden	31	2.52	(0-9.20)
Denmark	15	1.60	(0-8.89)
United Kingdom	184	1.12	(0.33-2.10)
Holland	24	0.94	(0-3.96)
Norway	7	0.73	(0-13.99)
Germany	9	0.06	(0-0.38)
Other	5	0.01	(0-0.20)
TOTAL	275	0.28	(0.16-0.65)

Table 5

Legionellosis among European tourists in Spain. Rates per 100 000 visitors, by country of origin and region visited, 1989-1995

		Sweden	Denmark	United Kingdom	Holland	Norway	Germany
Valencian region	Rates	-	-	2.01	1.77	-	-
	I.C. 95% / 95% C.I.	-	-	0-7.02	0-33.70	-	-
Balearic Isles	Rates	6.32	2.77	1.12	0.25	10.34	0.07
	I.C. 95% / 95% C.I.	0-42.99	0-26.17	0-2.8	0-13.11	0-127.40	0-0.79
Catalonia	Rates	2.03	0.41	2.07	1.69	1.34	-
	I.C. 95% / 95% C.I.	0-21.61	0-21.40	0-7.46	0-9.39	0-70.04	-
Canary Islands	Rates	24.77	11.83	3.54	36.77	-	0.30
	I.C. 95% / 95% C.I.	0-233.81	0-321.87	0-18.11	0-84.50	-	0-8.254
Andalusia	Rates	1.06	1.05	0.92	0.05	-	0.05
	I.C. 95% / 95% C.I.	0-28.96	0-28.73	0-3.88	0-1.50	-	0-2.71

the incubation period and the dates of 75 (25%) were unknown (figure 3). Nineteen cases had stayed at a total of 62 hotels, yet data on the tourist stays during the disease incubation period were available from only 21 of these establishments.

Two hundred and fifty-nine of the 281 reported cases had stayed at hotels. Of these, 240 had been at only one hotel, 19 at more than one (from two to five hotels; 62 in all), and 10 in more than one Autonomous Region. Six stayed in private homes, one in a caravan, and details of accommodation remained unknown for 15 patients.

In all, 186 hotels, 32 of which are classified as apartments on the EWGLI database, were associated with cases and 49 of these hotels were associated with between two and nine cases during the study period. Forty-three of the 49 hotels met the definition of single hotel case clustering in 12 of which all the cases occurred in the same year. When stays during the incubation period were analysed, however, only 38 hotels met the compatibility criteria (78% of all those associated with more than one case) and within this group, case clustering was considered to have occurred in 34 (table 2).

Distribution of cases by country of residence showed that Swedish tourists had the highest rate of illness (mean annual rate of 2.52 cases/100 000 European tourists) and Germans had the lowest (0.06/100 000). As with regional case distribution, rates by country of origin proved unstable from year to year, and statistical significance was observed only for British cases, with 184 diagnosed cases from 1989 to 1995 (70% of the total) and a rate of 1.12/100 000 (95% CI 0.33-2.10) (table 4). Both rates were calculated for years 1989-95, given that until 1989, only 6 cases had been reported.

Discussion

Cases of legionellosis among European visitors to Spain show a similar age and sex distribution as reported elsewhere (6).

The differences in rates of legionellosis between travellers from different European countries to the same region is remarkable (table 5), and suggests the existence of an information bias, due to differences in national surveillance systems or degrees of participation in the European system. We were unable to adjust rates we calculated for country of residence for lack of appropriate denominators distributed by age and sex. The crude rates showed that tourists visiting the Balearic Isles and the Valencian Region were the most greatly affected. As above, adjusted rates could not be calculated .

The high proportion of hotels associated with more than one case plus the appearance in many such establishments of patients in successive years suggested that their control measures are inadequate.

Recommendations

The results obtained highlight the need for EWGLI to adopt stricter case reporting criteria, especially with regard to the dates when cases stayed at particular places of accommodation, and dates of onset of symptoms, and the compatibility of those dates with the incubation period of legionellosis. Countries where the disease is diagnosed need to investigate risk factors more thoroughly and forward detailed information to the countries in which cases have travelled. Countries associated with the appearance of cases should monitor the maintenance of control measures over time, particularly in hotels repeatedly associated with cases. ELSS program records should be updated continually, by filing epidemiological and environmental data of interest. We would argue that all accommodation shown by subsequent investigation to be irrelevant to the development of the disease should be deleted from the registry. Similarly, if a patient has stayed at several establishments and environmental studies enable the case to be linked with just one of these, the establishments no longer under suspicion should be removed from the register.

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Harmonisation of the acute respiratory infection reporting system in the Czech Republic with the European community networks

J Kyncl[1] , W J Paget[2] , M Havlickova[1] , B Kriz[1]
1. Centre of Epidemiology and Microbiology, National Institute of Public Health, Prague, Czech Republic
2. European Influenza Surveillance Scheme Co-ordination Centre, Netherlands Institute for Health Services Research (NIVEL), Utrecht, the Netherlands

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Respiratory virus activity is detected in Europe each winter, yet the precise timing and size of this activity is highly unpredictable. The impact of influenza infection and/or acute respiratory infection in European countries is continuously monitored through a variety of surveillance systems. All of these sources of information are used to assess the nature and extent of activity of influenza and other respiratory viruses, and to offer guidance on the prevention and control of morbidity and mortality due to influenza at a local, national and international level.

The early warning system for a forthcoming influenza epidemic is mainly based on the use of a set of thresholds. In the Czech Republic, the acute respiratory infection (ARI) reporting system, with automated data processing, uses a statistical model for the early detection of unusual increased rates of the monitored indicators. The collected data consists of the number of ARI, the number of complications due to ARI and the population registered with the reporting general practitioners and paediatricians, all collected separately in five age groups. To improve the reporting system in the Czech Republic, clinical data on the weekly incidence of influenza-like illness (ILI) within the same population and the same age groups was started in January 2004. These data fit the European Commission's recently adopted ILI case definition and allows a better comparison of data with other countries in Europe, in particular those participating in EISS (European Influenza Surveillance Scheme).

Introduction

Information on the occurrence of infectious diseases is very important for maintaining public health in Europe. Every European country has its own national notification and surveillance system and legislation [1, 2]. National laboratories participate in many international surveillance programmes organised by the European Union, WHO and other organizations. Recently the Community network for epidemiological surveillance has been established in accordance with Decision No. 2119/98/EC of the European Parliament and of the Council.

Acute viral rhinitis, pharyngitis, laryngotracheitis, tracheobronchitis, bronchitis, bronchiolitis or pneumonia are associated with a large number of viruses, each of which is capable of producing a wide spectrum of acute respiratory illness, with different causes in children and adults [3].

Viral diseases of the respiratory tract may be characterised by fever and one or more systemic reactions, such as chills, headache, general aching, malaise and anorexia. Morbidity from acute respiratory diseases is particularly significant in children. In adults, the relatively high incidence and resulting disability, with consequent economic loss, make acute respiratory diseases a major health problem worldwide [3-5]. As a group, acute respiratory diseases are one of the leading causes of death from any infectious disease worldwide.

Influenza virus activity in Europe is detected each winter, yet the precise timing and magnitude of this activity remain highly unpredictable. The age groups of the population affected and the severity of illness that they experience depend on several factors including the virus types and subtypes that circulate during a given season. Clinical and virological data is collected and presented at a European level by the European Influenza Surveillance Scheme (EISS) through the internet [6]. EISS reported data for 22 countries during the 2003-2004 season; collaborators included 30 reference laboratories, at least 11 000 sentinel physicians and the surveillance covered a population of 445 million inhabitants [7].

FIGURE 1

Weekly ARI morbidity by age group per 100 000 population during the 2003-2004 season in the Czech Republic

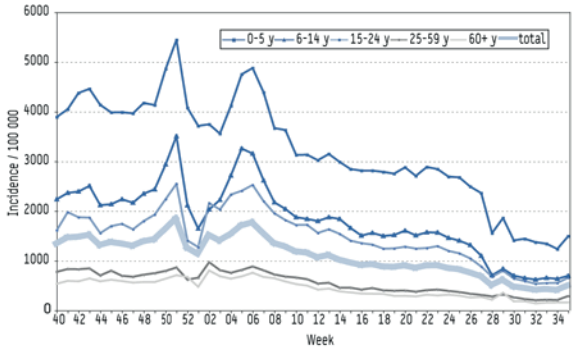
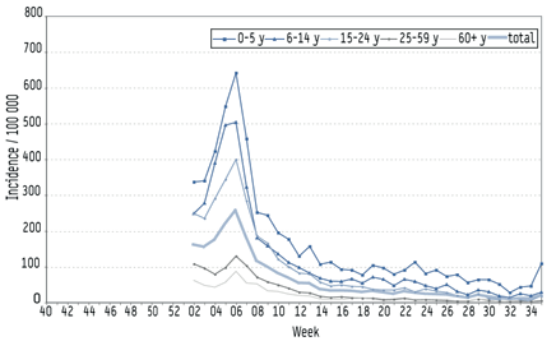


FIGURE 2

Weekly ILI morbidity by age group per 100 000 population during the 2003-2004 season in the Czech Republic



Epidemics of influenza are reported almost every year. Influenza pandemics occur at irregular intervals (three in the last century) and have been associated with unpredictable reassortments of genome segments of human, pig or avian viruses leading to surface antigens to which humans have no pre-existing immunity.

In an attempt to improve the health care information systems, substantial changes were made to the acute respiratory infection (ARI) reporting system from 2000 to 2002 in the Czech Republic [8]. The system (formerly based on sending the data by fax and entering them into a central database) was changed to a modern web-based system, which enables data to be entered at a local level with basic analysis in real time. Further changes were made in 2003 in accordance with the Commission Decision of 19 March 2002 laying down case definitions (Decision No. 253/2002/EC) for reporting communicable diseases to the Community network. The system was extended to enable the collection of age-specific incidence of influenza-like infections (ILI) as well.

Methods

The surveillance of influenza and other ARI is based mainly on clinical surveillance (morbidity reports and mortality statistics of influenza and respiratory infections as well as of all causes) and virological surveillance from the community and hospitals. The influenza morbidity monitoring program started in the Czech Republic in 1951. Since 1968, the age specific incidence of ARI and total incidence of complications have been monitored weekly. The system now includes approximately 2230 general practitioners (GP) and 1240 paediatricians and covers approximately 5 million inhabitants (half of the Czech population) in all 86 districts of the Czech Republic.

ILI is defined as: the clinical picture compatible with influenza, e.g. sudden onset of disease, cough, fever › 38 ºC, muscular pain and/or headache, in accordance with the EU case definition for influenza. ARI for reporting purposes is defined as every GP's clinical diagnosis of acute upper respiratory tract infection (as defined by the International Classification of Diseases, Tenth Revision (ICD-10), codes J00, J02, J04, J05, J06) and influenza (ICD-10 codes J10.1, J10.8, J11.1, J11.8).

Virological surveillance is performed by the Airborne Viral Infections Department at the National Institute of Public Health. The department is composed of two divisions: the National Reference Laboratory (NRL) for influenza and the NRL for non-influenza respiratory viruses. The virological surveillance program consists of a weekly assessment of routine laboratory test results of paired sera and nasopharyngeal swabs, provided by the collaborating virological laboratories. Test methods used are the complement fixation reaction (CFR), direct antigen detection from clinical specimens (ELISA) and isolation of the causative agent from a suitable cell culture. Lately, rapid diagnosis of the major causative agents of acute respiratory virus infections such as influenza virus of types A and B, respiratory syncytial virus, adenoviruses and parainfluenza viruses has been used within this program [9].

The data on morbidity from epidemiological surveillance are integrated with those from virological surveillance. After validation and assessment, the results are presented in a weekly bulletin. The bulletin is sent to the regional public health institutes, the Ministry of Health, collaborating laboratories and is also posted on the web page of the National Institute of Public Health [10]. Comprehensive outputs for international organisations such as EISS or WHO FluNet are provided by the National Reference Laboratory for influenza.

Results

Starting from the season 2001-2002, each regional public health service entered data from collaborating general practitioners and paediatricians into a central SQL database, using an encrypted web transfer with name and password controlled access. The district-specific data consists of the number of ARI, the number of complications due to an ARI and the population registered with the reporting GPs and paediatricians, all collected in five age groups (0-5, 6-14, 15-24, 25-59,

FIGURE 3

The first peak of ARI morbidity (week 51/2003). ARI incidence per 100 000 population, by district, Czech Republic

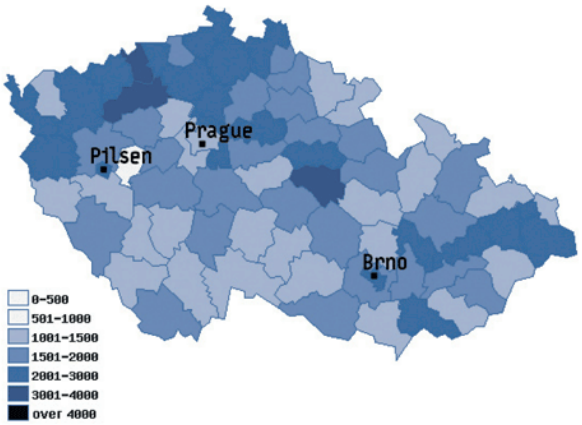
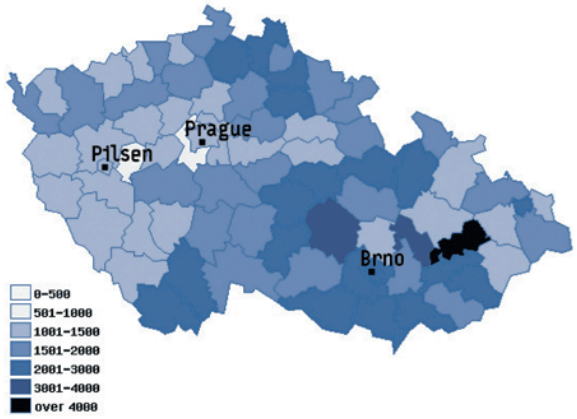


FIGURE 4

The second peak of ARI morbidity (week 6/2004). ARI incidence per 100 000 population, by district, Czech Republic



60+ years) [FIGURE 1*]. There is also space for comments. Pneumonia only is now considered as a complication of the infection. Starting from January 2004, clinical data on incidences of influenza-like illness (ILI) within the same population and the same age groups as in ARI have also been collected [FIGURE 2].

The basic data processing is automated and uses a statistical model for early detection of unusual increased rates of the indicators monitored, based on a general linear model for left-censored data. Usual weekly ARI incidence is modelled and this rate can only increase if a possible epidemic occurs. A threshold was established by averaging non-epidemic ARI incidences in the past years and applying an upper tolerance limit (covering 90% observations with 95% probability). The thresholds are available for the whole of the Czech Republic and also for each region. Direct standardisation and weighting for the size of the monitored population are also used to enable comparison of ARI and/or ILI morbidity among regions and districts. Figures 3 and 4 show the district distribution of ARI clinical incidence during two peak weeks.

Laboratory results for the season 2003-2004 (Figures 5 and 6) confirm that both regional outbreaks were caused by influenza. Weekly numbers of positive samples of the main circulating respiratory viruses of that season and the total ARI incidence is shown. Positive results for influenza can be seen to peak almost simultaneously with clinical illness incidence (positive results by the paired sera test are shown by the week when the second sample was tested and the results are therefore shifted by 2-3 weeks).

Discussion

Substantial changes have been made to the influenza reporting system in the Czech Republic in recent years. The changes started with an improvement of the ARI reporting system by making the system web-based during the 2001-2002 season. In January 2004, the reporting system also started a pilot ILI reporting project using an European Union adopted case definition. Data collected by the influenza reporting system in the Czech Republic have been reported to EISS since 1998 [11]. During the 2003-2004 season, 20 networks reported weekly ILI incidences and four networks (the Czech Republic, France, Germany and Romania) reported ARI incidences [7]. The pilot ILI incidences from the Czech Republic means that it is now possible to compare influenza activity with many more countries in Europe. The ILI rates in Europe varied considerably during the 2003-2004 season, with the peak incidences ranging from 12 per 100 000 population in Wales to 1885 per 100 000 population in the Slovak Republic. The peak ILI incidence in the Czech Republic was 256 per 100 000 population, much lower than in the neighbouring Slovak Republic. This difference may be due to a number of factors, including different case definitions, different health care systems and recent changes in the surveillance systems [12].

The age groups 0-5 and 6-14 were chosen because compulsory education starts at the age of 6 in the Czech Republic. Dividing children into school and preschool groups is relevant because of airborne spreading of respiratory infections.

Methods used for virological surveillance within EISS network were already published in 2004 [13]. Although only a small part of all clinical cases are analysed virologically each year, the virological results are of equal significance. Substantially more data are available for the specimens analysed, e.g. patient's age, clinical diagnosis, sampling date and onset of disease. First isolations of influenza virus and particularly an increase in their incidence may be predictive of the very beginning of an epidemic even before any change

FIGURE 5

Detection of ARI using an antigen detection: number of positive samples by week, season 2003-2004, Czech Republic

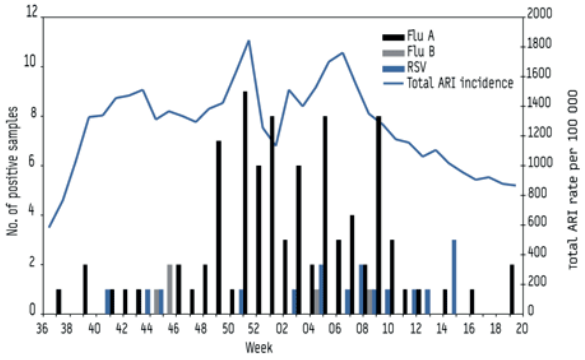
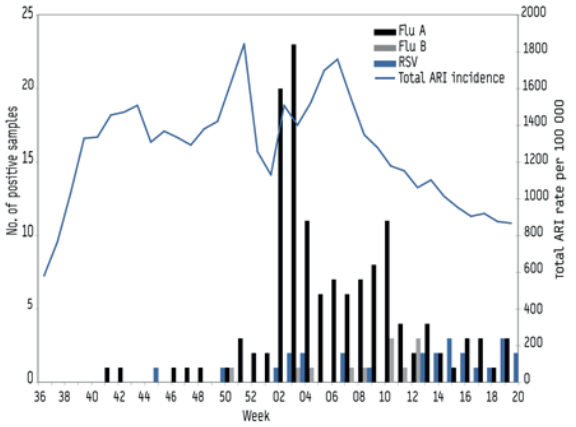


FIGURE 6

Detection of ARI using a serology: number of positive samples by week, season 2003-2004, Czech Republic



can be detected in the clinical morbidity rates. Routine detection of other viral respiratory pathogens yields complementary data which are useful in monitoring general trends in morbidity. Summary data are informative enough of the circulation of different agents in the population throughout the year. The virological results are also sometimes used to validate the clinical reports. For example, during the 2003-2004 season there were two ARI morbidity peaks in the Czech Republic [FIGURE 1]. This was caused by two regional influenza epidemics in different parts of the Czech Republic when the fast transmission was interrupted by the Christmas holidays [FIGURES 3 and 4.

The ARI / ILI reporting system of the Czech Republic is a modern and efficient surveillance system based on the collection of high quality data. The whole ARI / ILI reporting system is essential for pandemic planning in the Czech Republic. It can be linked with the system for crisis management to enable reporting and analysis on a daily basis. For efficient information at all levels, high quality local and national surveillance is necessary. Since using an internet-based platform, the reporting system in the Czech Republic as well as the EISS are easily accessible and provide timely information.

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*Editorial note-erratum

This figure, published on 19 April 2005, replaces Figure 1 with incorrect axis labelling originally published on 08 April

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A variant of *Chlamydia trachomatis* with deletion in cryptic plasmid: implications for use of PCR diagnostic tests

T Ripa[1], P Nilsson[1]
1. Epidemiology Service, Public Health Agency of Barcelona (PHAB), Barcelona, Spain

Correspondence: T Ripa (torvald.ripa@lthalland.se)

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A new variant of *Chlamydia trachomatis* with a deletion in the cryptic plasmid has been detected in Sweden, following an unexpected 25% decrease in *C. trachomatis* infections that was noted between November 2005 and August 2006 in Halland county, southwest Sweden. The number of patients tested during this period was similar to the number tested during the corresponding period one year earlier: 9055 compared with 8702.

For the past decade, laboratories in Sweden have used nucleic acid amplification tests (NAAT) to diagnose *C. trachomatis* infections. These NAAT tests use the cryptic plasmid (a non-chromosomal genetic element with unknown function found in all C. trachomatis strains) as the target area. If the current observed decrease in infections was not a true decrease in chlamydia incidence, it could be due to a change in the target area in the cryptic plasmid or a loss of the plasmid. Such a strain would obviously behave differently epidemiologically, because it would not be diagnosed by screening and no contact tracing would be performed from symptomatic index patients. Therefore, as part of the investigations into the decrease, we tested samples with alternative target areas of the plasmid as well as with a test specific for the major outer membrane protein (MOMP) area of the chromosome. The MOMP test was a commercial assay (Artus).

From mid-September to October 2006, the county microbiology laboratory in Halmstad, Halland county, tested 1700 consecutive incoming specimens with a MOMP-specific PCR in parallel with Abbotts m2000 plasmid PCR. In 13% of all diagnosed *C. trachomatis* cases in Halland county during this period (24/186), we found a variant strain that was only positive in MOMP tests. Clinical data indicates no difference from infections with the wild type strains.

The strain seems to be spread throughout the country, as it has also been found in northern, eastern and southern Sweden, although prevalence in these areas is still unknown. The findings throughout the country indicate that this strain is probably not a new phenomenon.

We have sequenced part of the plasmid from the variant strainand found a deletion of 377 base pairs in the target area for the *C. trachomatis* NAAT tests manufactured by Abbott and Roche. Twelve variant strains have now been sequenced and found to have the same deletion. Both companies have been informed and are currently working on a solution. This deletion does not affect the target area for the BD-ProbeTec test. At the laboratory in Halmstad, we are now developing a specific PCR test for the variant *C. trachomatis* strain.

The finding of a *C. trachomatis* variant does not, however, explain the decrease of 25% in *C. trachomatis* infections, especially if the variant has been circulating for some years. After 10 years of almost unbroken increase in reported number of *C. trachomatis* infections (from 332 cases in 1996 to 1000 cases in 2005), a decrease due to natural changes in incidence would not necessarily be surprising, but a decrease of 25% is much larger than expected. The authors would like to know how long this variant strain has been circulating undiagnosed, and whether it has occurred in other countries. If readers in other countries have recently experienced unexplained decreases in *C. trachomatis* infections, or have identified this variant, please contact the authors by emailing torvald.ripa@lthalland.se.

An outbreak of multi-resistant Shigella sonnei in Australia: possible link to the outbreak of shigellosis in Denmark associated with imported baby corn from Thailand

R Stafford , M Kirk , C Selvey , D Staines , H Smith , C Towner , M Salter , on behalf of the Outbreak Investigation Team

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An outbreak of shigellosis was recently reported in Denmark associated with the consumption of imported baby corn from Thailand [1]. We report a similar outbreak of shigellosis in Queensland, Australia that is possibly linked to the Danish outbreak through a common source in Thailand.

Queensland Health has investigated 11 laboratory-confirmed cases of Shigella sonnei (biotype g) with most cases having reported either consuming imported baby corn from Thailand or eating at a venue where imported baby corn was commonly served. These cases included two from another Australian State – Victoria – who had travelled to Queensland. Four cases were part of a larger outbreak among a film production crew where there were a further 43 probable cases (with symptoms including acute diarrhoea with or without vomiting, stomach cramps and fever between 9 and 14 August), although it was not possible to conduct a cohort study. Another two cases were infected while in hospital and a further two cases ate at a common holiday resort. All case isolates were resistant to augmentin, ampicillin, tetracycline, sulphonamides, trimethoprim, and streptomycin but susceptible to nalidixic acid, norfloxacin, ciprofloxacin, gentamicin, chloramphenicol, and ceftriaxone.

The dates of onset of illness among the 11 laboratory-confirmed cases were from 9 to 27 August, 2007. The median age of cases was 31 years (range 18-76 years) and seven cases were female.

Results of Pulsed Field Gel Electrophoresis (PFGE) testing of the human isolates from Queensland show a profile that is indistinguishable from that of human isolates from the outbreak in Denmark using the enzyme XbaI and the same running conditions as Denmark. We plan to run further gels on Australian S. sonnei isolates from the past three years to review the diversity of strains. We also plan to conduct further PFGE using a second enzyme BlnI (AvrII).

The traceback investigation to date shows that eight of the 11 cases may have eaten baby corn that was part of a very small consignment imported in late July by a single wholesaler in Queensland from an agent in Thailand. This Thai agent appears to be different from the Thai business that exported baby corn to Denmark, but the producer of the baby corn may still be the same, which remains to be investigated. Microbiological testing of baby corn from current batches is currently underway, although there was no leftover baby corn from the original consignment for testing.

Australia is attempting to trace the source of the baby corn with the assistance of Thai authorities. Onset date of illness for the last reported case was 27 August, and therefore no product recall has been initiated. Enhanced case surveillance has commenced to enable a more rapid response to the investigation of cases.

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Autochthonous chikungunya virus transmission may have occurred in Bologna, Italy, during the summer 2007 outbreak

T Seyler[1,2] , C Rizzo[1] , A C Finarelli[3] , C Po[3] , P Alessio[4] , V Sambri[5] , M L Ciofi Degli Atti[1] , S Salmaso[6]

1. Communicable Disease Epidemiology Unit, National Centre of Epidemiology, Surveillance and Health Promotion, Istituto Superiore di Sanità (National Public Health Institute, ISS), Rome, Italy
2. European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
3. Public Health Service, Emilia-Romagna Region, Bologna, Italy
4. Azienda Unità Sanitaria Locale (Local Health Unit, AUSL) of Bologna, Department of Public Health, Bologna, Italy
5. University of Bologna, Azienda Ospedaliera Universitaria di Bologna, Microbiologia, Bologna, Italy
6. National Centre of Epidemiology, Surveillance and Health Promotion, Istituto Superiore di Sanità (National Public Health Institute, ISS), Rome, Italy

Correspondence: T Seyler (thomas.seyler@iss.it)

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In Italy, a national surveillance system for chikungunya fever coordinated by the National Public Health Institute has been in place since August 2006. In summer 2007, an outbreak of chikungunya fever affected the Italian provinces of Ravenna, Cesena-Forli and Rimini [1-3]. As of 16 December 2007, health authorities identified 214 laboratory-confirmed cases with date of onset from 15 July to 28 September 2007. Most cases (161) occurred in the two neighbouring villages of Castiglione di Cervia and Castiglione di Ravenna, but limited local transmission also took place in the cities of Ravenna, Cesena, Cervia, and Rimini. In September 2007, two confirmed cases (two women aged 68 and 70) were reported among residents of the city of Bologna (373,026 inhabitants). Both had a history of travel in the affected areas (municipality of Cervia). No unusual increase in the density of Aedes albopictus mosquitoes in the Bologna area was noted at that time (September).

On 17 December 2007, the Regional Health Authority of Emilia-Romagna reported that three further residents of Bologna had tested positive for IgG and IgM antibodies against chikungunya virus by using a commercially available immunofluorescence test performed in Bologna on 14 December on blood samples taken on 5 December. Confirmation from the national laboratory at the National Public Health Institute is pending. The three patients (two women aged 78 and 79, and a boy aged 14) had developed fever, arthralgia and rash on 7, 18 and 23 September respectively, but had not been identified as suspected cases of chikungunya fever at that time. Blood samples were taken as one patient complained of persisting joint pain and the other two had had similar symptoms.

All three patients lived on the first floor of the same building, with a garden. The building is 2.5 km from the closest previously identified cases with a travel history to Cervia, reported in September. According to direct interviews, these three patients did not visit or stay in the area of the two imported cases, and vice versa. In addition, none of these last three cases reported having been abroad or having visited the affected areas at the time of the outbreak.

As these cases remained undetected at an early stage, no specific vector control measures were implemented in their premises. However, monthly routine preventive measures in Bologna from April to October included the use of larvicide in public areas. The apartment block was was not considered a “public area” for larvicide treatment.

This finding suggests that transmission may have occurred 75 km away from the initial cluster. This could be explained by the importation of the virus in the area where the three cases live through an undetected (asymptomatic) viraemic patient. Another possible explanation is passive vector mobility (e.g. infected mosquitoes transported by car from the initial cluster), since the flight range (active mobility) is usually considered to be less than 1 km, The sensitivity of the surveillance system relies on the continued dissemination of information to physicians regarding the clinical symptoms (i.e. fever and severe arthralgia) that should

prompt laboratory investigation for chikungunya virus infection. The present report highlights the need for reinforcing information and surveillance.

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Emergence of resistance to oseltamivir among influenza A(H1N1) viruses in Europe

A Lackenby[1], O Hungnes[2], S G Dudman[2], A Meijer[3,4], W J Paget[3], A J Hay[5], M C Zambon[1]
1. Health Protection Agency, Centre for Infection, London, United Kingdom
2. Norwegian Institute of Public Health, Oslo, Norway
3. EISS Coordination Centre, Nivel Institute, Utrecht, the Netherlands
4. National Centre for Public Health and the Environment, Bilthoven, the Netherlands
5. WHO Collaborating Centre, MRC National Institute of Medical Research, London, United Kingdom

Correspondence: M Zambon (Maria.Zambon@HPA.org.uk)

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Surveillance of the antiviral susceptibility of influenza viruses circulating in Europe has been established since 2004 through the European Union-funded European Surveillance Network for Vigilance against Viral Resistance (VIRGIL), in collaboration with the European Influenza Surveillance Scheme (EISS), the World Health Organization (WHO) and national influenza centres. Results from analysis of early winter (November 2007 – January 2008) A(H1N1) virus isolates has revealed that a significant proportion, approximately 14% of these European strains (see Table), are resistant to oseltamivir (Tamiflu), the most widely used anti-influenza drug, but retain sensitivity to zanamivir (Relenza) and amantadine/rimantadine.

As of week 03/2008, 16 European countries have reported significant influenza activity (Austria, Belgium, Bulgaria, France, Hungary, Ireland, Italy, Lithuania, Luxembourg, Northern Ireland, Poland, Portugal, Romania, Slovenia, Spain and Switzerland). Of the total virus detections since week 40/2007 (N=3447), 81% have been influenza A and 19% influenza B, and the predominant viruses circulating in most countries have been A(H1N1) similar to the A/Solomon Islands/3/2007 vaccine strain [1]. The presence of oseltamivir-resistant viruses circulating in the community in several European countries (Denmark, Finland, France, Germany, Netherlands, Norway, Portugal, Sweden and United Kingdom) is in marked contrast to the previous winter seasons of 2004/2005, 2005/2006, and 2006/2007, when oseltamivir resistance was detected in <1% of circulating strains from 24 countries.

A total of 437 influenza A(H1N1) viruses, isolated between November 2007 and January 2008, were tested using measurement of neuraminidase (NA) enzyme activity in the presence of oseltamivir to determine the drug-sensitivity (IC50) of the viral enzyme (2) in conjunction with sequence analysis of the viral neuraminidase gene. To date, oseltamivir-resistant viruses have been detected in nine countries (Table 1); in particular, 26 of 37 (70%) in Norway, 15 of 87(17%) in France, 3 of 43 (7.0%) in Germany and 8 of 162(5%) in the United Kingdom carry the same mutation, causing the substitution of histidine by tyrosine at residue 274 (H274Y) of the neuraminidase, which is known to confer a high level resistance to oseltamivir. Viruses bearing this mutation, when tested in enzyme assays, showed a reduction of approximately 400 fold in susceptibility to oseltamivir (IC50 values increased from approximately 1nM to more than 400nM). All these viruses remain sensitive to the other anti-neuraminidase drug zanamivir and to the anti-M2 drugs amantadine and rimantadine.

Table 1

A(H1N1) viruses resistant to Oseltamivir in Europe, winter season 07/08 (Nov 2007-Jan 2008)

Country	Total tested	Oseltamivir resistant by IC50(nM) or by 274Y	Percentage resistance with 95% confidence intervals
Austria	5	0	0% (0-43 %)
Denmark	10	1	10% (2-40%)
Finland	7	2	29% (8-64%)
France	87	15	17% (11-27%)
Germany	43	3	7% (2-19%)
Greece	5	0	0% (0-43%)
Hungary	5	0	0% (0-43%)
Italy	13	0	0% (0-23%)
Latvia	4	0	0% (0-49%)
Netherlands	16	1	6% (1-28%)
Norway	37	26	70% (54-83%)
Portugal	6	2	33% (10-70%)
Slovakia	5	0	0% (0-43%)
Slovenia	1	0	0% (0-79%)
Spain	11	0	0% (0-26%)
Sweden	13	1	8% (1-33%)
Switzerland	7	0	0% (0-35%)
United Kingdom	162	8	6% (3-9%)
Total	437	59	14% (11-17%)

The resistant (H274Y) viruses have been isolated from both adults and children, ranging from 1 month to 61 years in age, with the majority of viruses being isolated from adults. So far, there is no information that any of these viruses, in any country, has been obtained from a person who has either been treated or been in close contact with another individual who has been treated with oseltamivir. We therefore conclude that the identification of these oseltamivir-resistant viruses as a substantial proportion of circulating viruses, particularly in Norway, is the first clear evidence that influenza A(H1N1) virus with the H274Y mutation can readily transmit between individuals.

More extensive surveillance within Europe and in other parts of the world is required to establish the relative prevalence and geographical distribution of these resistant viruses, and to evaluate their potential impact on the effectiveness of drug use. The spectrum of clinical illness associated with infection by oseltamivir-resistant viruses remains to be fully determined, although limited information from initial clinical cases does not suggest unusual disease syndromes. Although the resistant viruses have been isolated from November through January, the ability of these viruses to persist throughout the influenza season, and from one season to the next, will require continuous world-wide surveillance by the WHO Global Influenza Surveillance Network. Determining the origins and genesis of these drug-resistant strains, which appear to have emerged in regions of the world where there is little drug pressure, will be important in understanding the emergence and persistence of oseltamivir resistance in relation to the evolution of influenza viruses and drug use.

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Influenza A(H1N1)v in Germany: the first 10,000 cases

A Gilsdorf (GilsdorfA@rki.de)[1], G Poggensee[1], on behalf of the working group pandemic influenza A(H1N1)v[1,2]
1. Department for Infectious Disease Epidemiology, Robert Koch Institute, Berlin, Germany
2. The members of the group are listed at the end of this article

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The analysis of the first 10,000 cases of influenza A(H1N1)v in Germany confirms findings from other sources that the virus is currently mainly causing mild diseases, affecting mostly adolescents and young adults. Overall hospitalisation rate for influenza A(H1N1)v was low (7%). Only 3% of the cases had underlying conditions and pneumonia was rare (0.4%). Both reporting and testing requirements have been adapted recently, taking into consideration the additional information available on influenza A(H1N1)v infections.

Introduction

After the first cases of influenza A(H1N1)v in the United States and Mexico became public, the Robert Koch Institute (RKI) established a case-based reporting of cases of influenza A(H1N1)v [1]. In the first weeks of the pandemic, data were reported to the national level by fax, phone and email in parallel with the routine electronic reporting system SurvNet [2]. Thereafter, this changed to exclusive electronic data reporting, including additional information relevant for the assessment of the epidemiological situation.

After the detailed examination of the first 100 cases in the early phase of the pandemic [1], we analyse here data of the first 9,950 cases in Germany, with a focus on information regarding the risk groups, hospitalisation frequency and other factors contributing to the impact this pandemic has on the healthcare system, in order to guide further public health measures.

Methods

As of 30 April 2009 the following information was collected through SurvNet with standardised free-text: classification of cases (possible, probable, confirmed, discarded case), in-country transmission, number of contacts (close as well as wider contacts), antiviral drug used. From 22 June 2009 onwards, the variables were changed in order to collect more detailed data on treatment (start of therapy, antiviral drug), risk groups, presence of pneumonia, hospitalisation and source of infection.

In order to take the age structure of the population into consideration, we calculated the incidence per 100,000 population per age group. From our data, we also calculated the time interval between date of symptom onset and diagnosis and start of therapy, respectively.

Categorical variables were presented as percentages with interquartile ranges when appropriate. Odds ratios were calculated including 95% confidence intervals where appropriate.

Results

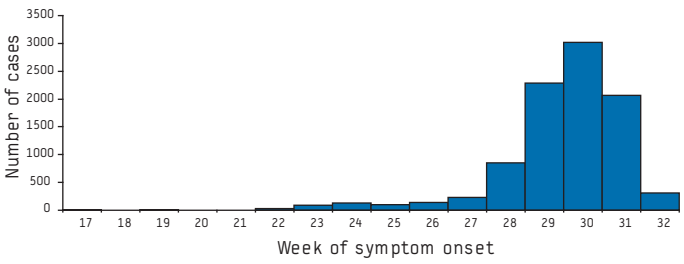
As of 25 August 2009, 14,940 cases of influenza A(H1N1)v have been reported in Germany. For the detailed report below we analysed the first 9,950 cases that were reported to the RKI until 10 August 2009.

The date of symptoms onset of the first German case was 20 April 2009. The person had travelled to Mexico and had already become symptomatic while staying in Mexico. Until the end of May, only sporadic cases were notified, usually associated with travel to North America. Most secondary infections with influenza A(H1N1)v which occurred in this period could be traced back to returning travellers. In June, the number of new cases rose to approximately 10 to 50 cases per day. Since mid-July we saw a considerable increase in cases in Germany (Figure 1) with a peak of up to 500 cases per day and 3,000 cases per week at the end of July. Since then, the number of new cases per day has decreased.

From the 9,950 cases, 54% were male. The median age was 19 years (range: 0-89 years). The majority of cases (77%) were from 10 to 29 years old. Two per cent of the cases were younger than five years, 3% were between five and nine years old, 17% were between 30 to 59 years old and less than 1% of the reported cases were 60 years old and older.

Figure 1

Notified cases of influenza A(H1N1)v by week of symptom onset, Germany, April-August 2009, (n=9,275 cases with available information on symptom onset)



Looking at the incidence (Figure 2), the 15 to 19 year-olds were most affected, with 90 cases per 100,000 population, followed by the 20 to 24 year-olds (43/100,000). In children up to two years old, there were 5.5 cases per 100,000 population. Persons 60 years old and older had less than one case per 100,000 population. The proportion of incidence by age group over the weeks 28 to 32 showed a stable age distribution over this time period (Figure 3).

For 2,141 cases (22%), Germany was indicated as the most likely country of infection. In the first weeks of the pandemic (May and June), most travel-associated cases had been returning travellers from North America. Since the first week in July, the proportion of infections associated with travel to European countries has risen sharply. In July, 80% of travel-associated infections were seen in travellers returning from Spain, followed by the United Kingdom (6%), Bulgaria (3%) and North America (2%). From week 29 to 32,

Figure 2

Incidence of notified cases of influenza A(H1N1)v, by age group, Germany, April-August 2009, (n=9,950)

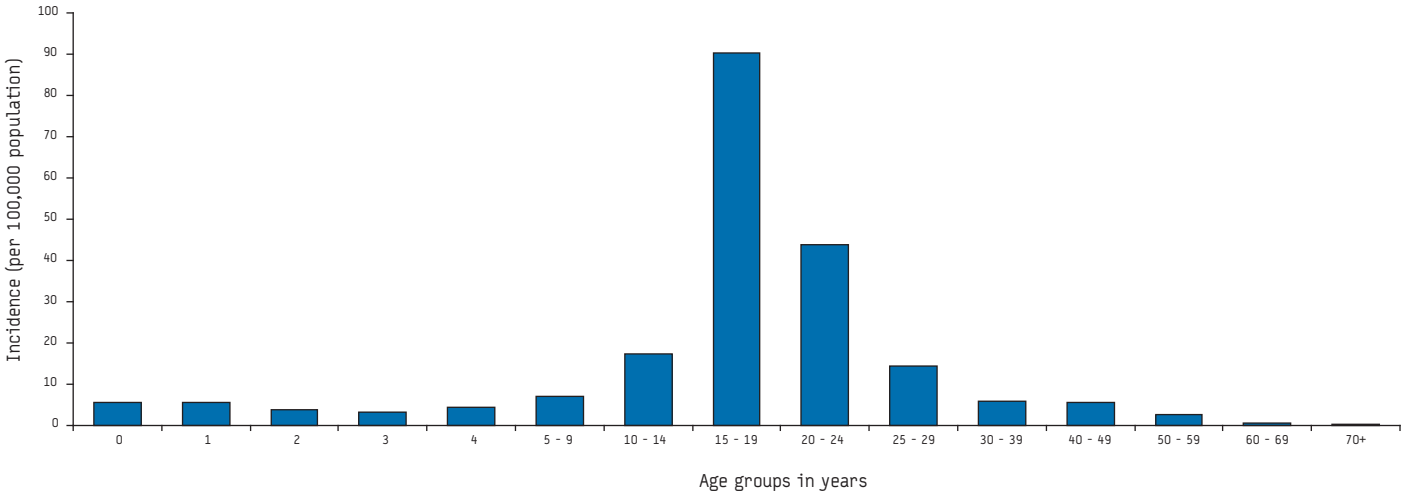
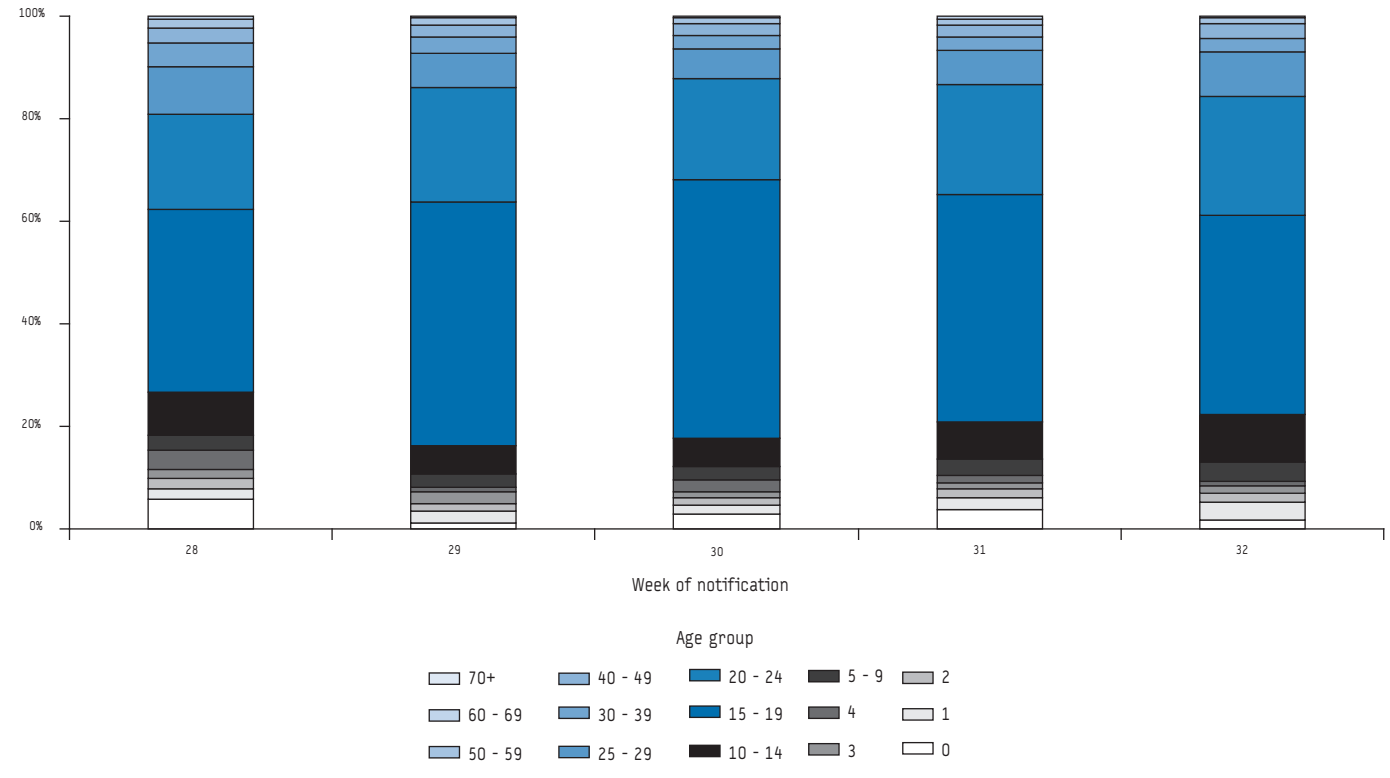


Figure 3

Proportion of incidences by age group and week of notification for notified cases of influenza A(H1N1)v, Germany, July-August 2009, (n=9,341)



the number of cases most likely infected in Germany rose steadily from 16% to 24%. For the cases without travel history, the proportion of infections without a known source increased between weeks 29 and 32 from 38% to 43% (n=1,039).

Symptoms were reported for all 9,950 cases. Cough was the most common symptom, present in 82% of the cases, followed by fever (78%).
Data were also collected on underlying health conditions and risk factors. The results are presented in the table.

The average time interval between date of symptom onset and diagnosis (n=7,955 cases for whom this information was available) was 3.6 days with an increasing trend from week 26 (2.4 days) to week 31 (3.8 days). The average time between date of symptom onset and start of therapy (n=1,810 cases for whom this information was available) was 2.2 days with a decreasing trend from week 28 (4.0 days) to week 32 (2.0 days). Cases with underlying conditions were more likely to receive treatment (72/134: 54%) than cases without underlying conditions (1,679/3,805: 45%; OR=1.44 [1.01; 2.07]). Information on presence of pneumonia at the time of notification was available for 6,460 cases. Pneumonia was reported for 26 cases (0.4%), out of which four belonged to a risk group (two had respiratory, two had unspecified risk factors) and eight were hospitalised.

From 3,630 cases for whom hospitalisation status was available, 263 (7%) persons were admitted to a hospital because of influenza, 122 cases (3%) were in hospital for other reasons, and for 42 cases (1%) the reason of hospitalisation was not known. The influenza hospitalisation rate changed from 11% in week 29 to 5% in week 31. We also looked for cases with information on their risk factors and their hospitalisation status (n=3,270). The proportion of people with risk factors who were hospitalised for influenza was 19% (20/108), while the proportion of people without risk factors that were hospitalised for influenza was 7% (220/3,162; OR = 3.04 [1.78; 5.16]). The median age was 19 years for both groups.

During the first phase of the pandemic, all contacts of cases in Germany were traced back by the local public health authorities and the number of contacts was reported to the national level. The trace back was done for 2,635 cases. On average, three contact persons per case were identified (upper and lower quartile: 2 to 6 contacts, range 0 to 330 contacts).

Table

Frequency of underlying health conditions for cases of influenza A(H1N1)v, Germany, April-August 2009, (n=5,885 cases for whom this information was available)

Underlying conditions*	Number of cases (%)	Proportion of all underlying conditions
No	5,690 (96.7%)	-
Yes	195 (3.3%)	-
Respiratory disease	87 (1.5%)	45%
Cardio-vascular disease	29 (0.5%)	15%
Diabetes	17 (0.3%)	9%
Obesity	11 (0.2%)	6%
Pregnancy	9 (0.2%)	5%
Immunsuppression	5 (0.1%)	3%
Others	34 (0.6%)	17%
Not specified	9 (0.2%)	5%

*Multiple answers were possible.

Discussion

The analysis of the first approximately 10,000 cases of influenza A(H1N1)v in Germany showed that after some sporadic cases and a slow increase in June 2009, a significant increase of newly reported cases was seen starting with July. This trend was also reported from other countries in Europe [3]. There seems to be a downward trend now in Germany, even taking into account a reporting delay of approximately one week. Whether this decrease is a true decline in incidence is not yet clear. A change in health-seeking behaviour might also play a role. The first anxiety about the new infection might have made more people with respiratory symptoms seek medical advice and therefore might have brought the cases to the attention of the of the public health authorities. However, other European countries, like the UK, also report signs that the potential first wave of the pandemic might be coming to an end [4].

The cumulative number of cases by age group clearly shows that there is a peak in the age group 15 to 19 years . Many of these cases were high-school graduates who travelled to Spain in large groups at the end of the school year. The incidence in the under two year old children is relatively low (5/100,000). Data from the United States showed a much higher incidence (22.9/100,000) in children up to five years old [5]. The very low incidence in people over 60 years of age is consistent with other investigations [4-7]. It is still unclear if this is due to a partial immunity from former infections with H1N1 influenza viruses or if this is because the virus has not yet been sufficiently introduced in this subpopulation. Looking at the proportion of affected age groups over weeks, no shift to the older (>60 years) or younger (<5 years) age groups can be seen yet.

The high proportion of cases imported from Spain does not necessarily indicate a relevant epidemic activity there, but probably rather reflects the travel patterns of German holiday makers during summer. The German Federal Office for Statistics reported that from June to August 2008 approximately 1.1 million people travelled every month from Germany to Spain by air [8]. Additionally, there are many organised bus tours to Spain that are especially favoured by high-school students. Closer physical contact, sharing of drinks and special party settings were discussed as possible risk factors, but they need to be validated by further research. Besides the high number of cases in travellers, we could see an increasing proportion of cases that had no travel history and no known source of infection in the last weeks.

Most cases of influenza A(H1N1)v currently seem to have uncomplicated influenza-like illnesses. Our data show that the most common symptoms were cough and fever, similarly to reports from other countries [6-9]. This was one of the reasons why we specified the list of symptoms for the physicians to notify a patient to the local health authorities.

A particular interest for the public health authorities is the protection of the vulnerable groups. These are people with underlying conditions, such as chronic diseases, but also pregnancy, who have a higher risk of developing complications during an influenza infection. From all notified cases in Germany for whom the information was available, only 3% had underlying conditions.

Nearly half of them had chronic respiratory tract diseases. Pregnancy was not often reported among the confirmed cases. Pneumonia at the time of notification was also very rarely reported.

With increasing numbers of cases and laboratory diagnoses, the time interval between date of onset of symptoms and date of diagnosis has increased considerably. In the beginning, both transport of specimens and laboratory testing were done very fast. Now diagnostics have become more routine work and the high number of samples has caused a backlog of samples to be tested. The time interval between onset of symptoms and start of therapy decreased from four to two days. That means physicians start therapy as recommended before the laboratory confirmation of the influenza infection. Treatment is started on average within 48 hours from symptom onset, when the antiviral drugs are supposed to be most effective.

The hospitalisation rate changed considerably over the weeks. During the first weeks, the majority of cases were hospitalised due to infection control measures. Even though that might still be the case for some patients, hospitalisation is now considered as a proxy for the severity of the disease in patients. In the last couple of weeks, the hospitalisation rate due to influenza in the notified cases halved to 5% in week 32. This is a relatively low proportion and does not constitute a high burden for the hospitals at this stage of the pandemic. When we looked closer at those cases with reported underlying conditions we could see that they had a hospitalisation rate more than two times higher than in cases without underlying conditions. Here precaution could have contributed to the referral to a hospital, but it still shows that these known groups with underlying conditions will present an important group when dealing with the pandemic.

Conclusion

As of August 2009, the majority of influenza A(H1N1)v cases reported in Germany are mainly imported from other European countries. However, the proportion of cases with in-country transmission is increasing.

Several factors might influence the characteristics of notified cases in the near future. Firstly, as of 18 August 2009, physicians have to notify possible cases only if the patient presents with cough and fever, therefore it is assumed that the number of cases reported to the national level will decrease. Since 17 August 2009, the costs of the laboratory confirmation have been paid by the statutory health insurances only for cases with severe disease or cases with the risk to develop severe disease. Therefore, the percentage of laboratory-confirmed cases among the notified cases will decrease. However, as long as the sentinel surveillance in Germany does not give a signal, the assessment of the epidemiological situation must rely on routine surveillance.

The public health strategy has changed in Germany from containment (follow-up of all contact persons) to the protection of vulnerable groups. Now, only contact persons who have occupational contacts to persons with a high risk to develop severe disease are followed up (e.g.: healthcare workers).

Until now, no fatalities due to influenza A(H1N1)v have been reported in Germany, which may be partly due to these strategies.
Germany wants to continue the current reporting system until the number of respiratory infections increases significantly, as can be expected in autumn again. Then it is planned to stop the case-based reporting by physicians and get the necessary information from the laboratory-based reporting of confirmed cases as it is done for seasonal influenza viruses and the sentinel surveillance.

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Members of the working group pandemic influenza A(H1N1)v:

Alpers, Katharina; Altmann, Doris; An der Heiden, Matthias; Bartels, Cornelius; Bätzing-Feigenbaum, Jörg; Becker, Anne; Behnke, Susanne; Bergholz, Andreas; Bernard, Helen; Bielecke, Jessica; Biere, Barbara; Böhmer, Merle; Brodhun, Bonita; Buchholz, Udo; Buda, Silke; Cai, Wie; Claus, Hermann; Dehnert, Manuel; Déleré, Yvonne; Dettmann, Marleen; Eckelmann, Fabian; Eckmanns, Tim; Faber, Mirko; Faensen, Daniel; Fahle, Caroline; Feig, Marcel; Frank, Christina; Gencaslan, Özlem; Ghassim, Parvin; Gilsdorf, Andreas; Gohlke-Micknis, Silvia; Gunsenheimer-Bartmeyer, Barbara; Haar, Karin; Haas, Walter; Hamouda, Osamah; Hellenbrand, Wiebke; Hermes, Julia; Herzhoff, Michael; Houareau, Claudia; Jansen, Andreas; Kalbhenn, Andrea; Kamga-Wambo, Oscar; Kappelmayer, Lutz; Kermer, Antje; Kirchner, Göran; Kleinkauf, Niels; Koch, Judith; Kollan, Christian; Köpke, Karla; Krause, Gérard; Kühne, Andrea; Laude, Gabi; Leuber, Michael; Lindemann, Christina; Liss, Ilka; Lohmann, Katrin; Maidhof, Heinrich; Männel, Andrea; Marcus, Uli; Matysiak-Klose, Dorle; Meyer, Birgit; Mohr, Oliver; Mücke, Inge; Neugebauer, Denise; Nielsen, Stine; Noll, Ines; Offergeld, Ruth; Pape, Ebi; Poggensee, Gabriele; Poorbiazar, Mona; Radun, Doris; Reinhardt, Bernd; Reiter, Sabine; Reuß, Annicka; Riedmann, Klaus; Ritter, Sabine; Riva, Alessandra; Rosner, Bettina; Sagebiel, Daniel; Sailer, Andrea; Sasse, Julia; Schenkel, Karl; Schirmack, Jaska; Schmidt, Axel; Schuppelius, Daniel; Schwarz, Franziska; Schweickert, Brigitta; Schweiger, Brunhilde; Siedler, Anette; Spackova, Michaela; Spielmann, Nadine; Stark, Klaus; Stegemann, Verena; Stöcker, Petra; Strobel, Hartmut; Süß, Thorsten; Trull, Heidi; Velasco, Edward; Voß, Lieselotte; Wadl, Maria; Walter, Dietmar; Weiß, Bettina; Werber, Dirk; Wessels, Guido; Wetzel, Sarah; Wiese-Posselt, Miriam; Zbinovcova-Dennis, Martina; Zimmermann, Ruth

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Listeriosis outbreak caused by acid curd cheese 'Quargel', Austria and Germany 2009

R Fretz (rainer.fretz@ages.at)[1], U Sagel[1,2], W Ruppitsch[1], A T Pietzka[1], A Stöger[1], S Huhulescu[1], S Heuberger[1], J Pichler[1], P Much[1], G Pfaff[3], K Stark[4], R Prager[4], A Flieger[4], O Feenstra[5], F Allerberger[1]

1. Austrian Agency for Health and Food Safety (AGES), Vienna, Austria
2. Binational Consiliar Laboratory for Listeria, Germany and Austria, Vienna, Austria
3. State Health Office (LGA) Baden-Württemberg, Stuttgart, Germany
4. Robert Koch Institute (RKI), Berlin and Wernigerode, Germany
5. Public Health Authority, Graz, Austria

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We report an outbreak of listeriosis in Austria and Germany due to the consumption of 'Quargel' cheese produced by an Austrian manufacturer. At the time of writing this report, the outbreak was known to account for 14 outbreak cases in 2009, including four cases with lethal outcome. On 23 January 2010, the cheese product was voluntarily withdrawn from the market.

On 14 August 2009, the binational Austrian-German Consiliar Laboratory for Listeria in Vienna noticed the occurrence of a new pulsed-field gel electrophoresis (PFGE) pattern in human isolates of *Listeria monocytogenes* serotype 1/2a. This consiliar laboratory receives all human isolates from Austria as required by law. In Germany, submission of isolates is voluntary. According to the available information at the time of writing this report, the outbreak clone accounted for 12 of the 46 Austrian cases in 2009 (serotype 1/2a (n=29), 4b (n=9), 1/2b (n=8)). Onset of illness is shown in the Figure. The 12 Austrian outbreak cases (two of them fatal) affected six of nine Austrian provinces. The mean age was 74.5 years (range: 58-88 years), eleven patients were male. In addition, two of 92 available human isolates from Germany in 2009 (total number of cases 389) showed this new PFGE-pattern. The German outbreak cases were two women in their 70s who died in November and December 2009 respectively. They had not visited Austria during the likely period of incubation (up to 70 days).

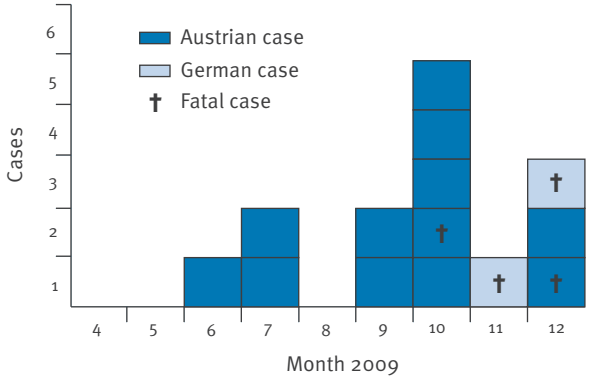
Since no reliable information was available on food consumed during the incubation period, all surviving Austrian outbreak cases were asked to collect grocery receipts for the three weeks after 3 December, i.e. after they were discharged from hospital, in order to collect information on routine food consumption behaviour. This epidemiological investigation revealed consumption of 'Quargel', a type of acid curd cheese available in different flavours, as a highly likely source of this outbreak. Three of seven outbreak cases providing receipts had bought product X produced by the Austrian manufacturer. Regular consumption of Quargel product X was confirmed by eight of nine participating outbreak cases, and consumption of Quargel cheese products was reported by heteroanamnesis for one German outbreak case (data on the second case remain unavailable).

Approximately 16 tons of Quargel per week are produced by the Austrian manufacturer. Fifty-three per cent of the product is exported to the German market and small amounts to the Czech Republic, Poland and Slovakia. This cheese is made of curdled milk, which ripens after addition of starter cultures for one day at 28°C, and after being sprayed with *Brevibacterium linens* for another two days at 14°C. The shelf life after packing and marketing is two months.

An environmental *L. monocytogenes* 1/2a isolate from the production plant, collected in December 2009, became available in January 2010 and proved indistinguishable from the outbreak strain by genotyping. Quargel cheese products sampled at the plant on January 13 yielded three different strains of

Figure

Outbreak cases of listeriosis by onset of illness, Austria and Germany, 2009 (n=14)



L. monocytogenes 1/2a, including the outbreak clone, in numbers of less than 100 colony-forming units (cfu) per gram. Food products collected on 18 January 2010 yielded greater than 100 cfu/g *L. monocytogenes*. The product was voluntarily withdrawn from the market on 23 January. On the same day, the public was informed about the incident and warned about cheese already bought. The plant stopped production. Investigation of the source of contamination is ongoing.

Conclusion

Industrial food production combined with international marketing of food and the low attack rate of *L. monocytogenes* hinder epidemiological outbreak investigation with traditional concepts [1]. Genotyping of *L. monocytogenes* isolates from clinical specimens can discriminate single-source clusters of food-borne infection and contribute to the identification and investigation of outbreaks. The outbreak described in this report probably would not have been identified without molecular typing [2]. The effectiveness of microbiological surveillance is entirely dependent upon the consistent and timely submission of all Listeria isolates from clinical laboratories to public health laboratories. In Austria, clinical laboratories are required by law to submit all clinical isolates of *L. monocytogenes* to AGES for PFGE analysis. In Germany, submission of *L. monocytogenes* isolates from clinical specimens by clinical laboratories is not required. The high case fatality ratio of listeriosis makes a strong case for the importance and priority of improved surveillance in Europe [3]. Our outbreak report underlines the value of routine molecular typing of Listeria isolates and also points out the considerable potential of cross-border cooperation for elucidating chains of infections.

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First two autochthonous dengue virus infections in metropolitan France, September 2010

G La Ruche (g.laruche@invs.sante.fr)[1], Y Souarès[1], A Armengaud[2], F Peloux-Petiot[3], P Delaunay[4], P Desprès[5], A Lenglet[6], F Jourdain[7], I Leparc-Goffart[8], F Charlet[3], L Ollier[4], K Mantey[6], T Mollet[6], J P Fournier[4], R Torrents[2], K Leitmeyer[6], P Hilairet[4], H Zeller[6], W Van Bortel[6], D Dejour-Salamanca[1], M Grandadam[5], M Gastellu-Etchegorry[1]

1. French Institute for Public Health Surveillance (Institut de Veille Sanitaire, InVS), Saint-Maurice, France
2. Regional office of the French Institute for Public Health Surveillance (Cire Sud), Marseille, France
3. Regional Health Agency of Provence-Alpes-Côte d'Azur, Marseille and Nice, France
4. Entomology-Parasitology, Virology and Emergency Medicine and Internal Medicine Departments, University Hospital of Nice, Nice, France
5. Institut Pasteur, National Reference Centre for arboviruses, Paris, France
6. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
7. Directorate General for Health, Ministry of Health, Paris, France
8. Institut de recherche biomédicale des armées, National Reference Centre for arboviruses associated laboratory, Marseille, France

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In September 2010, two cases of autochthonous dengue fever were diagnosed in metropolitan France for the first time. The cases occurring in Nice, south-east France, where Aedes albopictus is established, are evidence of dengue virus circulation in this area. This local transmission of dengue calls for further enhanced surveillance, active case finding and vector control measures to reduce the spread of the virus and the risk of an epidemic.

Dengue fever is the most important mosquito-borne viral disease in the world and is endemic in Africa, Asia, Caribbean and Latin America. According to the World Health Organization, there are annually more than 50 million cases and 22,000 deaths [1]. Dengue fever is caused by viruses of the Flaviviridae family and transmitted by mosquito vectors of the Aedes genus, mainly *Ae. aegypti* and *Ae. albopictus* [2].

In Europe, the last dengue epidemic was reported from 1927 to 1928 in Greece with high mortality and *Ae. aegypti* was implicated as the vector [3]. Since the 1970s, mainly through global trade of car tyres, *Ae. albopictus* has become increasingly established in European Union Member States, including France, Greece, Italy, the Netherlands (though only in greenhouses), Slovenia and Spain [4]. This mosquito species is also established in neighbouring countries such as Albania, Bosnia and Herzegovina, Croatia, Monaco, Montenegro, San Marino, Switzerland and Vatican City [2,5]. Imported cases of dengue fever in travellers returning from countries where dengue is endemic or where dengue epidemics are taking place have been frequently reported in European countries in recent years [6-10].

In metropolitan France, sporadic *Ae. albopictus* mosquitoes were first detected in Normandy in 1999 [11], but the mosquito is known to have been established since 2004 in south-east France [12]. Since 2006, and the widespread epidemic of chikungunya in Réunion which had posed an increased risk of importation of cases, enhanced surveillance is implemented each year from May to November in the departments where *Ae. albopictus* is established, as part of the national plan against the spread of chikungunya and dengue viruses in metropolitan France [13]. Enhanced surveillance, compared with routine surveillance, allows the reporting and confirmation of suspected cases to be accelerated. The laboratory network surveillance system, the most sensitive routine system in France, detected around 350–400 imported dengue cases per year between 2006 and 2009 in metropolitan France [14,15]. During the same four-year period, enhanced surveillance reported a total of 33 imported dengue cases (including 11 cases in 2009). Between 1 May and 17 September 2010 (i.e. the first 4.5 months of surveillance), 120 imported cases of dengue have been reported by the enhanced surveillance system [16], which represents an 11-fold increase when compared with the entire 2009 season. This increase in imported cases is mostly related to the ongoing epidemics in the French West Indies, Martinique and Guadeloupe, since the beginning of 2010. Here we report on the two first cases of autochthonous dengue virus infection ever diagnosed in metropolitan France and the public health measures subsequently implemented.

Case 1

The first case was detected through the routine enhanced surveillance system. The patient was a man

in his 60s, resident in Nice, Alpes-Maritimes department, who developed fever, myalgia and asthenia on 23 August 2010. He was hospitalised on 27 August 2010, but his clinical condition remained stable. A temporary thrombocytopenia with a minimal platelet count of 48,000/µl (norm: 150,000–400,000) on day five of the illness resolved without complications and he recovered within a few days after disease onset.

Laboratory findings

A panel of sera obtained during the acute and recovery phases on days five, seven, 11 and 25 of the illness was investigated by serological tests (in-house MAC-ELISA and direct IgG ELISA) and real-time RT-PCR. Moreover, a serum sample collected during a previous medical examination in May 2010 was tested retrospectively. Presence of IgM and IgG against dengue virus antigens was documented in all samples except for the serum sampled in May 2010. Antibody titration revealed sharp increases in IgM titres from 1:800 to 1:12,800 and in IgG titres from 1:32,000 to >1:128,000 over the 25 days follow-up. Anti-dengue virus IgA (Assure Dengue IgA rapid test, MP Biomedicals) were also detected on days five and seven. The dengue NS1 antigenic test (Dengue NS1 strip, Biorad) was positive on days five and seven but negative on day 11, demonstrating the active replication of a dengue virus during the symptomatic period. RT-PCR for dengue virus was positive on day five and negative thereafter. Molecular typing identified a dengue virus serotype 1.

It is of interest to note that high levels of specific anti-dengue IgG were detected during the acute phase of disease. Our hypothesis is that these IgG might result from activation of memory B cells (original antigenic sin) related to an ancient primary infection with a heterologous serotype of dengue virus. Seroneutralisation tests will be informative on the immunological status of the patient regarding a possible previous infection with a dengue virus of another serotype. Virus isolation and sequencing are also ongoing. No serum cross-reactions were observed with tick-borne encephalitis and West Nile viruses and no markers of chikungunya virus infection were found (absence of IgM and IgG antibodies, negative RT-PCR). The patient had been vaccinated against yellow fever 28 years ago.

Friends from the French West Indies had stayed with him since April 2010. He had no recent history of international travel or blood transfusion. Consequently, the patient was considered a confirmed autochthonous case of dengue virus infection.

Control measures

This classification prompted an immediate reaction of public health authorities to reduce the risk of further spread of the virus. Various measures were undertaken by health authorities as laid out in the national plan against the spread of dengue in France (level 2 of the plan) [13]: (i) 200 metres perifocal vector control activities centred on the case's residence, including spraying for adult mosquitoes and destruction of breeding sites; (ii) active case finding in the neighbourhood of the case's residence and in other areas visited by the case; (iii) providing information about dengue virus to health professionals, including incitation for screening suspected dengue cases and information to the public. The active case finding conducted by physicians and laboratories will be continued on a weekly basis up to 45 days after the onset of symptoms of the last autochthonous case.

The routine laboratory network surveillance system noticed that six recently imported confirmed dengue cases, including four with a RT-PCR positive for dengue, had been detected in Nice between 24 July and 23 August 2010. One of them had returned from Martinique and lives about 200 metres from the autochthonous case. This imported case was reported too late to implement vector control measures which routinely follow imported viraemic dengue cases in those departments where the vector is present, and could therefore be a potential source of infection of local *Ae. albopictus*. As of 24 September 2010, the active case finding has detected nine new suspected autochthonous cases of dengue fever in the neighbourhood of the index case. In four of them, no markers of dengue virus infection were found (absence of IgM and IgG antibodies, negative RT-PCR), results from epidemiological and laboratory investigations for further four patients are still pending. One case was confirmed to be infected by dengue virus; the latter patient is the second autochthonous dengue fever case ever diagnosed in metropolitan France.

Case 2

This second case is an 18 year-old man who had no recent history of international travel. He lives approximately 70 metres from the first autochthonous case. He developed fever, myalgia, headache and asthenia on 11 September 2010. He was hospitalised briefly because of fever of unknown origin and thrombocytopenia with a mild clinical disease. The thrombocytopenia (platelet count 53,000/µL on day seven of the illness) was temporary and moderate, and he has recovered fully.

Laboratory investigations

Laboratory tests conducted on an early serum sample on day three of illness indicate negative serology for IgG and IgM antibodies but strongly positive RT-PCR for dengue virus. Molecular typing identified a dengue virus serotype 1. The strain appears to be quite similar to those which currently circulate in Martinique; more detailed analyses are ongoing.

Discussion

The identification of two autochthonous cases of dengue fever which are clustered in space and time is strongly suggestive that a local transmission of dengue virus is ongoing. Therefore level 3 of the national plan against the spread of dengue virus has been activated [13]. It entails additional measures to those taken

at level 2: (i) active case finding of autochthonous cases in hospital emergency wards, at present in Nice and surrounding towns, (ii) implementation of vector control measures in hospitals, together with protection of potential viraemic patients against mosquito bites using electric light traps, electric diffusers for insecticides, and repellents, and vector control measures around the port and the international airport of Nice including enhanced entomological surveillance, and (iii) toxicovigilance related to the wide use of insecticides.

Based on the currently available information, these are the first confirmed cases of autochthonous transmission of dengue fever in metropolitan France and Europe, since the epidemic in Greece in the late 1920s and apart from one nosocomial case of dengue infection reported from Germany in 2004 [17]. The event is not entirely unexpected, as reflected in a specific preparedness plan and taking into account the increase in imported cases from the French West Indies and other endemo-epidemic areas. It is known that France, as well as other countries in Europe, has competent vectors for transmitting this flavivirus. The chikungunya outbreak in Italy that occurred in 2007, with over 300 cases reported, has shown that non-endemic arboviruses can be efficiently transmitted in continental Europe [18].

Whether the transmission of dengue virus in France followed a bite from an infectious mosquito imported to the area via airplanes or boats, or one already present in the area after biting a viraemic person residing or visiting Nice, remains to be determined. However, with the second confirmed case, the latter scenario is the most likely one. Therefore, taking into consideration the longest possible incubation period for dengue fever, 15 days, it can be considered that the conditions for successful transmission of dengue virus to humans existed in Nice during August 2010. To date, only two autochthonous cases of dengue fever have been detected in Nice, but the identification of new dengue cases in the near future cannot be excluded. The enhanced surveillance and strict vector control measures are expected to limit the risk for further spread as much as possible.

At this stage, the risk for further spread to humans in Europe, as well as the possibility of the establishment of dengue virus transmission in Nice or in neighbouring areas in France, may appear limited but needs to be closely monitored. Recent evidence demonstrates that compared with *Ae. aegypti*, which has been implicated in the majority of large dengue outbreaks worldwide, *Ae. albopictus* is a less efficient vector of this virus [2]. Nevertheless, it was involved in outbreaks in Japan from 1942 to 1945 [19], the Seychelles in 1977 [20], Hawaii from 2001 to 2002 [21] and Réunion island in 2004 [22]. Vertical transmission of dengue virus from mosquitoes to their offspring does not seem very efficient, and therefore overwintering of the virus in continental European *Ae. albopictus* populations is unlikely [2] but cannot be excluded [23,24]. The public health consequences of the presence of *Ae. albopictus*, in this context, appear to be more important for the transmission of chikungunya for example, for which experimentally better competence has been demonstrated, although the competence of local *Ae. albopictus* for dengue virus is far from being negligible [25]. It should also be noted, that the currently affected area of France as well as other countries in Europe is faced with a high number of imported dengue cases every year. However, despite this and established mosquito populations being potentially able to transmit arboviral diseases, local transmission of the dengue virus with *Ae. albopictus* as the vector in mainland Europe has never been observed before this reported emergence in the south-east of metropolitan France. The high vector density in Nice and the increase in the number of imported cases in this area in 2010, mainly due to intense epidemics in the French West Indies, are two major factors to explain this emergence and highlight the need to maintain an appropriate active surveillance.

In terms of blood safety, reported dengue infection following blood transfusion in dengue endemic areas is rare [26-28] but is also difficult to detect as a large proportion of the population would already have antibodies against the virus. However, as dengue infection is mild or asymptomatic in 40–80% of infected persons, depending on the area and the epidemiological context [29-31], it does pose a risk to blood safety. The two identified cases in Nice are suggestive that other infected persons may have lived in the city during the same period of exposure, without showing any symptoms. Asymptomatic carriers of dengue virus could pose a potential risk to blood safety if they donate blood while being viraemic. It is possible however, that the duration of viraemia in mild or asymptomatic cases is shorter and the virus titre is lower than in symptomatic persons, but this hypothesis is far from proven. Moreover, the limited extend of current virus dissemination, as shown by the actual clustering of confirmed autochthonous cases, does not indicate that such asymptomatic infections could have been spread around the whole city of Nice. At present, it is difficult to quantify this risk, and only a retrospective survey of blood supplies from Nice between July and September 2010 would allow to estimate it better. In France, authorities in charge of blood routinely exclude all febrile donors from donation. No additional exclusion measures have been implemented after the two neighbouring cases as the risk for dengue transmission has been considered very low.

Further investigations to identify the likely source of exposure of the two cases, as well as extensive comparison of the dengue virus genotypes between the locally identified viruses and the strains currently circulating in the French West Indies, will hopefully allow a better understanding of this event. The reactive surveillance in addition to the routine enhanced surveillance

is likely to identify new symptomatic cases in the area, determining also the potential geographic extension of the risk. Finally, better understanding is needed on how the vector abundance, activity and competence of *Ae. albopictus* for dengue transmission influence the risk for further transmission in the region [25,32].

Conclusion

The current clustering of cases of locally transmitted dengue fever in Nice is a significant public health event, but is not unexpected and more cases can be predicted. Such transmission was anticipated by the development of a national plan. Although this plan should be adjusted in the light of this experience, this event shows the advantage of such preparedness in order to implement rapid and proportionate measures of surveillance and control. Previous events, including a mosquito-borne arbovirus outbreak in Italy, the occurrence of vector-borne diseases around airports and other ports of entry and a previous risk assessment on dengue virus introduction in European Union countries [4] indicate that autochthonous transmission in continental Europe is possible, as confirmed by the present event. However, according to the available epidemiological information, the risk for establishment of dengue transmission in south-eastern France or further spread in Europe currently appears limited. Further data available in the near future will allow us to re-assess this likelihood.

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Outbreak of haemolytic uraemic syndrome and bloody diarrhoea due to *Escherichia coli* O104:H4, south-west France, June 2011

G Gault[1], F X Weill[2], P Mariani-Kurkdjian[3], N Jourdan-da Silva[4], L King[4], B Aldabe[1], M Charron[1], N Ong[1], C Castor[1], M Macé[2], E Bingen[3], H Noël[4], V Vaillant[4], A Bone (a.bone@invs.sante.fr)[4,5], B Vendrely[6], Y Delmas[6], C Combe[6], R Bercion[7], E d'Andigné[7], M Desjardin[7], H de Valk[4], P Rolland[1]

1. Cellule interrégionale d'épidémiologie (CIRE) Aquitaine, France
2. Institut Pasteur, Centre National de Référence des Escherichia coli et Shigella, Paris, France
3. Laboratoire associé au CNR des Escherichia coli et Shigella, Service de Microbiologie, Hôpital Robert Debré, Paris, France
4. French Institute for Public Health Surveillance (Institut de Veille Sanitaire, InVS), St Maurice, France
5. European Programme for Intervention Epidemiology (EPIET), European Centre of Disease Prevention and Control (ECDC), Stockholm, Sweden
6. Centre Hospitalier Universitaire de Bordeaux, Bordeaux, France
7. Hôpital des Instruction des Armées Robert Piqué, Villenave-D'ornon, France

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As of 12:00 28 June 2011, 15 cases of haemolytic uraemic syndrome (HUS) or bloody diarrhoea have been identified in the Gironde, south-west France. Investigations suggest the vehicle of transmission was sprouts, served at an event in Bègles on 8 June 2011. A strain of shiga toxin- producing *Escherichia coli* O104:H4 has been isolated from five cases. This strain is genetically related to the strain identified in the recent *E. coli* O104:H4 outbreak in Germany, and shares the same virulence and antimicrobial resistance characteristics.

Outbreak description

On 22 June 2011, the Cellule interrégionale d'épidémiologie (CIRE) Aquitaine, the regional office of the French Institute for Public Health Surveillance, was notified by the Robert Picqué Hospital in Bordeaux, south-west France, of eight cases of haemolytic uraemic syndrome (HUS) or bloody diarrhoea. Six of the cases lived in close proximity to one another in the commune of Bègles, in Bordeaux. Of these six cases, four were women (aged 41–78 years) and two were men (aged 34–41 years). Dates of symptom onset were between 15 and 20 June.

A case of HUS was defined as a person with acute renal failure and either microangiopathic haemolytic anaemia and/or thrombocytopenia. A possible outbreak case was defined as a case of HUS or a case of bloody diarrhoea without an alternative diagnosis in the French department (administrative region) of Gironde with a date of symptom onset since 10 June 2011. Active case finding has been carried out through contact with emergency, nephrology and intensive care departments of local hospitals, and general practitioners and out-of-hours doctors, and through the existing paediatric HUS surveillance network. Enhanced surveillance for cases of HUS or bloody diarrhoea in the rest of France has been implemented.

As of 12:00 28 June 2011, a further seven cases have been identified and investigated, bringing the total number of cases investigated to date to 15 cases of bloody diarrhoea, eight of whom have developed HUS.

Epidemiological investigations

The initial eight cases were interviewed using a standardised semi-structured questionnaire exploring food consumption, travel history and contact with other people with diarrhoea in the seven days before symptom onset. Initially no common food, visits to markets, restaurants or events, animal contact or leisure activity was identified. None of the cases reported eating sprouts. Only three of the cases shared the same municipal tap-water network. One of the cases had travelled away from home in France during the seven days before symptom onset and none had travelled abroad.

Given that a common exposure had not been identified, the predominance of adult women among the cases and the recent experience of the German sprout-related *Escherichia coli* O104:H4 outbreak in Germany [1,2], a second questionnaire was developed that included an in-depth exploration of vegetable consumption in the two weeks before illness.

Further questioning of the initial eight cases and seven newly identified cases indicated that 11 of these 15 cases had attended an open day at a children's

community centre on 8 June, at which a cold buffet was served consisting of crudités (raw vegetables), three dips, industrially produced gazpacho, a choice of two cold soups (carrot and cumin, and courgette), pasteurised fruit juices and individual dishes composed of white grapes, tomatoes, sesame seeds, chives, industrially produced soft cheese and fresh fruit. The soups were served with fenugreek sprouts, a small amount of which were also placed on the crudité dishes. Mustard and rocket sprouts, still growing on cotton wool, were used to decorate the crudité dishes. One of the 11 cases has not yet been fully questioned because of a deteriorating clinical condition, but is known to collect their grandchildren from the centre and may have attended the event. The remaining four cases had no obvious links to the centre.

Among the 11 cases with links to the centre, nine reported consuming sprouts at the event on 8 June; two cannot yet be fully questioned. Of these 11 cases, eight have HUS and three bloody diarrhoea. Seven are women aged 31–64 years and four are men aged 34–41 years. Dates of symptom onset are between 15 and 20 June (Figure). For the eight cases with a well-defined date of symptom onset, the incubation period ranges from 7 to 12 days (median: 9 days).

Microbiological investigations

A strain of *E. coli* O104:H4 possessing the stx2 gene, encoding Shiga toxin, has been isolated from five HUS cases, all of whom consumed sprouts at the event at the children's community centre. The strain is negative for the genes coding for intimin (*eae*), haemolysin A (*hlyA*) and EAST1 toxin (*astA*) and positive for the *aggR* gene which regulates the expression of aggregative adherence fimbriae. The antimicrobial resistance pattern of the strain is similar to that seen in the outbreak strain in recent *E. coli* O104:H4 outbreak in Germany [3](ampicillin resistant (R), cefotaxime R, ceftazidime R, imipenem sensitive (S), streptomycin R, kanamycin S, gentamicin S, sulfamethoxazole R, trimethoprim R, cotrimoxazole R, tetracycline R, chloramphenicol S, nalidixic acid R and ciprofloxacin S). Our PCR analysis indicates the presence of the extended-spectrum beta-lactamase (ESBL) $bla_{\text{CTX-M-15}}$ (group 1) gene and the penicillinase bla_{TEM} gene.

Strains of *E. coli* O104:H4 isolated from two imported cases in France linked to the *E. coli* O104:H4 outbreak in Germany in May and June 2011 were compared by two molecular techniques (Rep-PCR [4,5] and pulsed-field gel electrophoresis (PFGE), using a standardised PFGE using either *Xba*I or *Not*I [6]) with strains of *E. coli* O104:H4 isolated from three patients in the Bordeaux outbreak. The results of these analyses show the genetic relatedness of the outbreak strains in France and Germany. The profile of the outbreak strains in the two countries differs from the profiles of two *E. coli* O104:H4 stx2 strains isolated in 2004 and 2009 and from two other strains of serotypes *E. coli* O104:H21 and O104:H12. Comparison by whole-genome sequencing and optical maps will be performed in the coming days.

Food trace-back investigations

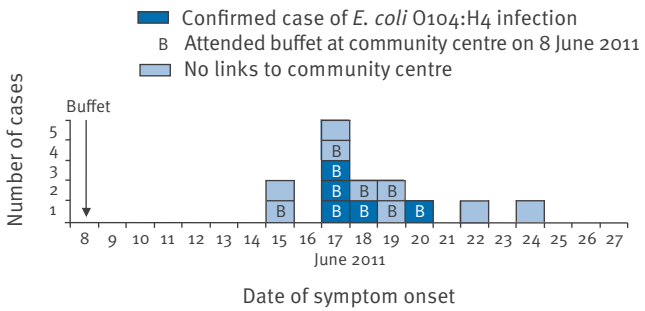
Food trace-back investigations were initiated on 24 June. The sprouts served at the event on 8 June had been grown from rocket, mustard and fenugreek seeds planted at the centre during 2 to 5 June. The fenugreek seeds were first soaked in tap water for 24 hours then placed in a jam jar topped with gauze and then rinsed with tap water two or three times a day. The mustard and rocket seeds were germinated on cotton wool moistened with tap water. They were harvested on 8 June to be served at the buffet. The seeds were purchased from a branch of a national chain of gardening retailers, having been supplied by a distributor in the United Kingdom. Leftover mustard and rocket seeds, gazpacho and tap water samples from the community centre have been sent for microbiological analysis, as have samples of rocket, mustard, fenugreek and other seeds from the French gardening retailer. Preliminary results are currently being analysed.

Control measures

Consumers have been advised by the French authorities not to eat raw sprouts, to thoroughly clean utensils used for germination and cooking, and to wash their hands thoroughly after contact with seeds and sprouts. Colleagues in other European countries were informed of this outbreak on 24 June via the Epidemic Intelligence Information System (EPIS) and Early Warning Response System (EWRS) of the European Centre of Disease Prevention and Control (ECDC). A European Food Standards Agency (EFSA) and ECDC joint rapid risk assessment has been carried out [7]. This assessment strongly recommends that consumers do not grow sprouts for their own consumption and do not eat sprouts or sprouted seeds unless thoroughly cooked.

Figure

Cases of HUS or bloody diarrhoea due to enterohaemorrhagic *Escherichia coli* O104:H4 with date of symptom onset since 10 June 2011, Gironde, France, June 2011 (n=14)



HUS: haemolytic uraemic syndrome.

Of the 15 cases of HUS or bloody diarrhoea, date of symptom onset was unavailable for one case, who attended the buffet on 8 June 2011.

Conclusions

Preliminary data indicate that this outbreak shares the same novel epidemiological, clinical and microbiological features identified in the *E. coli* O104:H4 outbreak in Germany [8], including a predominance of adult women among the cases, an unusually high proportion of HUS cases among identified possible outbreak cases, a longer median incubation period than expected for cases of Shiga toxin-producing *E. coli* infection, and a genetically related *E. coli* O104:H4 producing a CTX-M ESBL. The two outbreaks may share the same vehicle of transmission. A cohort study of those attending the event at the community centre and further epidemiological, microbiological and food trace-back investigations are underway. The possibility of similar outbreaks in France or elsewhere in Europe cannot be excluded.

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Four multifaceted countrywide campaigns to promote hand hygiene in Belgian hospitals between 2005 and 2011: impact on compliance to hand hygiene

M Costers (michiel.costers@health.fgov.be)[1], N Viseur[2], B Catry[2], A Simon[3]
1. Federal Public Service Health, Food Chain Safety and Environment, Belgian Antibiotic Policy Coordination Committee; Brussels, Belgium
2. Scientific Institute of Public Health, Healthcare Associated Infections and Antimicrobial Resistance; Brussels, Belgium
3. University Hospital Saint Luc UCL, Department of Microbiology and Infection Control; Brussels, Belgium

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Four consecutive one-month campaigns were organised to promote hand hygiene in Belgian hospitals between 2005 and 2011. The campaigns included a combination of reminders in wards, educational sessions for healthcare workers, promotion of alcohol-based hand rub use, increasing patient awareness, and audits with performance feedback. Prior and after each one month intervention period, the infection control teams measured hand hygiene compliance of healthcare workers by direct observation using a standardised observation roster. A total of 738,367 opportunities for hand hygiene were observed over the four campaigns. Compliance with hand hygiene significantly increased from 49.6% before to 68.6% after the intervention period for the first, from 53.2% to 69.5% for the second, from 58.0% to 69.1% for the third, and from 62.3% to 72.9% for the fourth campaign. The highest compliance rates were consistently observed in paediatric units. Compliance rates were always markedly lower among physicians than nurses. After patient contact and body fluid exposure risk, compliance rates were noticeably higher than before patient contact and performing aseptic procedures. We conclude that repeated countrywide campaigns to promote hand hygiene result in positive long-term outcomes. However, lower compliance rates among physicians compared with nurses, before patient contact, and before performing aseptic procedures remain challenges for future campaigns.

Introduction

Healthcare-associated infections (HAIs) place a tremendous burden on public health resources. A national point prevalence survey performed by the Belgian Health Care Knowledge Centre (KCE) in 2007 revealed a prevalence rate of infected patients of 6.2% in Belgian acute care hospitals, which amounts to an estimated 103,000 infected patients in this setting, annually [1]. Based on these data and matched cohort studies, the yearly excess in-hospital stay, healthcare payer cost and in-hospital mortality for patients with HAIs in Belgian acute care hospitals were estimated at 720,757 hospital-days, 384.3 million Euros and 2,625 deaths, respectively [2].

Transmission of microbial pathogens by the hands of healthcare workers (HCWs) during patient care plays a crucial role in the spread of HAIs [3]. Hence, it is not surprising that hand hygiene is generally regarded as the most effective measure to prevent these infections, with several reports showing a temporal relation between interventions to improve hand hygiene practices, higher compliance rates and/or reduced infection rates [4-8]. However, numerous reports indicate that hand hygiene compliance of HCWs remains disappointingly low, with mean baseline rates ranging from 5% to 89%, with an overall average of about 40% [4,5,9].

The Federal Platform for Infection Control (FPIC), with the support of the Belgian Antibiotic Policy Coordination Committee (BAPCOC), was able to procure funding of 125,000 Euros per campaign from the Belgian federal government for four multifaceted countrywide campaigns to improve hand hygiene compliance in Belgian hospitals. A multidisciplinary working group was created to organise these campaigns.

We describe the organisation of the Belgian campaigns and present their impact on compliance to hand hygiene by the HCWs.

Methods

Organisation of the campaigns

All Belgian acute care, chronic care and psychiatric hospitals were invited by the Federal Public Service Health, Food Chain Safety and Environment to voluntarily participate in the national campaigns. Psychiatric hospitals were invited from the second campaign onwards. The infection control (IC) teams of the participating

hospitals were responsible for the implementation of the campaign at their institution, and the working group organised workshops to inform the IC teams about the methodology of the campaigns and to provide training for measuring hand hygiene compliance.

Between 2005 and 2011, four campaigns were conducted, each lasting one month. The first campaign took place between 15 February and 15 March 2005, the second between 15 November and 15 December 2006, the third between 19 January and 13 February 2009, and the fourth between 14 February and 16 March 2011. The first three campaigns were launched by the Belgian Minister of Social Security and Public Health using press conferences. During the one-month intervention period of each campaign, the IC teams displayed or distributed campaign materials throughout their own institution and organised educational sessions for all HCWs. The IC teams were asked to measure hand hygiene compliance of HCWs by direct observation and to transfer these data to the Scientific Institute of Public Health (IPH). The observations before took place either in the weeks directly before the intervention (first campaign) or with an interval of one (second and third campaign) or two months (fourth campaign). The interval between the intervention and the observation of compliance after was one month (first and second campaign) or one and a half month (third and fourth campaign).

Campaign materials

The campaigns combined audits (with performance feedback), reminders in wards, educational sessions for HCWs, promotion of alcohol-based hand rub use, and information for patients. The campaign materials (Table 1) were provided free of charge to all participating institutions; they are available on the campaign website [10].

Measurement of hand hygiene compliance of healthcare workers by direct observation

Compliance to hand hygiene guidelines was measured by the IC teams by direct observation using a

TABLE 1

Materials used in four consecutive countrywide campaigns to promote hand hygiene in hospitals, Belgium, 2005–2011

Type of campaign material	Target group	Campaign number[a]
Posters with different topics		
Campaign slogan 'You are in good hands'	Healthcare workers and hospitalised patients	1, 2, 3, 4
Indications for hand hygiene–'When'	Healthcare workers and hospitalised patients	2, 3, 4
Correct hand hygiene technique using alcohol based hand rub–'How'	Healthcare workers and hospitalised patients	2, 3, 4
Rationale for hand hygiene–'Why'	Healthcare workers and hospitalised patients	3, 4
Deleterious effect on hand hygiene of jewels and bad nail hygiene	Healthcare workers and hospitalised patients	3, 4
Indications for glove use	Healthcare workers and hospitalised patients	3, 4
Role model for other healthcare worker	Healthcare workers and hospitalised patients	4
Leaflets for target groups		
Hospitalised patients' leaflets – first version	Hospitalised patients	1, 2, 3
Healthcare workers' leaflets	Healthcare workers	1
Physicians'leaflets	Physicians	3, 4
Hospitalised patients' leaflets – second version[b]	Hospitalised patients	4
Educational material		
Slide presentation for healthcare workers	Healthcare workers	1, 2, 3, 4
Slide presentation specifically targeted at physicians	Physicians	4
Gadgets with the campaign slogan[c]		
Pins	Healthcare workers	1
Badge holders	Healthcare workers	2, 3, 4
Bookmark	Hospitalised patients	3
Magnets	Healthcare workers	4
Web-based quiz on hand hygiene[d]	Healthcare workers[d]	2, 3, 4
Video clips on hand hygiene for hospital video circuit (n=2)	Healthcare workers and hospitalised patients	4
Questionnaire on hand hygiene	Healthcare workers	1

[a] Campaigns number 1, 2, 3, and 4 respectively took place in 2005, 2006, 2009, and 2011.

[b] The second version had more emphasis on patient empowerment.

[c] The campaign slogan was: 'You are in good hands'.

[d] The number of modules was gradually expanded, including modules specifically targeted at physicians, physiotherapists and healthcare workers in psychiatric hospitals.

standardised observation roster [11]. The opportunities for hand hygiene were counted and the actual episodes of hand hygiene were scored as hand hygiene with alcohol-based hand rub, hand hygiene with water and soap or no hand hygiene [12]. Compliance was stratified by indication (before patient contact, after patient contact, before an aseptic task, after body fluid exposure risk, after contact with patient surroundings) and by type of HCW (nurses, nursing assistants, physicians, physiotherapists, other). Thus, the metric used was the number of episodes divided by the number of opportunities. For each hospital unit included in the compliance survey, at least 150 opportunities had to be monitored both before and after the intervention period. Inclusion of the intensive care unit (ICU) was mandatory for the acute care hospitals, but otherwise the institutions were free to include any number or any type of (additional) hospital units in the compliance survey. If the hospitals sent their compliance data immediately to the IPH as suggested, they received feedback with benchmarking, defined as the position of the hospital in the national distribution, within a few days, allowing the IC teams to use this information as performance feedback to motivate HCWs in their institution.

Data management and statistical analysis

Data on hand hygiene compliance were entered in NSIHwin (MS Access application) [13], a software tool for data entry developed by the IPH and provided free of charge to participating institutions. This software tool also allows the user to generate some automatic reports for the hospital in question. Data from individual hospitals could be sent to the IPH to be appended to a national database. All data were processed and analysed using Stata 10.0 software. National results are given as a weighted mean, thus adjusting for varying numbers of observations between hospitals.

Results

Participation rates were good to excellent for the different types of hospitals, with at least 92% of acute care hospitals involved in each campaign, and at least 61% of chronic care hospitals and at least 61% of psychiatric hospitals, respectively (Table 2).

A total of 149,041 opportunities for hand hygiene (74,581 before and 74,460 after the intervention period) were observed during the first campaign, 196,685 (111,176 before and 85,509 after) during the second campaign, 223,719 (111,476 before and 112,243 after) during the third campaign, and 168,922 (89,553 before and 79,369 after) during the fourth campaign.

After each respective campaign, compliance with hand hygiene (national weighted mean for all hospital sites combined) increased significantly ($p<0.05$), from 49.6% before to 68.6% after the intervention for the first campaign (absolute increase in compliance rate, +19.0%), from 53.2% to 69.5% for the second campaign (+16.3%), from 58.0% to 69.1% for the third campaign (+11.1%), and from 62.3% to 72.9% for the fourth campaign (+10.6%).

The increase in compliance rates was observed in acute care hospitals, chronic care hospitals and psychiatric hospitals (Figure and Table 3). A wide distribution of the compliance rates of the different participating hospitals could be noticed (Figure).

Similarly to what could be observed at the hospitals and hospital type levels, compliance rates also improved significantly for all types of hospital units ($p<0.05$), with the highest compliance rates consistently being observed in paediatric units. Compliance rates were lowest for rehabilitation units during the first and fourth campaign and for surgical units during the second and third campaign.

Although compliance rates increased for all types of HCWs, it is remarkable that compliance was markedly lower (absolute difference in compliance rate, -13% to -20%, $p<0.05$) among physicians than nurses.

Compliance increased for all indications for hand hygiene but was much higher (absolute difference in compliance rate, often +20%, $p<0.05$) after patient contact and body fluid exposure risk than before patient contact and aseptic tasks, with compliance after contact with surroundings of patient somewhere in the middle (Table 3).

TABLE 2

Participation rate per type of hospital for four Belgian hand hygiene campaigns, Belgium, 2005–2011

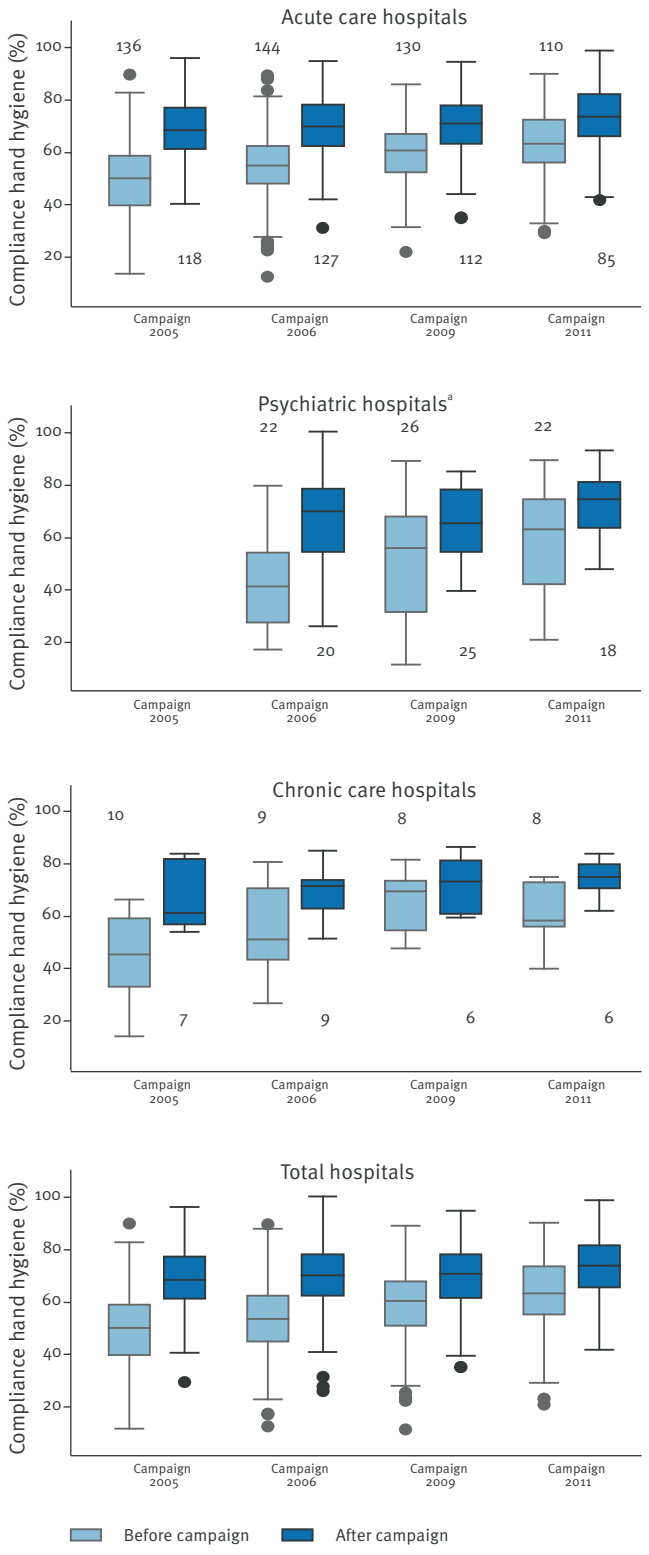
	Campaign 2005 n/N (%)	Campaign 2006 n/N (%)	Campaign 2009 n/N (%)	Campaign 2011 n/N (%)
Acute care hospitals	112/116 (97%)	113/116 (97%)	110/113 (97%)	98/107 (92%)
Chronic care hospitals	19/31 (61%)	22/30 (73%)	20/28 (71%)	16/24 (67%)
Psychiatric hospitals[a]	NA	43/68 (63%)	46/67 (69%)	41/67 (61%)
All hospitals	131/147 (89%)	178/214 (83%)	175/208 (84%)	156/198 (79%)

NA: Not available.

[a] Psychiatric hospitals were invited to participate in the study from the second campaign forth.

Figure

Distribution of compliance rates for acute care, chronic care and psychiatric hospitals[a] and for all hospital types combined before and after the Belgian hand hygiene campaigns, 2005–2011



The median (horizontal line in a box), inter-quartile range (box hight), as well as maximum and minimum limits (vertical whiskers) of the compliance rates are shown, as well as outliers (dots). The numbers above and below the box plots are the number of hospital sites that provided their compliance data to the Scientific Institute of Public Health.

[a] Psychiatric hospitals were invited to participate in the study from the second campaign onwards.

Overall, compliance with hand hygiene improved over the four campaigns. Furthermore, this improvement was partially sustained between campaigns: although compliance before the second, third and fourth campaign was lower than after the previous campaign, it was clearly higher than before the previous campaign. However, while before campaign compliance rates are steadily increasing over time from 49.6% to 62.3%, after campaign compliance rates seem to stabilise around 70%.

Discussion

In our study an increase in hand hygiene compliance was observed after each individual campaign to promote hand hygiene. Comparing the effect of the four campaigns over time also yielded an increased rate of compliance for all hospitals combined. The increase of compliance at the end of each campaign seemed to be partially sustained until the beginning of the next campaign. Although this suggests that the repeated campaigns resulted in an overall progressive improvement of hand hygiene, it is noteworthy that the participating hospitals may have varied between each campaign. The increase in hand hygiene compliance, however, was also observed for each type of hospitals, some of which, such as acute care hospitals, had a very high participation rate (over 92%). In this case, the hospitals participating in the different campaigns could not have varied much. The need for sustained or repeated interventions to obtain prolonged or permanent effects has moreover been documented previously [6,7,14,15].

The observation of a wide distribution of hand hygiene compliance rates of the different participating hospitals in this study can be partly explained by the type of hospital, the inclusion of different types of hospital units for measuring compliance, and inter-observer variability, but undoubtedly represents real differences between hospitals.

While the lower compliance to hand hygiene for physicians than for nurses confirms the findings of other authors [6,9,14-17], a study by Salemi et al. [18] shows that improvement of hand hygiene compliance among physicians is feasible .

That hand hygiene compliance for HCW is higher after patient contact and body fluid exposure than before patient contact and aseptic tasks has also been reported by others [6,9,14]. One explanation could be that HCWs are more inclined to protect themselves than their patients. Another possible interpretation is that HCWs are more likely to decontaminate their hands if they perceive them to be dirty [19].

Based on this study, the working group plans to repeat these national campaigns every two years with the fifth campaign scheduled for 2012–13. This forthcoming campaign will focus on hand hygiene before patient contact and aseptic tasks. Raising awareness among physicians of the importance of this deceptively simple

but crucial act also remains a priority. However, it could be that our national campaign approach, which is limited in time and not perfectly adapted to each specific setting, has reached its limits and that continuous initiatives more suited to the specific setting are needed to breach the ceiling of 70% compliance.

In 2009, twelve other European countries had also organised countrywide campaigns to promote hand hygiene [20]. However, national data demonstrating the impact of these campaigns on hand hygiene compliance and/or consumption of alcohol based hand rub solutions were not often collected or are not yet published. In fact, published data are at present only available for the United Kingdom: the NOSEC study (National Observational Study to Evaluate the cleanyourhands campaign) demonstrated a rise in the combined median use of alcohol-based hand rubs and soap from 13.2 to 31 mL/patient-bed-day, but there were no changes in HAI rates [21].

As with most studies in this research field, our study has several limitations. First, we used an uncontrolled before-and-after design so as to implement the campaign in a maximum number of institutions (no control group at the hospital level); and to limit the workload of the IC teams, we did not include control units (no control group at the hospital unit level). Second, although direct observation is considered the most appropriate method for measuring hand hygiene compliance rates, it still has several drawbacks including the 'Hawthorne effect', concerns with inter-observer reliability, and the fact that it only represents a sample of all hand hygiene opportunities [22,23]. Third, rates of HAIs were not evaluated. On the other hand, several studies have demonstrated a link between improvement of hand hygiene compliance and reduction of methicillin-resistant *Staphylococcus aureus* (MRSA) bacteraemia or HAI rates [24-30]. Finally, hand hygiene technique was not used as an outcome measure since standardised evaluation of this qualitative aspect is

TABLE 3

Compliance rate per type of hospital, type of hospital unit, type of healthcare worker and indication for hand hygiene during four Belgian hand hygiene campaigns, Belgium, 2005–2011

	Hand hygiene compliance (%)							
	Campaign 2005		Campaign 2006		Campaign 2009		Campaign 2011	
	Before	After	Before	After	Before	After	Before	After
Type of hospital								
All types	49.6	68.6	53.2	69.5	58.0	69.1	62.3	72.9
Acute care	50.4	69.0	54.8	70.2	58.9	69.8	63.2	73.1
Chronic care	45.5	67.6	56.6	70.0	66.0	72.9	61.6	74.7
Psychiatric	–	–	43.3	64.8	52.2	65.4	58.9	72.6
Type of hospital unit								
Intensive care unit	52.6	68.9	58.9	70.4	62.3	70.1	66.9	74.5
Surgery	49.5	69.6	51.4	65.7	55.7	67.5	61.4	70.7
Internal medicine	47.7	67.5	53.9	70.6	62.1	69.8	61.3	70.3
Paediatrics	60.1	76.1	65.8	76.9	65.7	74.4	71.2	80.4
Geriatrics	48.2	71.9	55.3	70.7	58.4	70.1	60.5	71.0
Rehabilitation	42.2	64.7	53.8	69.4	61.3	70.1	58.2	67.3
Type of healthcare worker								
Nurse	54.4	72.3	57.3	73.2	61.7	73.2	66.2	76.9
Nursing assistant	44.4	67.3	51.1	66.7	57.1	68.5	62.5	71.8
Physician	37.6	54.1	42.2	54.4	45.7	54.0	53.0	57.1
Physiotherapist	48.7	66.3	52.8	67.4	54.6	64.7	61.8	69.0
Other	33.2	61.4	40.2	56.5	48.8	58.0	52.6	63.8
Indication for hand hygiene								
Before patient contact	35.9	56.6	39.0	57.0	44.2	56.8	50.2	62.7
After patient contact	60.3	78.5	62.9	76.4	66.9	76.7	71.3	79.5
Before aseptic task	37.7	54.9	42.2	60.6	46.9	60.0	50.7	62.8
After body fluid exposure risk	61.4	76.4	65.0	79.6	69.1	78.9	72.8	82.9
After contact with surroundings of patient	47.8	68.2	49.6	66.6	53.9	64.8	57.3	69.3

All differences between compliance rates before and after each campaign are statistically significant ($p < 0.05$).

extremely difficult, especially when so many observers are involved [23].

On the other hand, our study has several unique strengths. It is the first publication of an intervention to improve hand hygiene on such a large countrywide scale, with a grand total of 738,367 opportunities observed. Furthermore, the scope is unprecedented with the participation of acute care, chronic care and psychiatric hospitals, and the observation of all types of HCWs over a broad range of different hospital units. Finally, we provide data for four successive campaigns over a six-year period.

We conclude that countrywide campaigns to promote hand hygiene are feasible and have positive short term and long term results when they are repeated regularly.

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Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction

V M Corman[1,2], I Eckerle[1], T Bleicker[1], A Zaki[3], O Landt[4], M Eschbach-Bludau[1], S van Boheemen[5], R Gopal[6], M Ballhause[4], T M Bestebroer[5], D Muth[1], M A Müller[1], J F Drexler[1], M Zambon[6], A D Osterhaus[5], R M Fouchier[5], C Drosten (drosten@virology-bonn.de)[1]

1. Institute of Virology, University of Bonn Medical Centre, Bonn, Germany
2. German Centre for Infection Research (DZIF), Germany
3. Virology Laboratory, Dr Soliman Fakeeh Hospital, Jeddah
4. TibMolbiol, Berlin, Germany
5. Department of Virology and Virosciences, Erasmus Medical Centre, Rotterdam, The Netherlands
6. Health Protection Agency (HPA), London, United Kingdom

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We present two real-time reverse-transcription polymerase chain reaction assays for a novel human coronavirus (CoV), targeting regions upstream of the E gene (upE) or within open reading frame (ORF)1b, respectively. Sensitivity for upE is 3.4 copies per reaction (95% confidence interval (CI): 2.5–6.9 copies) or 291 copies/mL of sample. No cross-reactivity was observed with coronaviruses OC43, NL63, 229E, SARS-CoV, nor with 92 clinical specimens containing common human respiratory viruses. We recommend using upE for screening and ORF1b for confirmation.

Introduction

Coronaviruses (CoV) are large positive-stranded RNA viruses causing mainly respiratory and enteric disease in a range of animals and in humans. Humans are known to maintain circulation of four different human coronaviruses (hCoV) at a global population level. These are part of the spectrum of agents that cause the common cold. The SARS-CoV constitutes a fifth hCoV, which was in circulation for a limited time during 2002 and 2003, when a novel virus appeared in humans and caused an outbreak affecting at least 8,000 people. Mortality was high, at ca. 10% [1]. Symptoms matched the clinical picture of acute primary viral pneumonia, termed severe acute respiratory syndrome (SARS).

During September 2012, health authorities were notified of two cases of severe hCoV infection caused by a novel virus type. Both patients had travelled, or resided, in Saudi Arabia. Laboratories dealing with each of these unlinked cases were situated in Jeddah, Rotterdam and London, respectively.

In a collaborative activity co-ordinated by major European and national epidemic response networks we have developed diagnostic real-time reverse-transcription polymerase chain reaction (RT-PCR) assays suitable for qualitative and quantitative detection of the new agent. Here we summarise the technical evaluation and analytical performance of these assays.

Materials and methods

Template for design of assays

A provisional genome sequence as well as an isolate of the new virus were obtained from author RM Fouchier on 24 September 2012, after public notification of the second case case, who was in the United Kingdom (UK), to be most probably infected by the same virus as the first case, yet unrelated. The sequence (GenBank accession number: JX869059 for the Rotterdam virus isolate, termed hCoV-EMC) served as the template for assay design, and the virus was used for initial validation experiments.

Clinical samples

Respiratory swab, sputum, and endotracheal aspirate material was obtained during 2010–2012 from several hospital wards of the University of Bonn Medical Centre.

Cell culture

Vero cells were infected with a the cell culture isolate (unpublished data) at two different doses (multiplicities of infection (MOI) of ca. 0.1 and ca. 10 TCID50 per cell) and harvested after 0, 12, 24, and 36 hours for RT-PCR analysis.

RNA extraction

RNA was extracted from the samples as described earlier [2] by using a viral RNA mini kit (Qiagen). Sputum samples were pretreated with 2× sputum lysis buffer (10 g of N-acetylcysteine/litre, 0.9% sodium chloride) for 30 minutes in a shaking incubator. Swabs were immersed in lysis buffer.

Real-time reverse-transcription polymerase chain reaction screening assay upstream of E gene (upE assay)

A 25-µl reaction was set up containing 5 µl of RNA, 12.5 µl of 2 X reaction buffer provided with the Superscript III one step RT-PCR system with Platinum Taq Polymerase (Invitrogen; containing 0.4 mM of each dNTP and 3.2 mM Magnesium sulfate), 1 µl of reverse transcriptase/Taq mixture from the kit, 0.4 µl of a 50 mM magnesium sulfate solution (Invitrogen – not provided with the kit), 1 µg of non-acetylated bovine serum albumin (Sigma), 400 nM concentrations of primer upE-Fwd (GCAACGCGCGATTCAGTT) and primer upE-Rev (GCCTCTACACGGGACCCATA), as well as 200 nM of probe upE-Prb (6-carboxyfluorescein [FAM])-CTCTTCACATAATCGCCCCGAGCTCG-6-carboxy-N,N,N,N´-tetramethylrhodamine [TAMRA]). All oligonucleotides were synthesized and provided by Tib-Molbiol, Berlin. Thermal cycling involved 55°C for 20 min, followed by 95°C for 3 min and then 45 cycles of 95°C for 15 s, 58°C for 30 s.

It should be mentioned that common one-step real-time RT-PCR kits formulated for application with probes should all provide satisfactory results with default reaction mix compositions as suggested by manufacturers. In the particular case of our formulation the bovine serum albumin can be omitted if using a PCR instrument with plastic tubes. The component only serves the purpose of enabling glass capillary-based PCR cycling.

Real-time reverse-transcription polymerase chain reaction confirmatory assay (open reading frame (ORF)1b gene)

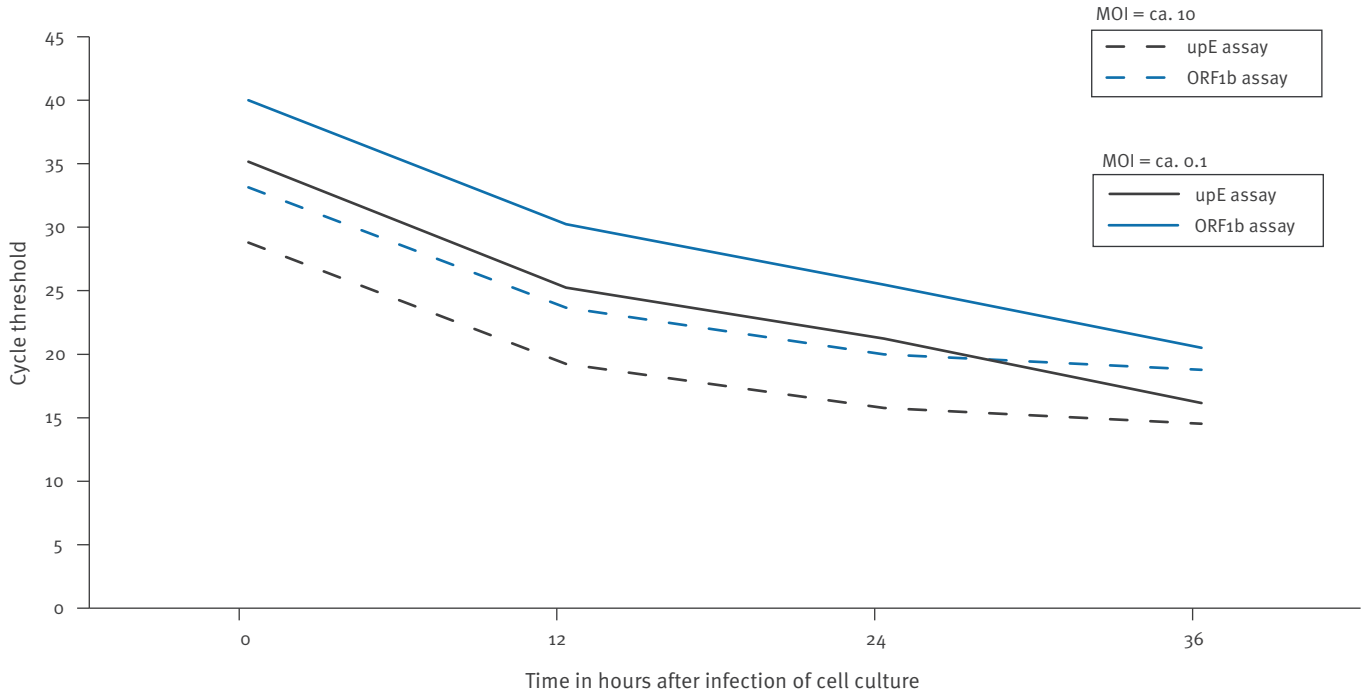
The assay had the same conditions as for the upE RT-PCR, except primer and probe sequences were ORF1b-Fwd (TTCGATGTTGAGGGTGCTCAT), primer ORF1b-Rev (TCACACCAGTTGAAAATCCTAATTG), and probe ORF1b-Prb (6-carboxyfluorescein [FAM])- CCCGTAATGCATGTGGCACCAATGT-6-carboxy-N,N,N,N´-tetramethylrhodamine [TAMRA]). This target gene did not overlap with those of known pan-CoV assays [3-5].

In-vitro transcribed RNA controls

PCR fragments covering the target regions of both assays, and some additional flanking nucleotides ('peri-amplicon fragments'), were generated using primers CTTCTCATGGTATGGTCCCTGT and AAGCCATACACACCAAGAGTGT for the upE assay, and CGAGTGATGAGCTTTGCGTGA and CCTTATGCATAAGAGGCACGAG for the ORF1b assay. Products were ligated into pCR 4 plasmid vectors and cloned in *Escherichia coli* by means of a pCR 4-TOPO TA

Figure 1

Replication of hCoV-EMC monitored by the upE and ORF1b RT-PCR assays, 2012



MOI : mulitplicity of infection (TCID50 per cell); RT-PCR: real-time reverse transcription-polymerase chain reaction; upE: upstream of the E gene; ORF1b: open reading frame 1b gene.

Vero cells were infected with hCoV-EMC at two different doses (MOI: ca. 10 and MOI: ca. 0.1) and standardised samples taken at different time points (after 0, 12, 24, and 36 hours) were tested by the upE and ORF1b RT-PCR assays.

cloning reagent set (Invitrogen). Plasmids were examined for correct orientation of inserts by PCR, purified, and re-amplified with plasmid-specific primers from the reagent set to reduce the plasmid background in subsequent in vitro transcription. Products were transcribed into RNA with the MegaScript T7 in vitro transcription reagent set (Ambion). After DNase I digestion, RNA transcripts were purified with Qiagen RNeasy columns and quantified photometrically. All transcript dilutions were carried out in nuclease-free water containing 10 µg/mL carrier RNA (Qiagen).

Determination of analytical sensitivities of real-time reverse-transcription polymerase chain reaction methods

Series of eight parallel reactions per concentration step were prepared and tested by the respective RT-PCR to determine concentration-dependent hit rates. Hit rates were subjected to probit regression analysis in StatgraphicsPlus software (version 5.0; Statistical Graphics Corp.).

Specificity of the assays

Assay specificity was determined using high-titred virus stock solutions, as well as clinical samples known to contain respiratory viruses. All material stemmed from the in-house strain and sample collection of University of Bonn, Institute of Virology. Identities and virus RNA concentrations were re-confirmed by specific real-time RT-PCRs for each virus before the experiment. Measured RNA concentrations are listed below along with the recorded stock virus titres.

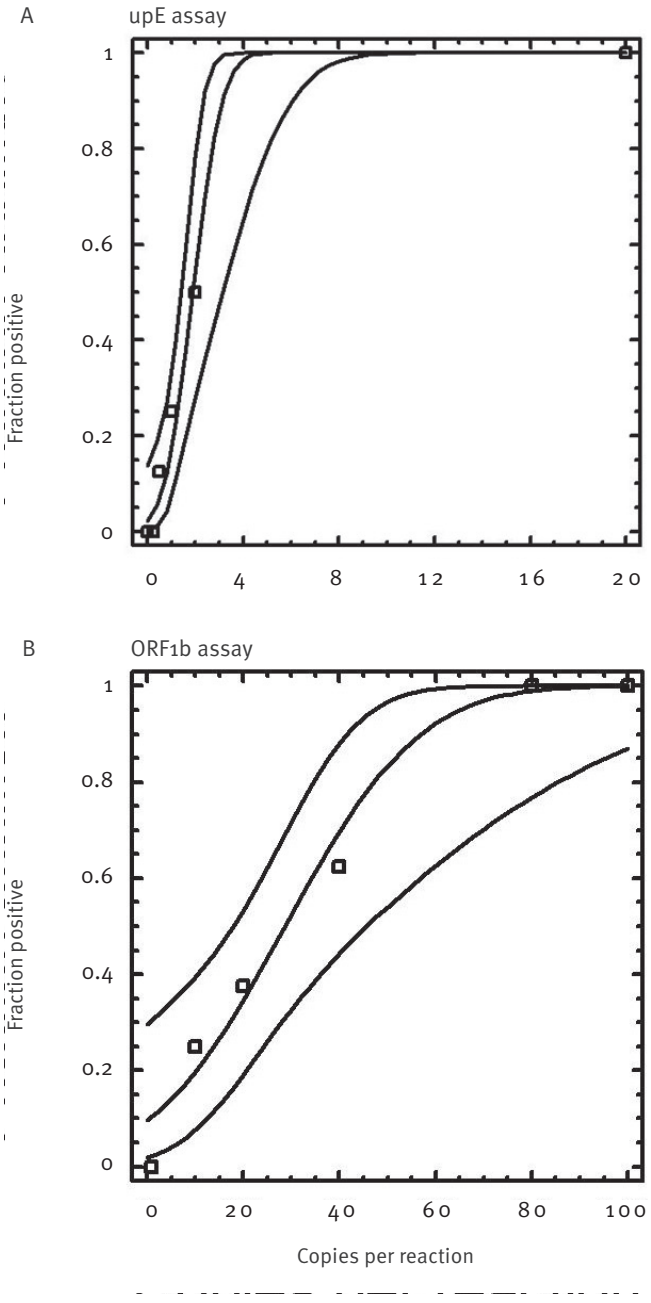
Results

Upon scanning of a provisional genome assembly, a region upstream of the putative E gene was identified as a particularly suitable target region for a real-time RT-PCR assay. The assay designed for this region is hereafter referred to as the upE-assay. A confirmatory test was designed in the open reading frame 1b (termed the ORF1b assay). This target gene did not overlap with those of known pan-CoV assays [3-5].

In order to obtain an estimate of the end point sensitivity of the assays, they were applied to cell culture-derived virus stock. The virus had a titre of 1.26 x 10^7 median tissue culture infective dose (TCID50)/mL. In limiting dilution experiments, the upE and ORF1b assays detected down to 0.01 and 0.1 TCID50 per reaction, respectively. The discrepancy between assays might be due to release of subgenomic RNA after onset of cytopathogenic effect (CPE) in cell culture, including the upE target fragment. As shown in Figure 1, PCRs on these samples indicated no divergence between the assays after onset of CPE (observed at 24h onwards). However, both assays deviated from each other by constant numbers of Ct values over the full duration of incubation, including time 0 (T0) when the cells were just infected and when no subgenomic RNA could have been present. It was concluded that the higher Ct values at each time point, and the lower dilution end point

Figure 2

Probit regression analysis to determine limit of detection for the upE and ORF1b assays, 2012



ORF: open reading frame of the frame; upE: upstream of the E gene.

The y-axis shows fractional hit-rates (positive reactions per reactions performed), the x-axis shows input RNA copies per reaction. Squares are experimental datum points resulting from replicate testing of given concentrations in parallels assays. The middle regression line is a probit curve (dose-response rule). The outer lines are 95% confidence intervals.

Table 1

Results of sensitivity and specificity tests for hCoV-EMC assays, 2012*

Experiment	upE assay	ORF1b assay
Detection end point for cell culture-derived virus	0.01 TCID50/reaction	0.1 TCID50/reaction
Technical LOD	3.4 RNA copies/reaction (95% CI: 2.5–6.9 copies/reaction)	64 RNA copies/reaction (95% CI: 47–126 copies/reaction)
Cross-reactivity with hCoV-229E	No reactivity with virus containing 10^5 PFU/mL (3×10^9 RNA copies/mL)	
Cross-reactivity with hCoV-NL63	No reactivity with virus containing 10^6 PFU/mL (4×10^9 copies/mL)	
Cross-reactivity with hCoV-OC43	No reactivity with virus containing 5×10^5 PFU/mL (3×10^{10} copies/mL)	
Cross-reactivity with SARS-CoV	No reactivity with virus containing 3×10^6 PFU/mL (5×10^{10} copies/mL)	

CI: confidence interval CoV: corona virus; LOD: limit of detection; ORF: open reading frame; PFU: plaque forming units; TCID50: median tissue culture infective dose; upE: upstream of the E gene.

for the ORF1b assay indicated that this assay had a lower sensitivity.

A more detailed assessment of technical sensitivity can be achieved using quantified, in-vitro transcribed RNA derived from the peri-amplicon region of each assay. These transcripts were generated and tested in serial ten-fold dilution experiments. Detection end points were two copies per reaction for the upE assay, and 10 copies per reaction for the confirmatory, ORF1b gene, assay. To obtain a statistically robust assessment of Limit Of Detection (LOD), transcripts were also tested in multiple parallel reactions in smaller dilution intervals above and below the end-point PCR limits. The results in terms of the fraction of positive reactions at each concentration were subjected to probit regression analysis and plotted as shown in Figure 2, where panel A shows the upE assay and panel B the ORF1b assay. The resulting LODs are summarised in Table 1. Based on the upE assay with a detection limit of 3.4 copies per reaction, and a cell-culture endpoint equivalent to 0.01 TCID50 per reaction, it was calculated that the RNA/infectious unit ratio of the virus stock must have been ca. 29 (100/3.4).

To exclude non-specific reactivity of oligonucleotides among each other, all formulations were tested 40 times in parallel with assays containing water and no other nucleic acids except the provided oligonucleotides. In none of these reactions was any positive signal seen. Cross-reactivity with known, heterospecific human CoVs was excluded by testing high-titred cell culture materials as summarised in Table 1. It should be noted that the unculturable hCoV-HKU1 was not included in these experiments.

To obtain a more clinically relevant figure on assay specificity, the assays were applied on 92 original clinical samples in which other respiratory viruses had already been detected during routine respiratory screening at Bonn University Medical Centre. These samples were prepared using the Qiagen Viral RNA kit, a formulation widely used to extract RNA in clinical laboratories. Of note, the tested panel included four samples containing hCoV-HKU1, which was not available as cultured virus stock. In total, none of the 92 original clinical samples as presented in Table 2, containing a wide range of respiratory viruses, gave any detection signal with either assay while positive controls were readily detected. It was concluded that the assay could be reliably applied to clinical samples.

Preliminary testing was also done on a patient hospitalised with acute infection during preparation of this report (Authors R Gopal and M Zambon, own unpublished observations). Both assays provided very clear amplification signal on various clinical samples. The upE assay again appeared more sensitive than the ORF1b assay.

Discussion

Here we provide the technical background data for RT-PCR assays developed in rapid response to the emergence of a novel human CoV (GenBank accession number: JX869059 for the Rotterdam virus isolate, termed hCoV-EMC).

Cell culture-derived virus is a useful source of reference material for the evaluation of molecular detection assays. However, detection end points determined on cell culture-derived virus are difficult to correlate to virus titre. Reasons include the discrepancy between

infectious viral particles and the number of copies of viral RNA, as well as the imbalance between viral genomic and subgenomic transcripts in the particular case of CoVs. This is important for laboratories using cell-cultured virus as reference, but also in the clinical setting. For example, SARS-CoV assays targeting structural protein genes tend to be slightly more sensitive than ORF1b-based assays when applied to clinical samples [6]. For the novel virus the ratio of RNA copies per infectious unit was ca. 29, while little imbalance seems to exist between genomic and subgenomic RNA in Vero cells up to 36 h post infection.

While we are not addressing the issue of quantitative PCR in this report, it should be mentioned that the availability of synthetic RNA standards enables immediate implementation of quantitative virus detection that is essential for case management and public health. Quantitative virus data can help assess the height and duration of virus excretion, and can also be useful as an early and robust parameter for the success of treatment [2,7,8]. Here we have used synthetic RNA to determine technical limits of detection in the style of standards applied by industry, taking inter-assay variation into account and providing statistically robust detection end points based on physically quantified target genes, which is impossible to achieve on cell-cultured virus. It is important to note that the detection limits we describe here are expressed as copies per reaction. We have chosen not to translate these numbers into other terms such as 'copies per ml of sputum', 'copies per swab sample', or 'copies per gram of faeces'. Such transformations vary greatly between different RNA extraction methods and clinical materials. However, we can project that the level of sensitivity, particularly for the upE assay, is very similar to those levels achieved with most advanced RT-PCR assays developed for the SARS-CoV [6,8]. For example, the Qiagen Viral RNA kit with an input volume of 140 µl of sample and an elution volume of 60 µl as recommended by the manufacturer involves a conversion factor of 85.7 between copies per reaction and copies per mL of sample. The upE assay should thus detect as little as ca. 291 copies per mL of sputum with 95% certainty. For solid samples such as swabs, which can be dipped into the lysis buffer, the resulting conversion factor is 12, resulting in a projected capability of the assay to detect as little as ca. 41 copies per swab with 95% certainty.

In this regard it is highly important to remember practical experiences made with SARS-CoV detection. Even with the highest levels of RT-PCR sensitivity it turned out that not all patients retrospectively shown to seroconvert could be diagnosed by RT-PCR in the acute phase of disease [6,8,9]. This has been ascribed to the fact the SARS-CoV replication occurs predominantly in the lower respiratory tract due to the anatomical localisation of its entry receptor, Angiotensin-converting enzyme 2 (ACE2). Should the novel virus use the same receptor, we might see a similar distribution of virus, and similar challenges in clinical application of molecular diagnostics. Studies of virus concentration in clinical samples are underway to address these highly critical issues.

Table 2

Known respiratory viruses in clinical samples used for testing the specificity of hCoV-EMC assays, 2012

Virus	Number of samples tested
Parainfluenza virus	
Parainfluenza 1 virus	5
Parainfluenza 2 virus	5
Parainfluenza 3 virus	8
Parainfluenza 4 virus	1
Respiratory syncytial virus	7
Human metapneumovirus	8
Coronavirus	
hCoV-NL63	6
hCoV-OC43	4
hCoV-229E	2
hCoV-HKU1	4
Rhinovirus	8
Enterovirus	9
Adenovirus	8
Human Parechovirus	
Type 1	5
Type 3	3
Influenza A (H_1N_1, H_3N_2)	9
Influenza B	2
Total	**92**

Specificity is a very important issue in rare, highly critical virus infections for which a broad number of differential diagnoses exist. The risk associated with false positive PCR results posed a challenge in development of the assays described here. First, real-time PCR can yield artificial signals due to technical interference of oligonucleotides involved in the assay (resembling primer dimers in which probe sequences participate). These may be observed at infrequent intervals due to the statistical nature of nonspecific random molecular interactions. We have taken care to exclude the occurrence of those signals by testing large series of water-containing assays. Second, any virus detection assay might cross-react with related viruses, and there is worldwide circulation of four different human CoVs. Viral stock solutions were tested in order to exclude cross-reactivity even on high-titred materials. In spite of the favourable outcome of this experiment, it should

be mentioned that of the two assays investigated, the target gene of the ORF1b-based assay was most conserved between CoV. The genetic range of known CoV from animals is larger than those human viruses tested here. Theoretical comparisons between genomes of these viruses and our ORF1b assay suggested no risk of significant cross-reactivity (not shown). However, in absence of further investigation we tend to recommend using the upE assay for case management. This is also due to the lower sensitivity of the ORF1b assay.

The final proof of assay specificity was provided in a set of clinical samples that was assembled to realistically reflect the composition of patient groups presenting with Acute respiratory infections (ARI). Of note, also the four 'common-cold coronaviruses' hCoV-NL63, -229E, -OC43, and -HKU1 were included in this panel. Consequentially, we can say from these data that typical human CoV will not cross-react with the assay, even under adverse conditions such as those created by the additional presence of patient-derived nucleic acid and other components typical of clinical samples that may all interfere with the performance of PCR.

The open availability of proven diagnostic assays early in an epidemic is useful in order to equip and prepare public health laboratories efficiently [10,11]. However, there is a number of caveats associated with the wide and largely uncontrolled provision of such technology during the very early phase of an epidemic. In this phase public health authorities around the world have to monitor the development of case statistics in order to make projections and attain epidemic risk assessment. The notification of false positive laboratory results can be highly detrimental during this phase of the epidemic.

The authors of this paper will provide in-vitro transcribed RNA controls to health professionals (refer to Acknowledgements section) but will not be able to provide intense technical advice. Authors will follow the policy of providing only one control, namely that for the upE assay, in order to minimise opportunities for accidental laboratory contamination. If laboratories find patient samples positive by the upE assay and control, they can conduct confirmatory testing using the ORF1b assay. A positive result in this test would most likely not be due to contamination. Of note, the target gene of our ORF1b assay does not overlap with that of other, so-called 'pan-CoV' assays [3-5], excluding the possibility of contaminating our assay with high-titred controls or PCR products from these assays.

In this light we should mention that we have been working on an N gene-based assay as well, but our experience with testing clinical material strongly suggests N-gene assays should not be used for diagnostic application for the time being, i.e., as long as no direct sequence information of the N gene is available from clinical samples.

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*Erratum:

Table 1 was corrected and replaced on 28 September 2012.

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Severe respiratory illness caused by a novel coronavirus, in a patient transferred to the United Kingdom from the Middle East, September 2012

A Bermingham[1], M A Chand (meera.chand@hpa.org.uk)[1], C S Brown[1,2], E Aarons[3], C Tong[3], C Langrish[3], K Hoschler[1], K Brown[1], M Galiano[1], R Myers[1], R G Pebody[1], H K Green[1], N L Boddington[1], R Gopal[1], N Price[3], W Newsholme[3], C Drosten[4], R A Fouchier[5], M Zambon[1]

1. Health Protection Agency (HPA), London, United Kingdom
2. Centre for Clinical Infection and Diagnostics Research, King's College London, London, England
3. Guy's and St Thomas' NHS Foundation Trust and King's Health Partners, London, United Kingdom
4. Institute of Virology, University of Bonn Medical Centre, Bonn, Germany
5. Department of Virology, Erasmus Medical Centre, Rotterdam, the Netherlands

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Coronaviruses have the potential to cause severe transmissible human disease, as demonstrated by the severe acute respiratory syndrome (SARS) outbreak of 2003. We describe here the clinical and virological features of a novel coronavirus infection causing severe respiratory illness in a patient transferred to London, United Kingdom, from the Gulf region of the Middle East.

Introduction

Coronaviruses are recognised causes of mild respiratory tract infections in humans, first identified in the 1960s [1]. These large RNA viruses affect a wide range of animals including domestic and companion animals and bats [2]. Limited surveillance data show that bats host the greatest diversity of coronaviruses, varying by region and species [3], suggesting that they may be the natural reservoir.

The severe acute respiratory syndrome (SARS) outbreak of 2003 – affecting over 8,000 people across three continents with a case fatality ratio of about 10% [4] – indicates the potential of an animal coronavirus to jump species and transmit from person to person causing severe illness. This experience has raised awareness of the potential threat from zoonotic coronaviral infections and the need to adopt strict infection control measures when such cases are found, especially in healthcare settings. We describe here the clinical features and diagnostic detection of a novel coronavirus infection in a severely ill adult transferred to London, United Kingdom, from the Gulf region of the Middle East for medical care.

Case history

On 14 September 2012, the United Kingdom Health Protection Agency (HPA) Imported Fever Service was notified of a case of unexplained severe respiratory illness in a London intensive care unit. The patient had recently transferred from Qatar and had a history of travel to Saudi Arabia.

He was a previously well 49 year-old man who developed a mild undiagnosed respiratory illness while visiting Saudi Arabia during August 2012, which fully resolved. He subsequently presented to a physician in Qatar on 3 September, with cough, myalgia and arthralgia, and was prescribed oral antibiotics. Five days later, he was admitted to a Qatari hospital with fever (38.4 °C) and hypoxia, with oxygen saturation of 91% on room air. A chest X-ray showed bilateral lower zone consolidation. He was treated with ceftriaxone, azithromycin and oseltamivir. After 48 hours, he required intubation and ventilation and was transferred by air ambulance to London. During transfer, he was clinically unstable, requiring manual ventilation.

On admission to intensive care in London, he remained severely hypoxic, achieving an arterial PaO_2 of 6.5 kPA (normal range: 11–13 kPA) on 100% oxygen with optimised pressure ventilation, and required low-dose norepinephrine to maintain blood pressure. His white blood cell count was 9.1 x 10^9/L (normal range: 4–11 x 10^9/L), C-reactive protein 350 mg/L (normal range: 0–10 mg/L) and creatinine 353 µmol/L (normal range: 53–97 µmol/L), with normal liver function and coagulation. He was treated with corticosteroids and broad-spectrum antibiotics, initially meropenem, clarithromycin and teicoplanin. Colistin and liposomal amphotericin B were subsequently added.

His condition deteriorated between 11 and 20 September, with progressive hypoxia. His C-reactive protein level peaked at 440 mg/L and procalcitonin at 68 ng/ml (normal level: ‹0.5 ng/ml). His renal function worsened and haemofiltration was initiated on 14

Table 1

Microbiological investigations performed on London patient with novel coronavirus infection, September 2012

Source	Sample	Date of investigation (September 2012)																
		9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Qatar	Broncho-alveolar lavage																	
London: ICU	Combined nose and throat swab																	
	Local bacterial/viral testing[a]																	
	Imported fever panel (blood/serum/urine/throat swab)[b]																	
	Sputum																	
	Nose swab																	
	Throat swab																	
	Tracheal aspirate																	
London: specialist ICU	Broncho-alveolar lavage[c]																	
	Cerebrospinal fluid																	
	Blood (EDTA/serum)																	
	Stool																	

EDTA: ethylenediaminetetraacetic acid; ICU: intensive care unit; PCR: polymerase chain reaction.

Red = coronavirus detected (pan-coronavirus assay and real-time PCR assay for UpE and ORF1b (specific for novel coronavirus)
Green = no pathogens detected, including testing by pan-coronavirus assay
Blue = negative for all pathogens (not tested by pan-coronavirus assay)

[a] Included multiple blood and sputum cultures; urinalysis; atypical pneumonia screen; blood-borne virus screen; Epstein–Barr virus, cytomegalovirus, and varicella zoster virus; respiratory virus screen; mycobacterial respiratory screen; and tracheostomy site culture.

[b] Included dengue virus; West Nile virus; chikungunya virus; hantavirus; Sindbis virus; Rift Valley fever virus; sandfly viruses; Rickettsiae; Coxiella burnettii; Burkholderia mallei and B. pseudomallei.

[c] Negative for respiratory bacterial culture and mycobacterial stain and respiratory Influenza A/B, parainfluenza 1-4, RSV A/B, human metapneumovirus, enterovirus, rhinovirus, adenovirus, human bocavirus, and the human coronaviruses (NL63, 229E, OC43, HKU1).

September. He was transferred to a specialist intensive care unit and on 20 September (day 17 of illness), extracorporeal membrane oxygenation (ECMO) was started. As of 2 October, he remains stable but fully dependent on ECMO after 13 days (day 30 of illness).

Diagnostic approach

Microbiological diagnostics in Qatar and London were used to look initially for common viral and bacterial causes of severe respiratory illness and subsequently for pathogens endemic in the Middle East (Table 1). By mid-September, the syndrome was considered most compatible with viral pneumonia. Upper and lower respiratory tract samples were sent to the HPA Respiratory Virus Unit for extended influenza testing; all were negative. On 20 September, a ProMED report described a novel human coronavirus recovered from an adult male Saudi Arabian who died in June 2012 following acute respiratory illness, pneumonia and renal failure [5]. The Erasmus Medical Center (the Netherlands) had sequenced the virus and identified it as a previously undescribed coronavirus, related to known bat coronaviruses. Given that the patient described in our report had travelled to Saudi Arabia, HPA, in consultation with local clinicians, decided to investigate samples from the patient for the presence of the novel coronavirus.

Detection of a novel coronavirus

We used real-time PCR on upper (nose and throat swabs) and lower respiratory tract samples (sputum and tracheal aspirates) to test for a range of coronaviruses: OC43, 229E, NL63 and SARS-CoV. We also used

Table 2

Real-time PCR results of coronavirus samples, September 2012

Sample/isolate	E Gene		ORF 1b Gene	
	Rotorgene (Ct)	ABI Taqman (Ct)	Rotorgene (Ct)	ABI Taqman (Ct)
Novel coronavirus isolated in the Netherlands (patient from Saudi Arabia) reported to ProMED				
Cultured virus (approximate titre 10^6/ml)	18.9	17.5	22.7	21.9
Samples from confirmed case in London				
Combined nose and throat swab 13/9/ 2012	30.5	28.8	35.6	35.4
Sputum 17/12/2012	28.3	26.6	32.8	31.7
Deep tracheal aspirate 19/12/2012	26.2	24.9	31.4	30.0

Ct: cycle threshold; PCR: polymerase chain reaction.

Results of specific real-time PCR assays [10] directed towards the upstream E gene (UpE) and the ORF 1b region of the new coronavirus tested against cultured virus from the patient who died in Saudi Arabia, and clinical material from the confirmed case of novel coronavirus in London.

a block-based pan-coronavirus PCR with degenerate primers targeted to the conserved RNA-dependent RNA polymerase (RdRp Pol) gene that detects all coronaviruses known to infect humans and a range of animal coronaviruses [6]. The pan-coronavirus assay yielded a band of the correct size in lower respiratory tract samples, but the assays for OC43, 229E, NL63 and SARS-coronaviruses were negative. Sanger sequencing of the pan-coronavirus PCR product (a 251 base pair fragment encompassing nucleotides 104–354 of the NSP12 gene) yielded a sequence that on BLAST analysis gave genetic identity of 81% to bat coronavirus/133/2005 (GenBank accession number DQ648794.1) and 75% identity to porcine haemagglutinating encephalomyelitis virus strain VW572 (GenBank accession number DQ011855.1) The sequence identified is available on the HPA website [7]. In response to this identification, a new set of real-time RT PCR assays were developed [8]. The results of these assays tested on novel coronavirus tissue culture material and clinical samples from this confirmed case are shown in Table 2.

On the basis of the sequence obtained, a maximum likelihood tree (Figure) showed that the virus belongs to the genus *Betacoronavirus*, with closest relationships to bat coronaviruses HKU4 and HKU5. Viruses that share more than 90% sequence identity in the conserved replicase domain are considered to belong to the same species by the International Committee on Taxonomy of Viruses (ICTV). Our sequence comparisons suggested that the virus nucleic acid fragment identified is derived from a novel coronavirus that is distinct from all coronaviruses described to date.

A total of 13 close contacts of the index case were identified who had developed mild self-limiting respiratory illnesses since exposure to the case [8]. Ten of these have had nose and throat swabs tested by pan-coronavirus assay and the novel coronavirus was not detected.

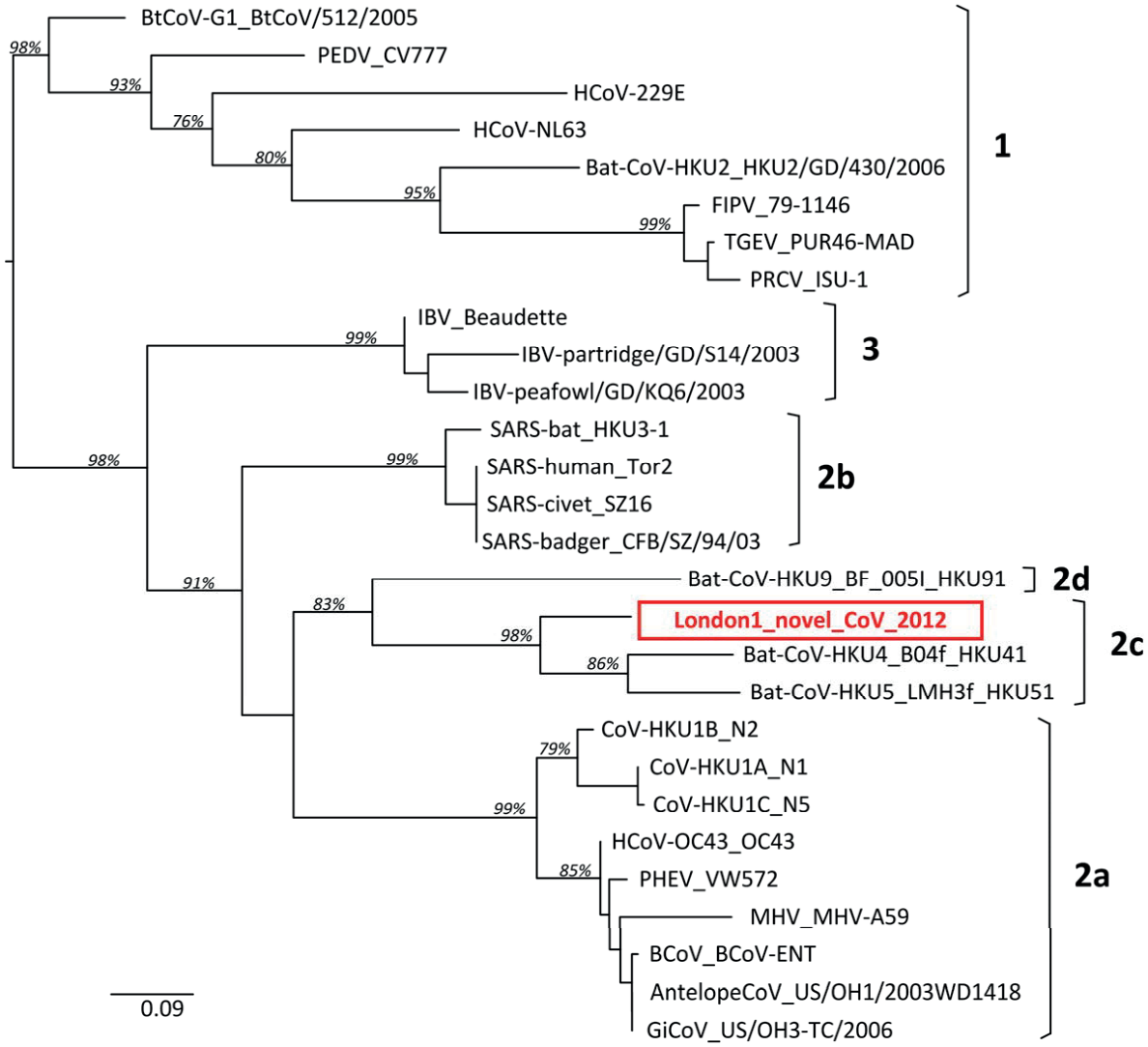
Discussion

Ascribing viral taxonomy on the basis of a small segment of sequence representing less than 1% of a viral genome is highly presumptive. However, the replicase genes are extremely conserved within coronaviruses, and the gene targeted by the pan-coronavirus assay is highly correlated with taxonomic classification based on the whole genome [9], confirming the choice of assay and the validity of the phylogeny (Figure). Final allocation of taxonomy and nearest neighbour relatedness will require more extensive sequence obtained either through genomic analysis of virus isolates cultured from the available clinical material, or more extensive partial genome sequence derived directly from clinical material if virus isolation is not possible.

While most coronaviral infections of humans cause mild illness, zoonotic transmission of animal coronaviruses such as SARS-CoV can cause severe illness and death. Preliminary data sharing (Ron Fouchier, personal communication, 23 September 2012) indicates 99.5% identity over the region of the replicase compared with the virus isolated from the patient in Saudi Arabia and described in ProMED. This is confirmed by the publication of the whole genome sequence (GenBank accession number JX869059.1). On the basis of the clinical and virological features, we believe that the fragment

Figure

Phylogenetic relationships of partial sequences from the polymerase gene (nsp12) of the coronavirus sequence obtained at the Health Protection Agency, together with representative coronaviruses from different groups



The sequence obtained at the Health Protection Agency has been tentatively named as London1_novel CoV 2012. The phylogenetic tree was constructed with fastTree software, using the maximum-likelihood method with general time-reversible model of nucleotide substitution. Bootstrap values were obtained with 1,000 replicates. Coronavirus groups are shown on the right hand side of the tree, with 1, 2 and 3 corresponding to Alpha, Beta and Gammacoronaviruses respectively.

of coronaviral sequence we have recovered represents a novel human coronavirus causing a severe respiratory illness.

The rapid development of sensitive and specific molecular diagnostics for new organisms is facilitated by sharing information and data between laboratories with different capabilities or reagents. The initial molecular approaches used in this case were part of a broad screening approach based on experience gained during the response to SARS. The development of specific diagnostics for the novel coronavirus will improve sensitivity and enable rapid exclusion or identification of potential clinical cases.

The origin for this novel virus is unknown. Epidemiological human and animal investigations in the region of origin are required to distinguish between an animal reservoir that either directly or indirectly transmits the virus occasionally to humans, and a previously unrecognised endemic infection of humans that causes severe outcomes in a few of those infected. Distinguishing between these possibilities will require wider application of more specific and sensitive molecular assays for coronaviruses, and greater awareness of the possible presence of coronaviruses in human acute severe respiratory illness. Extensive serological testing of potentially exposed human populations and contacts will be a key indicator of the extent of disease due to novel coronaviruses.

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Interim estimates of 2014/15 vaccine effectiveness against influenza A(H3N2) from Canada's Sentinel Physician Surveillance Network, January 2015

D M Skowronski (danuta.skowronski@bccdc.ca)[1,2], C Chambers[1], S Sabaiduc[1], G De Serres[3,4,5], J A Dickinson[6], A L Winter[7], S J Drews[8,9], K Fonseca[6,10], H Charest[3], J B Gubbay[7,11], M Petric[2], M Krajden[1,2], T L Kwindt[1,2], C Martineau[3], A Eshaghi[7], N Bastien[12], Y Li[12,13]

1. British Columbia Centre for Disease Control, Vancouver, Canada
2. University of British Columbia, Vancouver, Canada
3. Institut National de Santé Publique du Québec (National Institute of Health of Quebec), Québec, Canada
4. Laval University, Quebec, Canada
5. Centre Hospitalier Universitaire de Québec (University Hospital Centre of Quebec), Québec, Canada
6. University of Calgary, Calgary, Canada
7. Public Health Ontario, Toronto, Canada
8. University of Alberta, Edmonton, Canada
9. Alberta Provincial Laboratory, Edmonton, Canada
10. Alberta Provincial Laboratory, Calgary, Canada
11. University of Toronto, Toronto, Canada
12. National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada
13. University of Manitoba, Winnipeg, Canada

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The 2014/15 influenza season to date in Canada has been characterised by predominant influenza A(H3N2) activity. Canada's Sentinel Physician Surveillance Network (SPSN) assessed interim vaccine effectiveness (VE) against medically attended, laboratory-confirmed influenza A(H3N2) infection in January 2015 using a test-negative case–control design. Of 861 participants, 410 (48%) were test-positive cases (35% vaccinated) and 451 (52%) were test-negative controls (33% vaccinated). Among test-positive cases, the majority (391; 95%) were diagnosed with influenza A, and of those with available subtype information, almost all influenza A viruses (379/381; 99%) were A(H3N2). Among 226 (60%) A(H3N2) viruses that were sequenced, 205 (91%) clustered with phylogenetic clade 3C.2a, considered genetically and antigenically distinct from the 2014/15 A/Texas/50/2012(H3N2)-like clade 3C.1 vaccine reference strain, and typically bearing 10 to 11 amino acid differences from the vaccine at key antigenic sites of the haemagglutinin protein. Consistent with substantial vaccine mismatch, little or no vaccine protection was observed overall, with adjusted VE against medically attended influenza A(H3N2) infection of –8% (95% CI: –50 to 23%). Given these findings, other adjunct protective measures should be considered to minimise morbidity and mortality, particularly among high-risk individuals. Virus and/or host factors influencing this reduced vaccine protection warrant further in-depth investigation.

Background

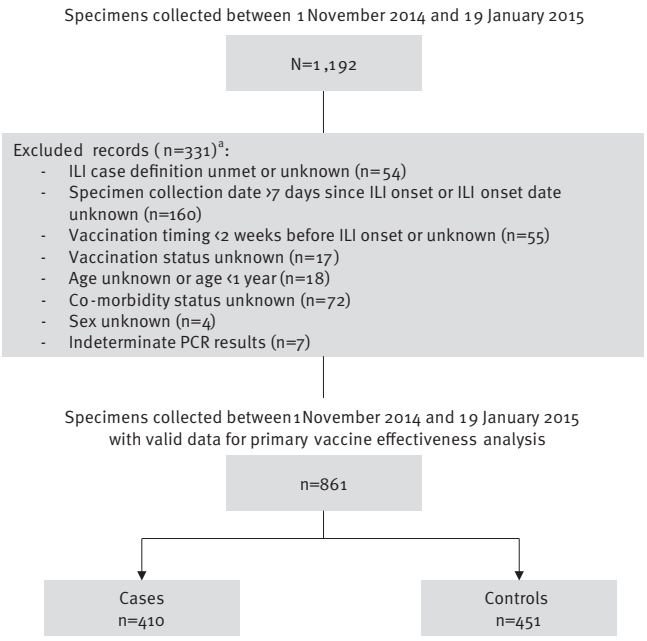
In Canada, the 2014/15 influenza season has been distinguished by an early and intense epidemic due almost exclusively (›90%) to influenza A(H3N2) subtype viruses. Virtually all (›99%) of these A(H3N2) viruses have been characterised as genetically and/or antigenically distinct from the A/Texas/50/2012(H3N2)-like (clade 3C.1) vaccine reference strain used for both the current 2014/15 and prior 2013/14 northern hemisphere influenza vaccines [1].

This profile of dominant influenza A(H3N2) activity is in sharp contrast to the 2013/14 season, when an early epidemic peak also occurred, but was instead due to predominant but antigenically well-conserved A(H1N1) pdm09 viruses [2]. The 2014/15 season more closely resembles that of 2012/13, although the predominant vaccine-mismatched influenza A(H3N2) activity in that season in Canada was related to a different combination of vaccine-virus divergence, notably mutations in that season's egg-adapted vaccine strain used for manufacturing, rather than antigenic drift in circulating viruses [3,4]. In some parts of Canada, an unprecedented number of influenza outbreaks in long-term care facilities (LTCF) were reported in association with vaccine mismatch in 2012/13 [4,5], but the mid-season tally for 2014/15 has already exceeded even that of 2012/13 in some jurisdictions [5].

In response to surveillance signals suggesting suboptimal vaccine performance, Canada's Sentinel Physician

Figure 1

Specimen inclusion and exclusion criteria, interim 2014/15 influenza vaccine effectiveness evaluation, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 861)



ILI: influenza-like illness.

[a] Exclusions are not mutually exclusive; specimens may have >1 exclusion criterion that applies. Counts for each criterion will sum to more than the total number of specimens excluded.

Surveillance Network (SPSN) assessed interim influenza vaccine effectiveness (VE) in January 2015. VE findings are presented in the context of in-depth genetic and antigenic characterisation of contributing sentinel influenza A(H3N2) viruses, relevant to the upcoming selection of vaccine strains in February 2015 by the World Health Organization (WHO) for the 2015/16 northern hemisphere influenza vaccine. Findings are also considered in relation to virus-host interactions, notably the effects of influenza vaccination in the previous season on protection by the current season's vaccine.

Methods

Epidemiological estimation of influenza vaccine effectiveness

As previously described [2-4,6,7], a test-negative case–control design was used to estimate VE. Inclusion and exclusion criteria applied to the current dataset are shown in Figure 1. Patients presenting to community-based practitioners at sentinel sites across participating provinces (British Columbia, Alberta, Ontario and Quebec) within seven days of onset of influenza-like illness (ILI) and testing positive for influenza were considered cases; those testing negative were considered controls. ILI was defined as acute onset of respiratory illness with fever and cough and one or more of the following symptoms: sore throat, arthralgia, myalgia, or prostration. Fever was not an eligibility requirement for elderly adults 65 years and older.

As annual influenza immunisation campaigns typically commence in October across Canada, and increased influenza virus circulation (exceeding 10% test-positivity) typically begins in early November, nasal or nasopharyngeal specimens collected from 1 November 2014 (week 44) were eligible for inclusion in the primary VE analysis. Epidemiological information was obtained from consenting patients or their parent/guardian using a standard questionnaire at specimen collection. Ethics review boards in participating provinces approved this study.

Specimens were tested for influenza A (by subtype) and B viruses at provincial reference laboratories using real-time RT-PCR. Odds ratios (OR) for medically attended, laboratory-confirmed influenza by self-reported vaccination status were estimated by multivariable logistic regression. VE was calculated as $(1 - OR) \times 100\%$. Vaccine was administered to participants during the seasonal immunisation campaign. Non-adjuvanted, inactivated, split trivalent influenza vaccine (TIV) is primarily used in Canada. Live attenuated influenza vaccine (LAIV) is approved for individuals two to 59 years-old, including the trivalent but for the first time in Canada also the quadrivalent formulation, and was publicly funded in the SPSN provinces of British Columbia, Alberta and Quebec. An adjuvanted subunit TIV is approved for elderly Canadians and publicly funded in British Columbia and Ontario. Participants who received seasonal 2014/15 influenza vaccine at least two weeks before ILI onset were considered vaccinated. Those for whom vaccination timing was unknown or less than two weeks before ILI onset were excluded from primary analysis but explored in sensitivity analyses, as were participants whose comorbidity status was unknown. The effects of prior 2013/14 influenza vaccine receipt on current vaccine protection were explored through indicator variable analysis.

Influenza vaccine manufacturers require an egg-adapted, high-growth reassortant (HGR) version of the reference strain recommended by WHO for further high-yield propagation in embryonated hens' eggs. The HGR version of the WHO-recommended A/Texas/50/2012(H3N2) reference strain [8] used by manufacturers for both the 2014/15 and 2013/14 northern hemisphere influenza vaccines is called X-223A and differs from the A/Texas/50/2012(H3N2) prototype by three amino acids (aa) in antigenic sites of the haemagglutinin (HA) protein.

Laboratory characterisation of contributing sentinel viruses

The HA1 and HA2 regions of the HA gene from a convenience sample of sentinel influenza A(H3N2) viruses

Table 1

Reference haemagglutinin sequences obtained from the EpiFlu database of the Global Initiative on Sharing All Influenza Data and used in phylogenetic analysis, 2014/15 Canadian Sentinel Physician Surveillance Network (n = 13)

Segment ID	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors
EPI539806	Hong Kong (SAR)	30 Apr 2014	A/Hong Kong/5738/2014	Government Virus Unit	National Institute for Medical Research	
EPI539576	Hong Kong (SAR)	26 Feb 2014	A/Hong Kong/4801/2014	Government Virus Unit	National Institute for Medical Research	
EPI426061	Hong Kong (SAR)	11 Jan 2013	A/Hong Kong/146/2013	Government Virus Unit	National Institute for Medical Research	
EPI530647	Norway	3 Feb 2014	A/Norway/466/2014	WHO National Influenza Centre	National Institute for Medical Research	
EPI460558	Russian Federation	12 Mar 2013	A/Samara/73/2013	WHO National Influenza Centre Russian Federation	National Institute for Medical Research	
EPI360950	Germany	3 Jul 2011	A/Berlin/93/2011	National Institute for Medical Research	Centers for Disease Control and Prevention	
EPI530687	Switzerland	6 Dec 2013	A/Switzerland/9715293/2013	Hopital Cantonal Universitaire de Geneves	National Institute for Medical Research	
EPI543062	Switzerland	1 Jan 2013	A/Switzerland/9715293/2013 X-247	New York Medical College	Centers for Disease Control and Prevention	
EPI551814	Australia	1 Jan 2014	IVR-176(A/ Switzerland/9715293/2013)	CSL Ltd	WHO Collaborating Centre for Reference and Research on Influenza	Deng,Y-M.; Iannello,P.; Spirason,N.; Jelley,L.; Lau,H.; Komadina,N.
EPI377499	United States	15 Apr 2012	A/Texas/50/2012	Texas Department of State Health Services -Laboratory Services	Centers for Disease Control and Prevention	
EPI407126	United States	1 Jan 2012	A/Texas/50/2012 X-223A	New York Medical College	Centers for Disease Control and Prevention	
EPI349103	Australia	24 Oct 2011	A/Victoria/361/2011	Melbourne Pathology	WHO Collaborating Centre for Reference and Research on Influenza	Deng,Y-M; Caldwell,N; Iannello,P; Komadina,N
EPI358038	Australia	1 Jan 2011	IVR-165(A/Victoria/361/2011)	WHO Collaborating Centre for Reference and Research on Influenza	Centers for Disease Control and Prevention	

WHO: World Health Organization.

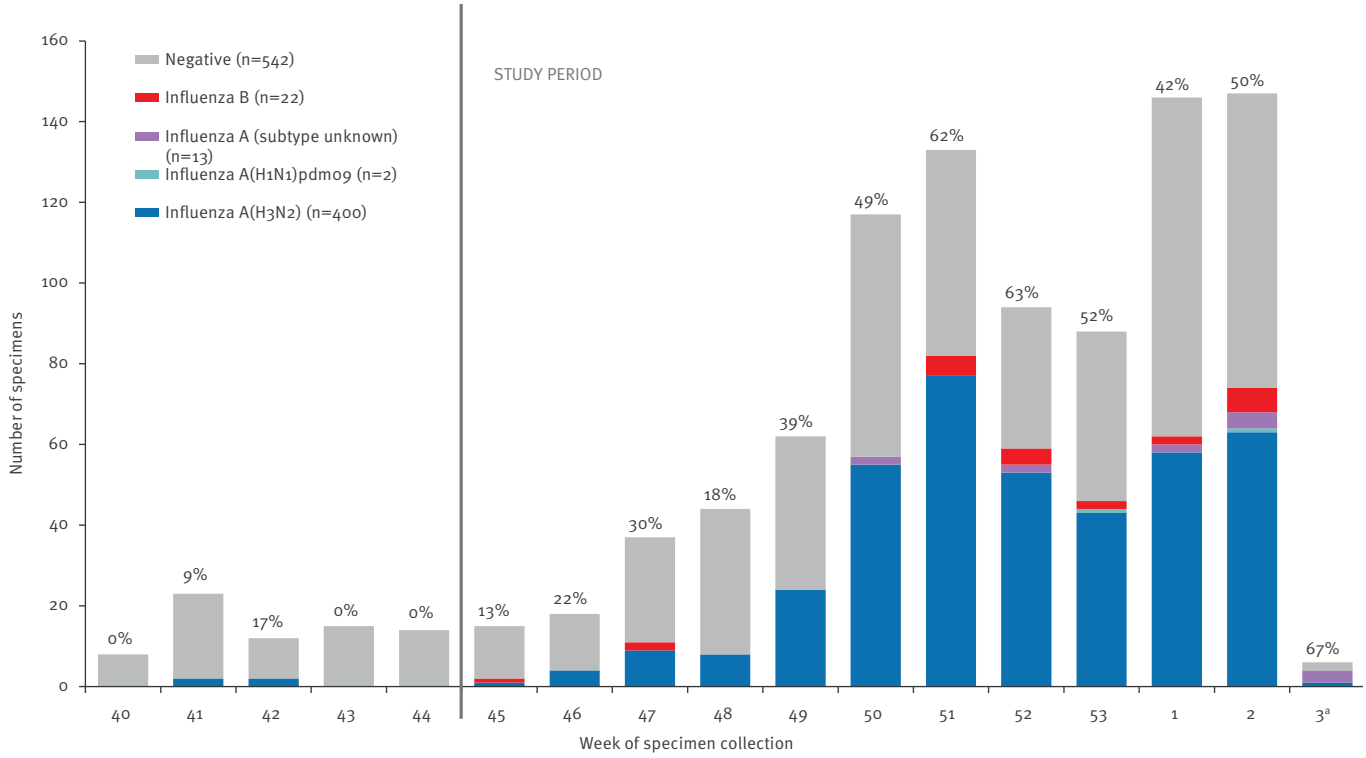
from original patient specimens contributing to VE analysis were sequenced for phylogenetic and pairwise aa identity analysis based on antigenic maps spanning the 131 aa residues across HA1 antigenic sites A–E [4,6,7,9]. The approximate likelihood method was used to generate the phylogenetic tree of aligned translated sequences in FastTree [10], visualised in FigTree [11], including representative vaccine reference, HGR and clade-specific HA sequences shown in Table 1, kindly made available by the Global Initiative on Sharing All Influenza Data (GISAID), and using clade nomenclature specified by the European Centre for Disease Prevention and Control (ECDC) [12].

Historically, each new significant antigenic drift variant has, in general, had at least four aa substitutions located in at least two antigenic sites [13]. However, substitutions at antigenic sites A, B and D of the H3 globular head located closest to the receptor-binding site (RBS) are typically considered most influential [14], with site B being emphasised as particularly immunodominant among more recent influenza A(H3N2) strains [15]. Substitutions at just seven antigenic site positions, located in antigenic site A (position 145) and B (positions 155, 156, 158, 159, 189 and 193) have been emphasised in relation to all major A(H3N2) antigenic cluster transitions since 1968 [16]. Substitutions associated with gain or loss of glycosylation may also influence antibody binding [17]. Sequencing findings among sentinel influenza A(H3N2) viruses are thus interpreted within these key antigenic considerations.

A convenience sample of influenza-positive specimens was also inoculated into Madin Darby Canine Kidney (MDCK) (British Columbia, Alberta, Quebec) or Rhesus Monkey Kidney (Ontario) cell culture for virus isolation.

Figure 2

Laboratory detections of influenza by week and type/subtype, interim 2014/15 influenza vaccine effectiveness evaluation, Canadian Sentinel Physician Surveillance Network, 28 September 2014–19 January 2015 (n = 978)



[a] Based on partial week.

Influenza percent positivity by week is shown above bars.

One participant in week 1 had co-infection with influenza A(H3N2) and influenza B; subtotals for influenza A and B will add to more than the total number of influenza positives.

Of the 1,286 nasal or nasopharyngeal specimens collected between week 40 (starting 28 September 2014) and week 3 (starting 18 January 2015), we excluded 308 specimens from the epidemic curve: those failing to meet the influenza-like illness (ILI) case definition or for whom it was unknown (n=58), those whose specimens were collected more than seven days after ILI onset or for whom the interval was unknown (n=173), those whose age was unknown or who were younger than one year (n=20), those with unknown comorbidity status (n=80), those with unknown sex (n=4) and those for whom influenza test results were unavailable or indeterminate (n=9). Specimens were included regardless of the patient's vaccination status or timing of vaccination. Missing collection dates were imputed as the laboratory accession date minus two days, the average time period between collection date and laboratory accession date for records with valid data for both fields.

Note that the epidemic curve displays specimen collection and influenza detections from week 40 and regardless of the patient's vaccination status or timing; as such, tallies do not match those in the text.

Aliquots of virus isolates were submitted to the National Microbiology Laboratory (NML), Canada's influenza reference laboratory, for antigenic characterisation by haemagglutination inhibition (HI) assay using guinea pig erythrocytes [4,18] in relation to the cell-passaged A/Texas/50/2012(H3N2)-like clade 3C.1 vaccine reference strain and the A/Switzerland/9715293/2013(H3N2)-like clade 3C.3a reference strain recommended for the 2015 southern hemisphere vaccine [8]. To address potential neuraminidase-mediated binding of influenza A(H3N2) viruses to erythrocytes, the HI assay was conducted in the presence of 20 nM oseltamivir carboxylate following re-growth of viruses in MDCK-SIAT1 cells [19]. HI titres were recorded as the reciprocal of the highest ferret serum dilution at which inhibition of haemagglutination was detected. Previously, a ≥ 4-fold reduction in post-infection ferret HI-antibody titre was considered a signal of antigenic distinction between the field isolate and vaccine reference strain, but this has more recently been revised to a ≥ 8-fold titre reduction [18]. Due to difficulties this season in growing influenza A(H3N2) viruses to sufficient titres for antigenic characterisation by HI assay in the presence of oseltamivir carboxylate, genetic characterisation by sequencing at the NML and provincial public health laboratories was performed to infer antigenic properties of sentinel viruses, as also reported in national laboratory-based surveillance summaries in the United States [20] and Canada [1] for the current 2014/15 season.

Results

Epidemiological findings

A total of 1,192 specimens were submitted within the VE study period, of which 861 (72%) were included in primary VE analyses with collection dates between 3

Table 2

Influenza virus characterisation by type and subtype, interim 2014/15 influenza vaccine effectiveness evaluation, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 861)

Specimen	Alberta n (%)	British Columbia n (%)	Ontario n (%)	Quebec n (%)	Overall n (%)
Total	**262**	**156**	**228**	**215**	**861**
Influenza-negative	128 (49)	89 (57)	130 (57)	104 (48)	451 (52)
Influenza-positive	134 (51)	67 (43)	98 (43)	111 (52)	410 (48)
Influenza A[a]	131 (98)	63 (94)	96 (98)	101 (91)	391 (95)
A(H3N2)	130 (99)	57 (90)	95 (99)	97 (96)	379 (97)
A(H1N1)pdm09	0 (0)	0 (0)	1 (1)	1 (1)	2 (1)
Subtype unknown	1 (1)	6 (10)	0 (0)	3 (3)	10 (3)
Influenza B[a]	3 (2)	4 (6)	2 (2)	11 (10)	20 (5)
Antigenic characterisation of A(H3N2) sentinel viruses by HI assay[b]					
Total	**6**	**1**	**0**	**0**	**7**
A/Texas/50/2012-like[c]	**0**	**0**	**0**	**0**	**0**
< 4-fold reduced titre	0	0	0	0	0
≥ 4-fold reduced titre	5	0	0	0	5
≥ 8-fold reduced titre	5	0	0	0	5
Insufficient volume for HI assay	1	1	0	0	2
A/Switzerland/9715293/2013-like[c]	**6**	**1**	**0**	**0**	**7**
< 4-fold reduced titre	3	1	0	0	4
≥ 4-fold reduced titre	3	0	0	0	3
≥ 8-fold reduced titre	0	0	0	0	0
Genetic characterisation of A(H3N2) sentinel viruses by sequencing					
Total	**104**	**30**	**28**	**64**	**226**
Clade 3C.2a	98 (94)	17 (57)	27 (96)	63 (98)	205 (91)
Clade 3C.3x	5 (5)	13 (43)	0 (0)	1 (2)	19 (8)
Clade 3C.3	1 (1)	0 (0)	1 (4)	0 (0)	2 (1)

HI: haemagglutination inhibition.

[a] One participant in Quebec had co-infection with influenza A(H3N2) and influenza B; subtotals for influenza A and B will add to more than the total number of influenza positives.

[b] 37 additional specimens (34 Alberta, 3 Quebec) submitted to the National Microbiology Laboratory for antigenic characterisation had insufficient titre to characterise by HI assay.

[c] In two-way HI assay, anti-sera raised to the cell-passaged A/Switzerland/9715293/2013(H3N2) referent virus inhibited the homologous antigen at a titre of 320, equivalent to the titre in inhibiting the heterologous cell-passaged A/Texas/50/2012(H3N2) antigen. Conversely, anti-sera raised to the A/Texas/50/2012(H3N2) referent strain inhibited homologous antigen at an HI titre of 1280 and the heterologous A/Switzerland/9715293/2013(H3N2) antigen at a titre of 80, a 16-fold titre reduction. These referent strains are antigenically distinct.

November 2014 (week 45: 2–8 November 2014) and 19 January 2015 (week 3: 18–24 January 2015) (Figure 1, Figure 2). Of these, 410 (48%) were test-positive cases and 451 (52%) were test-negative controls. Among test-positive cases, the majority (n = 391; 95%) were influenza A, and of those with subtype information available, almost all (379/381; 99%) were A(H3N2) (Figure 2, Table 2).

As in previous SPSN publications, adults 20–49 years-old contributed the largest proportion of specimens (40%) (Table 3) [2-4,6,7]. However, compared with the 2013/14 mid-season analysis [2], a significantly lower proportion of participants in 2014/15 were 20–49 years-old (40% vs 50%; $p < 0.01$), more notable among cases (36% vs 53%; $p < 0.01$) than controls (44% vs 48%; $p > 0.05$). Conversely, a greater proportion of participants were elderly adults ≥ 65 years and older (16% vs 8%; $p < 0.01$), again more notable among cases (16% vs 4%; $p < 0.01$) than controls (15% vs 12%; $p > 0.05$) [2]. The proportion of female participants (62%) and those with chronic comorbidity (24%) were comparable to observations in the 2013/14 mid-season analysis (63% and 22%, respectively) [2].

When vaccination status was assessed without regard to timing of ILI onset, 166 of 470 (35%) controls self-reported receipt of the 2014/15 influenza vaccine, comparable to the 2013/14 mid-season analysis (32%) [2] and the most recent influenza immunisation coverage survey for the general adult population in Canada (37%) [21]. Overall, 291 (34%) participants self-reported

Table 3A

Profile of participants included in interim 2014/15 influenza vaccine effectiveness evaluation, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 861)

	Distribution by case status n (%)				Vaccination coverage within strata n (%) vaccinated[a]			
	Overall	Cases	Controls	p value[b]	Overall	p value[b]	Cases	Controls
N (%)	861	410 (48)	451 (52)		291 (34)		144 (35)	147 (33)
Age group (years)				0.08		< 0.01		
1–8	102 (12)	48 (12)	54 (12)		18 (18)		12 (25)	6 (11)
9–19	109 (13)	62 (15)	47 (10)		19 (17)		13 (21)	6 (13)
20–49	344 (40)	146 (36)	198 (44)		93 (27)		36 (25)	57 (29)
50–64	172 (20)	87 (21)	85 (19)		64 (37)		36 (41)	28 (33)
≥ 65	134 (16)	67 (16)	67 (15)		97 (72)		47 (70)	50 (75)
Median (range)	39 (1–103)	39 (1–103)	39 (1–94)	0.98	NA		NA	NA
Sex				< 0.01		< 0.01		
Female	533 (62)	228 (56)	305 (68)		201 (38)		90 (39)	111 (36)
Male	328 (38)	182 (44)	146 (32)		90 (27)		54 (30)	36 (25)
Co-morbidity[c]				0.43		< 0.01		
No	655 (76)	307 (75)	348 (77)		180 (27)		86 (28)	94 (27)
Yes	206 (24)	103 (25)	103 (23)		111 (54)		58 (56)	53 (51)
Province				0.11		< 0.01		
Alberta	262 (30)	134 (33)	128 (28)		107 (41)		58 (43)	49 (38)
British Columbia	156 (18)	67 (16)	89 (20)		39 (25)		14 (21)	25 (28)
Ontario	228 (26)	98 (24)	130 (29)		87 (38)		42 (43)	45 (35)
Quebec	215 (25)	111 (27)	104 (23)		58 (27)		30 (27)	28 (27)

ILI: influenza-like illness; LAIV: live attenuated influenza vaccine; NA: not applicable.

[a] Participants who received seasonal 2014/15 influenza vaccine at least two weeks before ILI onset were considered vaccinated; participants who received seasonal 2014/15 influenza vaccine less than two weeks before ILI onset were excluded from primary analysis but explored in sensitivity analysis. Vaccination status was based on self/parent/guardian report. Details related to special paediatric dosing requirements was not sought.

[b] Differences between cases and controls or vaccinated and unvaccinated participants (based on overall sample to explore potential confounding) were compared using the chi-squared test or Wilcoxon rank-sum test.

[c] Chronic co-morbidities that place individuals at higher risk of serious complications from influenza as defined by Canada's National Advisory Committee on Immunization, including heart, pulmonary, renal, metabolic, blood, cancer and immunocomprising conditions or those that compromise management of respiratory secretions, or morbid obesity. Questionnaire answers were 'yes,' 'no,' or 'unknown' without specifying the co-morbidity.

receipt of the 2014/15 vaccine at least two weeks before ILI onset and were considered vaccinated for the purpose of VE analysis. Among vaccinated participants reporting vaccine type, the proportion that received LAIV was 10% (16/165) in those two to 59 years-old and 47% (16/34) in those two to 19 years-old (i.e. all LAIV recipients were two to 19 years-old) (Table 3). The proportion of vaccinated participants overall did not differ significantly between cases and controls (35% vs 33%; p = 0.43). As observed in previous publications of the SPSN [2-4,6,7], the vast majority of vaccinated participants in 2014/15 were repeat recipients, including 251 of 283 (89%) who had also been vaccinated in 2013/14 and 237 of 269 (88%) also vaccinated in 2012/13.

Crude VE against influenza A was −17% (95% CI: −55 to 12%), and −21% (95% CI: −61 to 9%) against the dominant circulating A(H3N2) viruses (Table 4). With full adjustment for covariates, VE estimates increased to −4% (95% CI: −45 to 25%) and −8% (95% CI: −50 to 23%) for influenza A and A(H3N2), respectively. Calendar time was the covariate most influential on adjusted VE. In sensitivity analyses, adjusted VE estimates remained within 10% of the primary analysis with confidence intervals slightly wider but consistently overlapping zero (Table 4). Among participants immunised in 2014/15 only, crude and adjusted VE estimates were higher at ca 40–50% (vs unvaccinated participants) compared with those immunised in 2013/14 only or in 2013/14 and 2014/15 (<10%); however, confidence intervals were wide and overlapping with the further reduced sample size (Table 4).

Laboratory findings

In total, 44 of 379 (12%) influenza A(H3N2)-positive specimens were submitted to Canada's NML, of which just seven of 44 (16%), collected between 17 November and 18 December 2014, had sufficient titre for antigenic characterisation by HI assay when tested in the presence of oseltamivir carboxylate. All viruses were considered antigenically distinct from the cell-passaged A/Texas/50/2012-like vaccine reference strain and

Table 3B

Profile of participants included in interim 2014/15 influenza vaccine effectiveness evaluation, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 861)

	Distribution by case status n (%)				Vaccination coverage within strata n (%) vaccinated[a]			
	Overall	Cases	Controls	p value[b]	Overall	p value[b]	Cases	Controls
N (%)	861	410 (48)	451 (52)		291 (34)		144 (35)	147 (33)
Collection interval				<0.01		0.51		
≤4 days	642 (76)	337 (82)	305 (68)		213 (33)		118 (35)	95 (31)
5–7 days	219 (25)	73 (18)	146 (32)		78 (36)		26 (36)	52 (36)
Median (range)	3 (0–7)	3 (0–7)	3 (0–7)	<0.01	NA		NA	NA
Calendar time[d]				<0.01		0.06		
Week 45–46	31 (4)	5 (1)	26 (6)		5 (16)		1 (20)	4 (15)
Week 47–48	72 (8)	16 (4)	56 (12)		17 (24)		3 (19)	14 (25)
Week 49–50	173 (20)	78 (19)	95 (21)		57 (33)		31 (40)	26 (27)
Week 51–52	217 (25)	135 (33)	82 (18)		84 (39)		51 (38)	33 (40)
Week 53–1	221 (26)	102 (25)	119 (26)		74 (33)		32 (31)	42 (35)
Week 2–3	147 (17)	74 (18)	73 (16)		54 (37)		26 (35)	28 (38)
Received 2014/15 influenza vaccine[a]								
Any vaccination[e]	326/896 (36)	160/426 (38)	166/470 (35)	0.49	NA		NA	NA
≥2 weeks before ILI onset	291 (34)	144 (35)	147 (33)	0.43	NA		NA	NA
Received LAIV[f]	16/165 (10)	11/85 (13)	5/80 (6)	0.15	NA		NA	NA
Received adjuvanted vaccine[g]	27/51 (53)	11/21 (52)	16/30 (53)	0.95	NA		NA	NA
Prior vaccination history								
Received 2013/14 vaccine[h]	358/804 (45)	177/388 (46)	181/416 (44)	0.55	251/358 (70)	<0.01	131/177 (74)	120/181 (66)
Received 2012/13 vaccine[i]	343/761 (45)	178/377 (47)	165/384 (43)	0.24	237/343 (69)	<0.01	127/178 (71)	110/165 (67)

[a] Participants who received seasonal 2014/15 influenza vaccine at least two weeks before ILI onset were considered vaccinated; participants who received seasonal 2014/15 influenza vaccine less than two weeks before ILI onset were excluded from primary analysis but explored in sensitivity analysis. Vaccination status was based on self/parent/guardian report. Details related to special paediatric dosing requirements was not sought.

[b] Differences between cases and controls or vaccinated and unvaccinated participants (based on overall sample to explore potential confounding) were compared using the chi-squared test or Wilcoxon rank-sum test.

[c] Chronic co-morbidities that place individuals at higher risk of serious complications from influenza as defined by Canada's National Advisory Committee on Immunization, including heart, pulmonary, renal, metabolic, blood, cancer and immunocomprising conditions or those that compromise management of respiratory secretions, or morbid obesity. Questionnaire answers were 'yes,' 'no,' or 'unknown' without specifying the co-morbidity.

[d] Based on week of specimen collection. Missing collection dates were imputed as the laboratory accession date minus two days, the average time period between collection date and laboratory accession date for records with valid data for both fields. Week 3 of 2015 based on partial week.

[e] Participants who received seasonal 2014/15 influenza vaccine less than two weeks before ILI onset or for whom vaccination timing was unknown were excluded from the primary analysis. They were included for assessing 'any' immunisation, regardless of timing, for comparison with other sources of vaccination coverage. The denominator is shown for 'any' immunisation.

[f] Among participants 2–59 years-old who received 2014/15 influenza vaccine at least two weeks before ILI onset and had valid data for type of vaccine. All 16 participants who received LAIV were 2–19 years of age. Among vaccinated participants 2–19 years-old, 16 of 34 (47%) overall received LAIV including 11 of 24 cases (46%) and five of 10 controls (50%).

[g] Among participants 65 years and older who received 2014/15 influenza vaccine at least two weeks before ILI onset and had valid data for receipt of adjuvanted vaccine.

[h] Children younger than two years in 2014/15 were excluded from 2013/14 vaccine uptake analysis as they may not have been eligible for vaccination during the immunisation campaign in autumn 2013 on the basis of age under six months.

[i] Children younger than three years in 2014/15 were excluded from 2012/13 vaccine uptake analysis as they may not have been eligible for vaccination during the immunisation campaign in autumn 2012 on the basis of age under six months.

were instead antigenically similar to the cell-passaged A/Switzerland/9715293/2013-like reference strain (Table 2). Based on phylogenetic analysis, five of these viruses clustered with clade 3C.2a and two with an emerging clade of viruses awaiting official ECDC clade-level designation and thus temporarily labelled in the current analysis as 3C.3*x*. Both clade 3C.3*x* viruses had an L157S substitution in antigenic site B and an N122D substitution in antigenic site A, as discussed below.

Of the 379 sentinel A(H3N2) viruses collected between 11 November 2014 and 10 January 2015, 226 (60%) were sequenced; 205 (91%) belonged to clade 3C.2a, 19 (8%) to our provisionally named clade 3C.3*x*, and two (1%) to clade 3C.3 (Table 2, Figure 3, Figure 4).

Table 4A

Interim 2014/15 influenza vaccine effectiveness evaluation, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 861)

Model	Influenza (any)	Influenza A	Influenza A(H3N2)
	VE (95% CI)	VE (95% CI)	VE (95% CI)
Primary analysis			
N [n case (% vac); n control (% vac)]	861 [410 (35); 451 (33)]	842 [391 (36); 451 (33)]	830 [379 (37); 451 (33)]
Unadjusted	−12 (−49 to 16)	−17 (−55 to 12)	−21 (−61 to 9)
Age group (1–8, 9–19, 20–49, 50–64, ≥ 65 years)	−11 (−51 to 18)	−17 (−60 to 14)	−22 (−67 to 10)
Sex (female/male)	−19 (−58 to 11)	−24 (−65 to 7)	−29 (−73 to 4)
Comorbidity (no/yes)	−10 (−47 to 18)	−15 (−54 to 14)	−19 (−60 to 12)
Province (Alberta, British Columbia, Ontario, Quebec)	−12 (−49 to 16)	−15 (−54 to 14)	−19 (−59 to 11)
Collection interval (≤ 4/5–7 days)	−14 (−52 to 14)	−19 (−59 to 11)	−23 (−65 to 8)
Calendar time (2-week interval)	0 (−34 to 25)	−4 (−39 to 23)	−8 (−45 to 20)
Age, sex, comorbidity, province, interval, time	−1 (−40 to 28)	−4 (−45 to 25)	−8 (−50 to 23)
Sensitivity analysis – vaccination timing			
Vaccination defined without regard to vaccination timing (i.e. any vaccination)			
N [n case (% vac); n control (% vac)]	896 [426 (38); 470 (35)]	876 [406 (38); 470 (35)]	861 [391 (39); 470 (35)]
Unadjusted	−10 (−45 to 16)	−14 (−51 to 13)	−16 (−54 to 12)
Fully adjusted[a]	0 (−37 to 27)	−2 (−41 to 26)	−5 (−44 to 24)
Participants vaccinated < 2 weeks before ILI onset recoded as 'unvaccinated'			
N [n case (% vac); n control (% vac)]	887 [422 (34); 465 (32)]	867 [402 (35); 465 (32)]	853 [388 (36); 465 (32)]
Unadjusted	−12 (−48 to 15)	−17 (−55 to 12)	−22 (−62 to 8)
Fully adjusted[a]	1 (−38 to 28)	−3 (−43 to 26)	−8 (−51 to 22)
Participants vaccinated < 2 weeks before ILI onset recoded as 'vaccinated'			
N [n case (% vac); n control (% vac)]	887 [422 (37); 465 (35)]	867 [402 (38); 465 (35)]	853 [388 (38); 465 (35)]
Unadjusted	−11 (−46 to 16)	−15 (−52 to 13)	−18 (−56 to 11)
Fully adjusted[a]	−2 (−41 to 26)	−4 (−44 to 24)	−7 (−48 to 23)
Sensitivity analysis – comorbidity			
N [n case (% vac); n control (% vac)]	910 [433 (35); 477 (31)]	890 [413 (36); 477 (31)]	878 [401 (37); 477 (31)]
Includes participants with unknown comorbidity			
Unadjusted	−17 (−54 to 11)	−22 (−61 to 8)	−26 (−67 to 5)
Fully adjusted[b]	−7 (−47 to 23)	−10 (−52 to 20)	−14 (−58 to 18)
Participants with unknown comorbidity recoded as 'no'			
Unadjusted	−17 (−54 to 11)	−22 (−61 to 8)	−26 (−67 to 5)
Fully adjusted[a]	−5 (−46 to 24)	−9 (-51 to 21)	−13 (−56 to 19)
Participants with unknown comorbidity recoded as 'yes'			
Unadjusted	−17 (−54 to 11)	−22 (−61 to 8)	−26 (−67 to 5)
Fully adjusted[a]	−6 (−46 to 23)	−9 (−51 to 21)	−13 (−57 to 18)

CI: confidence interval; ILI: influenza-like illness; VE: vaccine effectiveness; % vac: percentage vaccinated.

[a] Adjusted for age group, sex, comorbidity, province, collection interval, and calendar time.

Clade 3C.2a viruses comprised the majority (>90%) of viruses in all contributing SPSN provinces, with the exception of British Columbia, where there was more equal contribution of clade 3C.2a (17/30; 57%) and clade 3C.3*x* (13/30; 43%). None of the 226 sentinel A(H3N2) viruses contributing to the VE analysis that were sequenced belonged to the northern hemisphere 2014/15 A/Texas/50/2012(H3N2) vaccine clade 3C.1, nor to the 2015 southern hemisphere A/Switzerland/9715293/2013(H3N2) vaccine clade 3C.3a. However, as described above, all seven viruses that could be characterised by HI assay were considered antigenically similar to the A/Switzerland/9715293/2013(H3N2) strain, even though none of those seven viruses clustered within clade 3C.3a.

Relative to the X-223A HGR, sentinel clade 3C.2a viruses typically differed by 10 or 11 antigenic site aa substitutions as itemised in Figure 3. In addition to the

Table 4B

Interim 2014/15 influenza vaccine effectiveness evaluation, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 861)

Model	Influenza (any)	Influenza A	Influenza A(H3N2)
	VE (95% CI)	VE (95% CI)	VE (95% CI)
Stratified analysis – restricted to non-elderly adult participants 20–64 years old			
N [n case (% vac); n control (% vac)]	516 [233 (31); 283 (30)]	506 [223 (32); 283 (30)]	496 [213 (33); 283 (30)]
Unadjusted	−4 (−52 to 29)	−11 (−62 to 24)	−16 (−71 to 20)
Fully adjusted[a]	11 (−35 to 41)	6 (−43 to 38)	2 (−49 to 36)
Stratified analysis – restricted to specimens collected from week 50 onward			
N [n case (% vac); n control (% vac)]	699 [365 (36); 334 (36)]	682 [348 (37); 334 (36)]	670 [336 (38); 334 (36)]
Unadjusted	1 (−34 to 28)	−4 (−42 to 24)	−8 (−48 to 21)
Fully adjusted[c]	−3 (−47 to 28)	−9 (−55 to 24)	−13 (−61 to 21)
Indicator variable analysis – effect of prior 2013/14 influenza vaccine receipt on 2014/15 VE[d]			
Unvaccinated both seasons			
N [n case (%); n control (%)]	414 [201 (52); 213 (51)]	400 [187 (51); 213 (51)]	392 [179 (50); 213 (51)]
Unadjusted/fully adjusted	Reference	Reference	Reference
Current 2014/15 influenza vaccine only			
N [n case (%); n control (%)]	32 [10 (3); 22 (5)]	32 [10 (3); 22 (5)]	32 [10 (3); 22 (5)]
Unadjusted	52 (−4 to 78)	48 (−12 to 76)	46 (−17 to 75)
Fully adjusted[a]	49 (−15 to 78)	46 (−24 to 76)	43 (−29 to 75)
Prior 2013/14 influenza vaccine only			
N [n case (%); n control (%)]	107 [46 (12); 61 (15)]	105 [44 (12); 61 (15)]	105 [44 (12); 61 (15)]
Unadjusted	20 (−23 to 48)	18 (−27 to 47)	14 (−33 to 44)
Fully adjusted[a]	8 (−47 to 42)	8 (−47 to 43)	4 (−54 to 40)
Both 2013/14 and 2014/15 influenza vaccine			
N [n case (%); n control (%)]	251 [131 (34); 120 (29)]	248 [128 (35); 120 (29)]	247 (127 (35); 120 (29)]
Unadjusted	−16 (−58 to 15)	−21 (−67 to 12)	−26 (−73 to 8)
Fully adjusted[a]	−8 (−56 to 26)	−11 (−62 to 23)	−15 (−67 to 21)

CI: confidence interval; ILI: influenza-like illness; VE: vaccine effectiveness; % vac: percentage vaccinated.

[a] Adjusted for age group, sex, comorbidity, province, collection interval, and calendar time.

[b] Adjusted for age group, sex, province, collection interval, and calendar time; not adjusted for comorbidity.

[c] Adjusted for age group, sex, comorbidity, province, and collection interval; not adjusted for calendar time.

[d] Based on same exclusion criteria as primary analysis, with further restriction to participants aged ≥ 2 years in 2014/15 and those with data for 2013/14 and 2014/15 influenza vaccine receipt.

N145S site A cluster-transition substitution distinguishing all clade 3C.2 (and 3C.3) viruses generally, differences between clade 3C.2 viruses and X-223A include N128T (gain of glycosylation) and P198S site B substitutions. The latter two substitutions are the result of having switched the vaccine prototype strain from A/Victoria/361/2011(H3N2) (a clade 3C virus) in 2012/13 to A/Texas/50/2012(H3N2) (a clade 3C.1 virus) since the 2013/14 season. Clade 3C.2 viruses also differ from X-223A at positions 186 (site B), 219 (site D) and 226 (site D) due to mutations in the egg-adapted HGR. Sentinel viruses within the dominant 3C.2a subgroup were further distinguished through an N144S (site A) substitution associated with loss of glycosylation, an additional F159Y (site B) cluster-transition mutation and an adjacent K160T (site B) substitution associated with the gain of a potential glycosylation site, as well as Q311H (site C) and N225D substitutions, the latter within the RBS (but not within defined antigenic sites A–E [4,6,9]). Other substitutions relative to X-223A were scattered through antigenic sites A, C and E.

The provisionally named clade 3C.3*x* sentinel viruses typically differed from X-223A by 12 antigenic site aa substitutions, as also shown in Figure 3. Of note, in addition to the L157S substitution at antigenic site B that distinguishes this emerging subgroup, 18 of 19 clade 3C.3*x* viruses also bore an N122D antigenic site A substitution associated with loss of glycosylation.

Discussion

Interim VE estimates from the Canadian SPSN show little or no protection from the 2014/15 influenza vaccine against the A(H3N2) epidemic strain. The disappointing 2014/15 mid-season VE of −8%, with 95% confidence intervals (CI) overlapping zero and extending to just

Figure 3A

Influenza A(H3N2) haemagglutinin (HA1) antigenic site pairwise sequence and per cent amino acid identity comparisons, relative to the 2014/15 high growth reassortant vaccine strain X-223A, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 217)

Antigenic site		C		E							A	B	A						B				A	D	B			D							E	C					Clade	# aa (% identity)[a,b]
Amino acid number HA1		48	53	62	63	78	83	88	91	94	122	128	137	138	140	142	144	145	156	157	159	160	168	171	186	192	198	207	208	213	214	219	226	261	278	279	309	311	312		
A/Victoria/361/2011 (MDCK)[c]		I	D	E	N	G	K	V	S	Y	N	T	S	A	I	R	N	N	H	L	F	K	M	N	G	I	S	K	R	V	I	S	I	R	N	S	V	Q	S	3C	6 (95.4%)
2012-13 HGR: A/Victoria/361/2011 (IVR-165)[d]		I	D	E	N	G	K	V	S	Y	N	T	S	A	I	R	N	N	Q	L	F	K	M	N	V	I	S	K	R	V	I	Y	I	R	N	S	V	Q	S	3C	6 (95.4%)
A/Texas/50/2012 (MDCK)[e]		I	D	E	N	G	K	V	S	Y	N	N	S	A	I	R	N	N	H	L	F	K	M	N	G	I	P	K	R	V	I	S	I	R	K	S	V	Q	S	3C.1	3 (97.7%)
HGR: A/Texas/50/2012 (X-223A)[f]		**I**	**D**	**E**	**N**	**G**	**K**	**V**	**S**	**Y**	**N**	**N**	**S**	**A**	**I**	**R**	**N**	**N**	**H**	**L**	**F**	**K**	**M**	**N**	**V**	**I**	**P**	**K**	**R**	**V**	**I**	**F**	**N**	**R**	**K**	**S**	**V**	**Q**	**S**	**3C.1**	-
A/Switzerland/9715293/2013 (MDCK)[g]		I	D	E	N	G	K	V	S	Y	N	A	S	S	I	G	N	S	H	L	S	K	M	N	G	I	S	K	R	V	I	S	I	R	K	S	V	Q	S	3C.3a	9 (93.1%)
2015 HGR: A/Switzerland/9715293/2013 (IVR-176)[h]		I	D	E	N	G	K	V	S	Y	N	A	S	S	R	G	N	S	R	L	S	K	M	N	V	I	S	K	R	V	I	S	I	R	K	S	V	Q	S	3C.3a	10 (92.4%)
2015 HGR: A/Switzerland/9715293/2013 (X-247)[h]		I	D	E	N	G	K	V	S	Y	N	A	S	S	R	G	N	S	H	L	S	K	M	N	V	I	S	K	R	V	I	F	I	R	K	S	V	Q	S	3C.3a	8 (93.9%)
British Columbia	n																																									
A/British Columbia/81/2014	7											T					S	S			Y	T			G		S					S	I					H		3C.2a	10 (92.4%)
A/British Columbia/94/2014	4											T					S	S			Y	T			G		S					S	I	L				H		3C.2a	11 (91.6%)
A/British Columbia/89/2014	1											T					S	S			Y				G		S					S	I	L				H		3C.2a	10 (92.4%)
A/British Columbia/93/2014	2											T					S	S			Y	T			G		S					S	I					H	N	3C.2a	11 (91.6%)
A/British Columbia/97/2014	2											T					S	S			Y	T			G		S		G			S	I					H		3C.2a	11 (91.6%)
A/British Columbia/10/2015	1							R				T					S	S			Y	T			G		S					S	I					H		3C.2a	11 (91.6%)
A/British Columbia/67/2014	11			K			R				D	A				G		S		S					G		S					S	I	Q						3C.3x	12 (90.8%)
A/British Columbia/83/2014	1			K			R				D	A				G		S		S					G		S					Y	I	Q						3C.3x	12 (90.8%)
A/British Columbia/100/2014	1		N									A				G		S		S					G		S				T	S	I							3C.3x	10 (92.4%)

HGR: high-growth reassortant; MDCK: Madin Darby Canine Kidney cell-passaged virus; WHO: World Health Organization.

Analysed viruses were a convenience sample of those collected by the Canadian Sentinel Physician Surveillance Network, contributing to vaccine effectiveness analyses and fully sequenced across all antigenic sites.

The comparator virus specified in bold is the 2014/15 influenza A(H3N2) HGR X-223A vaccine strain used by manufacturers. Sentinel influenza A(H3N2) viruses (n = 217, total of all four provinces) are compared against this strain with respect to antigenic site aa substitutions. Only antigenic site residues with substitutions in sentinel or vaccine viruses relative to the anchoring X-223A HGR are displayed. The aa residues 145, 156 and 159 shaded in black are recognised H3 antigenic cluster transition sites.

Viruses labelled clade 3C.3x bear the L157S substitution +/– N122D substitution but have not yet received official clade level-specific designation. They are temporarily labelled clade 3C.3x for this manuscript.

[a] #aa signifies the number of aa substitutions between the sentinel virus sequence and the X-223A HGR at H3 antigenic sites, A–E.

[b] % identity calculated as [1 – (number of aa substitutions in antigenic sites) / (total number of antigenic site aa residues)] × 100%, relative to the X-223A HGR. The total number of A–E antigenic site aa residues is 131 for H3 viruses.

[c] A/Victoria/361/2011 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the northern hemisphere's 2012/13 influenza vaccine.

[d] IVR-165 is the egg-adapted HGR version of A/Victoria/361/2011 used by vaccine manufacturers.

[e] A/Texas/50/2012 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the northern hemisphere's 2014/15 influenza vaccine

[f] X-223A is the egg-adapted HGR version of A/Texas/50/2012 used by manufacturers, shown in bold as the strain against which sentinel influenza A(H3N2) virus antigenic site aa are compared.

[g] A/Switzerland/9715293/2013 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the southern hemisphere's 2015 influenza vaccine.

[h] IVR-176 and X-247 are egg-adapted HGR versions of A/Switzerland/9715293/2013 for vaccine manufacturers.

Figure 3B

Influenza A(H3N2) haemagglutinin (HA1) antigenic site pairwise sequence and per cent amino acid identity comparisons, relative to the 2014/15 high growth reassortant vaccine strain X-223A, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 217)

Antigenic site		C		E							A	B	A						B				A	D	B			D							E	C				Clade	# aa (% identity)[a,b]
Amino acid number HA1		48	53	62	63	78	83	88	91	94	122	128	137	138	140	142	144	145	156	157	159	160	168	171	186	192	198	207	208	213	214	219	226	261	278	279	309	311	312		
A/Victoria/361/2011 (MDCK)[c]		I	D	E	N	G	K	V	S	Y	N	T	S	A	I	R	N	N	H	L	F	K	M	N	G	I	S	K	R	V	I	S	I	R	N	S	V	Q	S	3C	6 (95.4%)
2012-13 HGR: A/Victoria/361/2011 (IVR-165)[d]		I	D	E	N	G	K	V	S	Y	N	T	S	A	I	R	N	N	Q	L	F	K	M	N	V	I	S	K	R	V	I	Y	I	R	N	S	V	Q	S	3C	6 (95.4%)
A/Texas/50/2012 (MDCK)[e]		I	D	E	N	G	K	V	S	Y	N	N	S	A	I	R	N	N	H	L	F	K	M	N	G	I	P	K	R	V	I	S	I	R	K	S	V	Q	S	3C.1	3 (97.7%)
HGR: A/Texas/50/2012 (X-223A)[f]		**I**	**D**	**E**	**N**	**G**	**K**	**V**	**S**	**Y**	**N**	**N**	**S**	**A**	**I**	**R**	**N**	**N**	**H**	**L**	**F**	**K**	**M**	**N**	**V**	**I**	**P**	**K**	**R**	**V**	**I**	**F**	**N**	**R**	**K**	**S**	**V**	**Q**	**S**	**3C.1**	-
A/Switzerland/9715293/2013 (MDCK)[g]		I	D	E	N	G	K	V	S	Y	N	A	S	S	I	G	N	S	H	L	S	K	M	N	G	I	S	K	R	V	I	S	I	R	K	S	V	Q	S	3C.3a	9 (93.1%)
2015 HGR: A/Switzerland/9715293/2013 (IVR-176)[h]		I	D	E	N	G	K	V	S	Y	N	A	S	S	R	G	N	S	R	L	S	K	M	N	V	I	S	K	R	V	I	S	I	R	K	S	V	Q	S	3C.3a	10 (92.4%)
2015 HGR: A/Switzerland/9715293/2013 (X-247)[h]		I	D	E	N	G	K	V	S	Y	N	A	S	S	R	G	N	S	H	L	S	K	M	N	V	I	S	K	R	V	I	F	I	R	K	S	V	Q	S	3C.3a	8 (93.9%)
Alberta	n																																								
A/Alberta/57/2014	48											T					S	S			Y	T			G		S					S	I					H		3C.2a	10 (92.4%)
A/Alberta/56/2014	11											T					S	S			Y	T			G		S		G			S	I					H		3C.2a	11 (91.6%)
A/Alberta/77/2014	16							I				T					S	S			Y	T			G		S					S	I					H		3C.2a	11 (91.6%)
A/Alberta/113/2014	1							I				T					S	S			Y				G		S					S	I					H		3C.2a	10 (92.4%)
A/Alberta/55/2014	1											T					S	S			Y	T	I		G		S					S	I					H		3C.2a	11 (91.6%)
A/Alberta/60/2014	1				K							T					S	S			Y	T			G		S					S	I					H		3C.2a	11 (91.6%)
A/Alberta/67/2014	1									H		T					S	S			Y	T			G		S					S	I					H		3C.2a	11 (91.6%)
A/Alberta/100/2014	1					D						T					S	S			Y	T			G		S					S	I	L				H		3C.2a	12 (90.8%)
A/Alberta/119/2014	1		N									T					S	S			Y	T			G		S					S	I					H		3C.2a	11 (91.6%)
A/Alberta/120/2014	3											T	A				S	S			Y	T			G		S					S	I					H		3C.2a	11 (91.6%)
A/Alberta/127/2014	1											T					S	S			Y	T			G		S					S	I	P				H		3C.2a	11 (91.6%)
A/Alberta/133/2014	3										S	T					S	S			Y	T			G		S					S	I					H		3C.2a	11 (91.6%)
A/Alberta/138/2014	1											T					S	S			Y	T			G		S					S	I			F		H		3C.2a	11 (91.6%)
A/Alberta/159/2014	1							I				T					S	S			Y	T			G		S			I		S	I					H		3C.2a	12 (90.8%)
A/Alberta/166/2014	2	T										T					S	S			Y	T			G		S					S	I					H		3C.2a	11 (91.6%)
A/Alberta/124/2014	1											A				G		S							G		S					S	I	Q						3C.3	8 (93.9%)
A/Alberta/88/2014	4			K			R				D	A				G		S		S					G		S					S	I	Q						3C.3x	12 (90.8%)

HGR: high-growth reassortant; MDCK: Madin Darby Canine Kidney cell-passaged virus; WHO: World Health Organization.

Analysed viruses were a convenience sample of those collected by the Canadian Sentinel Physician Surveillance Network, contributing to vaccine effectiveness analyses and fully sequenced across all antigenic sites.

The comparator virus specified in bold is the 2014/15 influenza A(H3N2) HGR X-223A vaccine strain used by manufacturers. Sentinel influenza A(H3N2) viruses (n = 217, total of all four provinces) are compared against this strain with respect to antigenic site aa substitutions. Only antigenic site residues with substitutions in sentinel or vaccine viruses relative to the anchoring X-223A HGR are displayed. The aa residues 145, 156 and 159 shaded in black are recognised H3 antigenic cluster transition sites.

Viruses labelled clade 3C.3x bear the L157S substitution +/– N122D substitution but have not yet received official clade level-specific designation. They are temporarily labelled clade 3C.3x for this manuscript.

[a] #aa signifies the number of aa substitutions between the sentinel virus sequence and the X-223A HGR at H3 antigenic sites, A–E.

[b] % identity calculated as [1 – (number of aa substitutions in antigenic sites) / (total number of antigenic site aa residues)] × 100%, relative to the X-223A HGR. The total number of A–E antigenic site aa residues is 131 for H3 viruses.

[c] A/Victoria/361/2011 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the northern hemisphere's 2012/13 influenza vaccine.

[d] IVR-165 is the egg-adapted HGR version of A/Victoria/361/2011 used by vaccine manufacturers.

[e] A/Texas/50/2012 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the northern hemisphere's 2014/15 influenza vaccine

[f] X-223A is the egg-adapted HGR version of A/Texas/50/2012 used by manufacturers, shown in bold as the strain against which sentinel influenza A(H3N2) virus antigenic site aa are compared.

[g] A/Switzerland/9715293/2013 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the southern hemisphere's 2015 influenza vaccine.

[h] IVR-176 and X-247 are egg-adapted HGR versions of A/Switzerland/9715293/2013 for vaccine manufacturers.

Figure 3C

Influenza A(H3N2) haemagglutinin (HA1) antigenic site pairwise sequence and per cent amino acid identity comparisons, relative to the 2014/15 high growth reassortant vaccine strain X-223A, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 217)

Antigenic site		C		E							A	B	A						B					A	D	B			D								E	C				Clade	# aa (% identity)[a,b]
Amino acid number HA1		48	53	62	63	78	83	88	91	94	122	128	137	138	140	142	144	145	156	157	159	160	168	171	186	192	198	207	208	213	214	219	226	261	278	279	309	311	312		
A/Victoria/361/2011 (MDCK)[c]		I	D	E	N	G	K	V	S	Y	N	T	S	A	I	R	N	N	H	L	F	K	M	N	G	I	S	K	R	V	I	S	I	R	N	S	V	Q	S	3C	6 (95.4%)
2012-13 HGR: A/Victoria/361/2011 (IVR-165)[d]		I	D	E	N	G	K	V	S	Y	N	T	S	A	I	R	N	N	Q	L	F	K	M	N	V	I	S	K	R	V	I	Y	I	R	N	S	V	Q	S	3C	6 (95.4%)
A/Texas/50/2012 (MDCK)[e]		I	D	E	N	G	K	V	S	Y	N	N	S	A	I	R	N	N	H	L	F	K	M	N	G	I	P	K	R	V	I	S	I	R	K	S	V	Q	S	3C.1	3 (97.7%)
HGR: A/Texas/50/2012 (X-223A)[f]		**I**	**D**	**E**	**N**	**G**	**K**	**V**	**S**	**Y**	**N**	**N**	**S**	**A**	**I**	**R**	**N**	**N**	**H**	**L**	**F**	**K**	**M**	**N**	**V**	**I**	**P**	**K**	**R**	**V**	**I**	**F**	**N**	**R**	**K**	**S**	**V**	**Q**	**S**	**3C.1**	-
A/Switzerland/9715293/2013 (MDCK)[g]		I	D	E	N	G	K	V	S	Y	N	A	S	S	I	G	N	S	H	L	S	K	M	N	G	I	S	K	R	V	I	S	I	R	K	S	V	Q	S	3C.3a	9 (93.1%)
2015 HGR: A/Switzerland/9715293/2013 (IVR-176)[h]		I	D	E	N	G	K	V	S	Y	N	A	S	S	R	G	N	S	R	L	S	K	M	N	V	I	S	K	R	V	I	S	I	R	K	S	V	Q	S	3C.3a	10 (92.4%)
2015 HGR: A/Switzerland/9715293/2013 (X-247)[h]		I	D	E	N	G	K	V	S	Y	N	A	S	S	R	G	N	S	H	L	S	K	M	N	V	I	S	K	R	V	I	F	I	R	K	S	V	Q	S	3C.3a	8 (93.9%)
Ontario	n																																									
A/Ontario/44/2014	14											T					S	S			Y	T			G		S					S	I					H		3C.2a	10 (92.4%)
A/Ontario/42/2014	8											T					S	S			Y	T			G		S					S	I	L				H		3C.2a	11 (91.6%)
A/Ontario/41/2014	1	T								H		T					S	S			Y	T			G		S					S	I					H		3C.2a	12 (90.8%)
A/Ontario/58/2014	1											T					S	S			Y	T			G		S					S	I				I	H		3C.2a	11 (91.6%)
A/Ontario/68/2014	1											T					S				Y	T			G		S					S	I					H		3C.2a	9 (93.1%)
A/Ontario/71/2014	1											T					S	S			Y	T		K	G		S					S	I	L				H		3C.2a	12 (90.8%)
A/Ontario/74/2014	1	T										T					S	S			Y	T			G		S					Y	I					H		3C.2a	11 (91.6%)
A/Ontario/61/2014	1											A				G		S							G		S					S	I							3C.3	7 (94.7%)

HGR: high-growth reassortant; MDCK: Madin Darby Canine Kidney cell-passaged virus; WHO: World Health Organization.

Analysed viruses were a convenience sample of those collected by the Canadian Sentinel Physician Surveillance Network, contributing to vaccine effectiveness analyses and fully sequenced across all antigenic sites.

The comparator virus specified in bold is the 2014/15 influenza A(H3N2) HGR X-223A vaccine strain used by manufacturers. Sentinel influenza A(H3N2) viruses (n = 217, total of all four provinces) are compared against this strain with respect to antigenic site aa substitutions. Only antigenic site residues with substitutions in sentinel or vaccine viruses relative to the anchoring X-223A HGR are displayed. The aa residues 145, 156 and 159 shaded in black are recognised H3 antigenic cluster transition sites.

Viruses labelled clade 3C.3x bear the L157S substitution +/– N122D substitution but have not yet received official clade level-specific designation. They are temporarily labelled clade 3C.3x for this manuscript.

[a] #aa signifies the number of aa substitutions between the sentinel virus sequence and the X-223A HGR at H3 antigenic sites, A–E.

[b] % identity calculated as [1 – (number of aa substitutions in antigenic sites) / (total number of antigenic site aa residues)] × 100%, relative to the X-223A HGR. The total number of A–E antigenic site aa residues is 131 for H3 viruses.

[c] A/Victoria/361/2011 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the northern hemisphere's 2012/13 influenza vaccine.

[d] IVR-165 is the egg-adapted HGR version of A/Victoria/361/2011 used by vaccine manufacturers.

[e] A/Texas/50/2012 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the northern hemisphere's 2014/15 influenza vaccine

[f] X-223A is the egg-adapted HGR version of A/Texas/50/2012 used by manufacturers, shown in bold as the strain against which sentinel influenza A(H3N2) virus antigenic site aa are compared.

[g] A/Switzerland/9715293/2013 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the southern hemisphere's 2015 influenza vaccine.

[h] IVR-176 and X-247 are egg-adapted HGR versions of A/Switzerland/9715293/2013 for vaccine manufacturers.

Figure 3D

Influenza A(H3N2) haemagglutinin (HA1) antigenic site pairwise sequence and per cent amino acid identity comparisons, relative to the 2014/15 high growth reassortant vaccine strain X-223A, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 217)

Antigenic site		C		E							A	B	A						B				A	D	B			D								E	C					Clade	# aa (% identity)[a,b]
Amino acid number HA1		48	53	62	63	78	83	88	91	94	122	128	137	138	140	142	144	145	156	157	159	160	168	171	186	192	198	207	208	213	214	219	226	261	278	279	309	311	312		
A/Victoria/361/2011 (MDCK)[c]		I	D	E	N	G	K	V	S	Y	N	T	S	A	I	R	N	N	H	L	F	K	M	N	G	I	S	K	R	V	I	S	I	R	N	S	V	Q	S	3C	6 (95.4%)
2012-13 HGR: A/Victoria/361/2011 (IVR-165)[d]		I	D	E	N	G	K	V	S	Y	N	T	S	A	I	R	N	N	Q	L	F	K	M	N	V	I	S	K	R	V	I	Y	I	R	N	S	V	Q	S	3C	6 (95.4%)
A/Texas/50/2012 (MDCK)[e]		I	D	E	N	G	K	V	S	Y	N	N	S	A	I	R	N	N	H	L	F	K	M	N	G	I	P	K	R	V	I	S	I	R	K	S	V	Q	S	3C.1	3 (97.7%)
HGR: A/Texas/50/2012 (X-223A)[f]		**I**	**D**	**E**	**N**	**G**	**K**	**V**	**S**	**Y**	**N**	**N**	**S**	**A**	**I**	**R**	**N**	**N**	**H**	**L**	**F**	**K**	**M**	**N**	**V**	**I**	**P**	**K**	**R**	**V**	**I**	**F**	**N**	**R**	**K**	**S**	**V**	**Q**	**S**	**3C.1**	-
A/Switzerland/9715293/2013 (MDCK)[g]		I	D	E	N	G	K	V	S	Y	N	A	S	S	I	G	N	S	H	L	S	K	M	N	G	I	S	K	R	V	I	S	I	R	K	S	V	Q	S	3C.3a	9 (93.1%)
2015 HGR: A/Switzerland/9715293/2013 (IVR-176)[h]		I	D	E	N	G	K	V	S	Y	N	A	S	S	R	G	N	S	R	L	S	K	M	N	V	I	S	K	R	V	I	S	I	R	K	S	V	Q	S	3C.3a	10 (92.4%)
2015 HGR: A/Switzerland/9715293/2013 (X-247)[h]		I	D	E	N	G	K	V	S	Y	N	A	S	S	R	G	N	S	H	L	S	K	M	N	V	I	S	K	R	V	I	F	I	R	K	S	V	Q	S	3C.3a	8 (93.9%)
Quebec	n																																									
A/Quebec/55/2014	15											T					S	S			Y	T			G		S					S	I					H		3C.2a	10 (92.4%)
A/Quebec/36/2014	26											T					S	S			Y	T			G		S					S	I	L				H		3C.2a	11 (91.6%)
A/Quebec/110/2014	1											T					S	S			Y				G		S					S	I	L				H		3C.2a	10 (92.4%)
A/Quebec/35/2014	13	T										T					S	S			Y	T			G		S					S	I					H		3C.2a	11 (91.6%)
A/Quebec/112/2014	1	T										T					S	S			Y	T			G							S	I	Q				H		3C.2a	11 (91.6%)
A/Quebec/59/2014	1								N			T					S	S			Y	T			G		S					S	I					H		3C.2a	11 (91.6%)
A/Quebec/76/2014	2											T					S	S			Y	T			G		S	R				S	I					H		3C.2a	11 (91.6%)
A/Quebec/109/2014	1											T					S	S			Y	T			G	A	S					S	I					H		3C.2a	11 (91.6%)
A/Quebec/117/2014	1					S						T					S	S			Y	T			G		S					S	I	L				H		3C.2a	12 (90.8%)
A/Quebec/44/2014	1			K							D	A				G		S		S					G		S					S	I							3C.3x	10 (92.4%)

HGR: high-growth reassortant; MDCK: Madin Darby Canine Kidney cell-passaged virus; WHO: World Health Organization.

Analysed viruses were a convenience sample of those collected by the Canadian Sentinel Physician Surveillance Network, contributing to vaccine effectiveness analyses and fully sequenced across all antigenic sites.

The comparator virus specified in bold is the 2014/15 influenza A(H3N2) HGR X-223A vaccine strain used by manufacturers. Sentinel influenza A(H3N2) viruses (n = 217, total of all four provinces) are compared against this strain with respect to antigenic site aa substitutions. Only antigenic site residues with substitutions in sentinel or vaccine viruses relative to the anchoring X-223A HGR are displayed. The aa residues 145, 156 and 159 shaded in black are recognised H3 antigenic cluster transition sites.

Viruses labelled clade 3C.3x bear the L157S substitution +/– N122D substitution but have not yet received official clade level-specific designation. They are temporarily labelled clade 3C.3x for this manuscript.

[a] #aa signifies the number of aa substitutions between the sentinel virus sequence and the X-223A HGR at H3 antigenic sites, A–E.

[b] % identity calculated as [1 – (number of aa substitutions in antigenic sites) / (total number of antigenic site aa residues)] × 100%, relative to the X-223A HGR. The total number of A–E antigenic site aa residues is 131 for H3 viruses.

[c] A/Victoria/361/2011 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the northern hemisphere's 2012/13 influenza vaccine.

[d] IVR-165 is the egg-adapted HGR version of A/Victoria/361/2011 used by vaccine manufacturers.

[e] A/Texas/50/2012 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the northern hemisphere's 2014/15 influenza vaccine

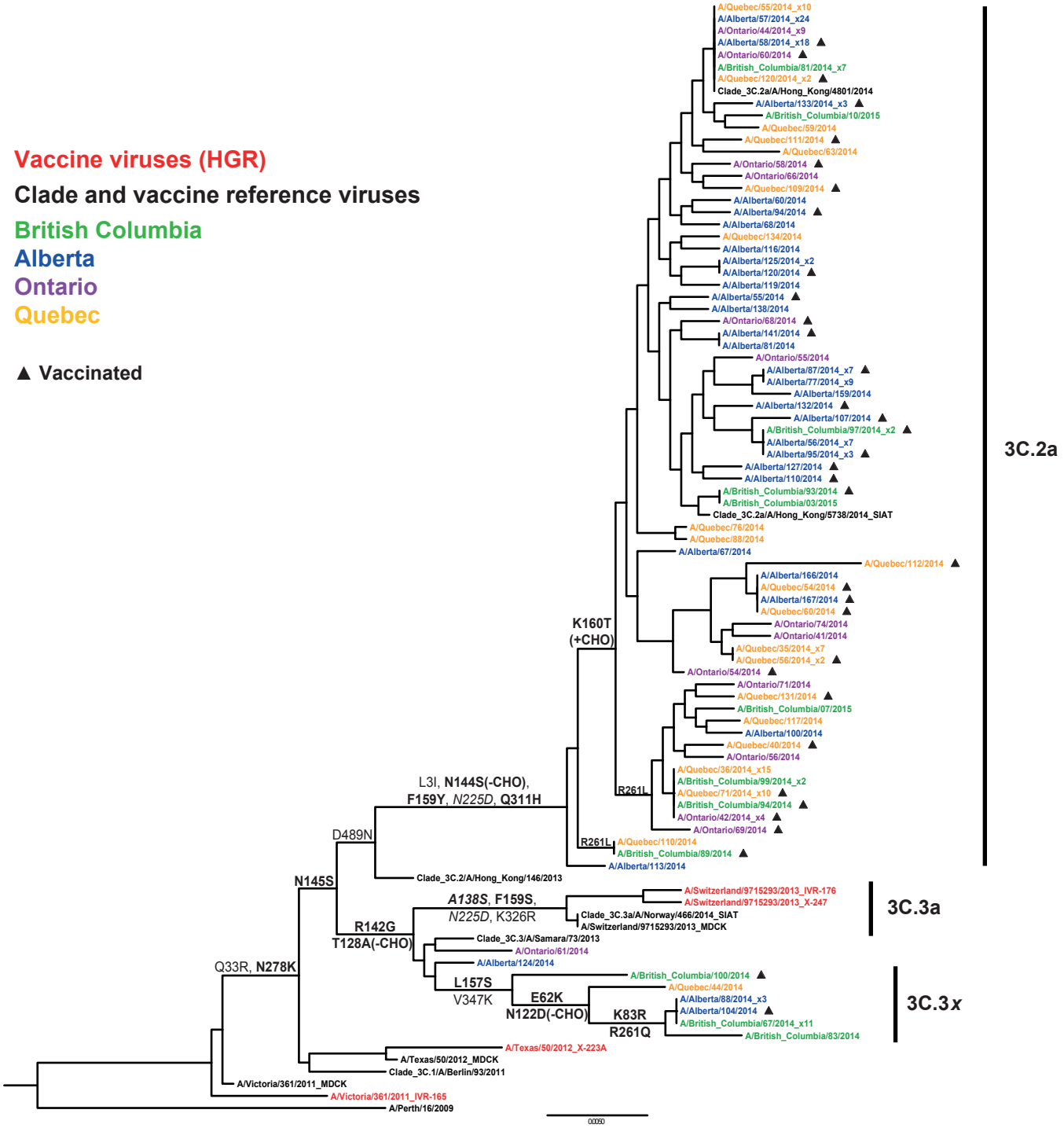
[f] X-223A is the egg-adapted HGR version of A/Texas/50/2012 used by manufacturers, shown in bold as the strain against which sentinel influenza A(H3N2) virus antigenic site aa are compared.

[g] A/Switzerland/9715293/2013 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the southern hemisphere's 2015 influenza vaccine.

[h] IVR-176 and X-247 are egg-adapted HGR versions of A/Switzerland/9715293/2013 for vaccine manufacturers.

Figure 4

Phylogenetic tree of influenza A(H3N2) viruses 2014/15, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 215)



The phylogenetic tree was constructed by alignment of 215 Canadian sentinel translated sequences covering the 514 residues of the extracellular domain against sequences representative of emerging viral clades as described by the European Centre for Disease Prevention and Control (n=6) [12], and recent vaccine A(H3N2) prototype and high-growth reassortant strains (n=8) (Table 1). Substitutions in bold are in antigenic sites and italicised substitutions are in the receptor-binding site.

23%, is in striking contrast to the 2013/14 mid-season VE analysis. During that season's interim analysis with comparable sample size, we measured substantial and statistically significant VE of 74% (95% CI: 58–83%) against the dominant but antigenically well-conserved A(H1N1)pdm09 epidemic strain [2]. The VE point estimate reported here for the 2014/15 seasonal vaccine is the lowest component-specific estimate reported by the Canadian SPSN against any seasonal strain of the past 10 years, including other recent influenza A(H3N2) vaccine-mismatched seasons in 2012/13 (VE=45% mid-season [3], 41% end-of-season [4]) or 2010/11 (VE=39%) [7].

Consistent with the low VE we report for 2014/15, virtually all (99%) of the sentinel influenza A(H3N2) viruses contributing to VE analysis showed genetic and/or antigenic evidence of vaccine mismatch. Although only seven SPSN viruses contributing to VE analysis grew to sufficient titre for antigenic characterisation by HI assay, the high proportion of vaccine-mismatched viruses reported here is similar to reports from national laboratory-based surveillance summaries for Canada [1]. Of the 62 A(H3N2) viruses HI-characterised in the presence of oseltamivir carboxylate and reported to date nationally by Canada's NML (including non-SPSN viruses), 61 (98%) have shown reduced titres to the A/Texas/50/2012(H3N2) vaccine strain [1]. The majority of these viruses have clustered with clade 3C.2a, and the remainder with what we have provisionally labelled here as clade 3C.3*x*. Nationally, based on genetic characterisation of viruses unable to grow to sufficient titre for HI assay, 393 of 395 (99%) viruses to date have been found to belong to one of these two genetic groups (foremost clade 3C.2a) and are considered antigenically distinct from the vaccine strain [1]. The approach used this season to impute vaccine mismatch based on phylogenetic findings follows that established by the United States Centers for Disease Control and Prevention (US CDC) where only 64% of circulating A(H3N2) viruses so far this season have been considered antigenically distinct from the vaccine strain [20]. This substantial difference between Canada and the US in the proportion of A(H3N2) viruses that are considered vaccine-mismatched may explain the higher (albeit still suboptimal) VE estimate reported in mid-season analysis by the US CDC (22%) [22]; however, other methodological, demographic or immunological differences should also be considered.

As in previous seasons, non-elderly adults contributed most (60%) to our VE analyses, although elderly participants were slightly more represented (16%) this season compared to previous years (10% or less) [2-4,6,7]. The adult predominance in our sample may be relevant to consider when comparing our 2014/15 mid-season VE estimates to those from the US CDC, where there was a greater paediatric contribution (43% of the overall sample) [22]. Children are less likely to have had prior influenza vaccine or virus exposure history and are more likely to have received LAIV. LAIV has been associated with better efficacy than inactivated vaccine in the very young [23-27], although the opposite was observed against influenza A(H1N1)pdm09 in the US during the 2013/14 season [28] and relative effectiveness in the context of substantial vaccine mismatch or with history of prior repeat immunisation is uncertain. Our VE estimate against influenza A(H3N2) in non-elderly adults of 2% is comparable to (within 10% of) the US mid-season VE estimate for adults 18–49 years-old (12%), although neither country's estimate in adults is statistically significant and confidence intervals overlap. More nuanced evaluation of age and other influences on VE will be important to explore with larger sample size in end-of-season analyses.

At the genetic level, vaccine-virus divergence in 2014/15 was defined among Canadian SPSN viruses by a substantial number of aa differences (10–11) in the dominant (>90%) clade 3C.2a viruses relative to the vaccine component, including substitutions at pivotal antigenic, cluster-transition and receptor-binding sites and/or in association with potential gain or loss of glycosylation, each of which may influence antibody recognition. Substitutions evident in the vaccine strain, notably associated with egg-adaptation and HGR generation, may also have compounded the effects of antigenic drift in circulating viruses [4]. The emerging but as yet minor subgroup of viruses bearing the L157S +/- N122D mutation (here labelled clade 3C.3*x*) also warrants close monitoring. Although position 157 has not been identified historically as a cluster-transition residue, it is within the same pocket as other key residues (i.e. 155, 156, 158, 159) and may be of emerging significance [16]. The loss of glycosylation associated with the N122D substitution may also be influential [17]. Clade 3C.3 viruses with this particular combination of aa substitutions have not previously been identified by the Canadian SPSN, but were detected in Spain during the 2013/14 season, cited in association with the low VE (13%) against A(H3N2) viruses in mid-season analysis from that country [29]. Compared with Spanish sequences from 2013/14, clade 3C.3*x* viruses characterised by the Canadian SPSN in 2014/15 have acquired an additional three aa mutations in antigenic site E, an antigenic site distant from the RBS and not typically considered immuno-dominant but possibly relevant to overall virus fitness.

As published previously by the Canadian SPSN [4,6] and US CDC and other investigators [30-33], we observed variability in VE by prior vaccination history. In particular, VE against influenza A(H3N2) among those who received the 2014/15 influenza vaccine without prior vaccination in 2013/14 was higher (43%) than among participants who were vaccinated with the same A(H3N2) vaccine component in both 2013/14 and 2014/15 (–15%). Although none are statistically significant, these substantial differences in VE based on prior immunisation are consistent with the antigenic distance hypothesis articulated by Smith et al. [34]. That hypothesis suggests that negative interference from

prior immunisation may be more pronounced when the antigenic distance is small between successive vaccine components but large between vaccine and circulating strains. Such is the scenario for the current 2014/15 season for which the identical A(H3N2) vaccine component was used as during the 2013/14 season, poorly matched to the 2014/15 epidemic strain. However, limited sample size precludes definitive conclusions, particularly since a large proportion (nearly 90%) of vaccinated SPSN participants are repeat vaccine recipients [2-4,6,7]. There may also be other unrecognised differences across subgroups of participants with differing immunisation histories. Further evaluation is required across additional study settings and seasons and with greater sample size to confirm these findings, assess possible underlying immunological interactions, and inform implications for vaccine reformulation and policy recommendation.

There are limitations to this study, notably related to sample size, in particular in subgroup analyses. Mid-season analysis was undertaken with the recognition that sample size was sufficient to provide 80% statistical power to detect a VE of at least 40%, given vaccine coverage typically spanning 30 to 40% in our setting. The absence of statistical significance with much lower VE is not unexpected given that in order to measure a VE of 10% in either direction from zero with the same statistical power would require more than 10,000 participants and more than 1 million participants would be required to show a significant VE of 1%. Our findings are thus consistent with a VE close to zero, where a precise estimate may never be resolved statistically. Higher VE may be observed in final end-of-season analyses, particularly if other influenza types or subtypes for which the trivalent vaccine is a better match circulate through the remainder of the 2014/15 season. Vaccine status in this study was based on self-reporting which may introduce some misclassification bias. However, this information was collected at the time of specimen collection, before the test result was known, minimising differential misclassification. As in prior seasons' analyses by the SPSN, the predominance of adults and repeat influenza vaccine recipients among our study participants is relevant to consider in the generalisation of our findings to other settings where the population profile may differ. Although we uniquely characterised more than half of our sentinel A(H3N2) viruses to the level of clade specification, and our virological profile reflected that of national surveillance summaries for Canada [1], we cannot rule out systematic differences in viruses available for genetic or antigenic characterisation, a problem for all laboratory-based surveillance. The validity of VE estimates derived by the test-negative approach has been previously demonstrated [35,36] but the design remains observational and bias and confounding cannot be ruled out.

In summary, interim VE findings from the Canadian SPSN indicate that the 2014/15 influenza vaccine has provided little or no protection against medically attended illness due to predominant and substantially mismatched A(H3N2) viruses this season. Given limited vaccine protection, other adjunct protective measures should be considered to minimise associated morbidity and mortality, particularly among high-risk individuals. The virological and/or host factors influencing reduced vaccine protection against influenza A(H3N2) during the 2014/15 season warrant further in-depth investigation.

GenBank Accession Numbers

Viruses from original specimens with complete or partial sequences of the haemagglutinin (HA) gene (HA1 and HA2) provided by provincial laboratories and contributing to the 2014/15 interim influenza vaccine effectiveness analysis by the Canadian Sentinel Physician Surveillance Network were deposited in GenBank with accession numbers KP701523 –KP701743.

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Conflict of interest

Within 36 months of manuscript submission, GDS received research grants from GlaxoSmithKline (GSK) for unrelated vaccine studies. JG has received a research grant from Pfizer. MK has received research grants from Roche, Merck, Gen-Probe and Siemens. SS and TLK are funded by the Canadian Institutes of Health Research Grant (TPA-90193). The other authors declare that they have no competing interests to report.

Authors' contributions

Principal investigator (epidemiology): DMS (National and British Columbia); GDS (Québec); JAD (Alberta); ALW (Ontario). Investigators (laboratory): JBG (Ontario); HC and CM (Québec); MP and MK (British Columbia); SD and KF (Alberta); YL and NB (national). National database coordination: TLK. Data analysis: CC and DMS (epidemiology); SS and AE (phylogenetic). Preparation of first draft: DMS. Draft revision and approval: all.

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Emergence of a novel GII.17 norovirus – End of the GII.4 era?

M de Graaf (m.degraaf@erasmusmc.nl)[1], J van Beek[1,2], H Vennema[2], A T Podkolzin[3], J Hewitt[4], F Bucardo[5], K Templeton[6], J Mans[7], J Nordgren[8], G Reuter[9], M Lynch[10], L D Rasmussen[11], N Iritani[12], M C Chan[13], V Martella[14], K Ambert-Balay[15], J Vinjé[16], P A White[17], M P Koopmans[1,2]

1. Erasmus MC, Department of Viroscience, Rotterdam, the Netherlands
2. Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), the Netherlands
3. Central Research Institute of Epidemiology, Moscow, Russia
4. Institute of Environmental Science and Research, Porirua, New Zealand
5. Department of Microbiology, University of Leon, Nicaragua
6. Department of Medical Microbiology, Royal Infirmary of Edinburgh, Edinburgh, United Kingdom
7. Department of Medical Virology, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa
8. Division of Molecular Virology, Department of Clinical and Experimental Medicine, Linköping University, Sweden
9. Regional Laboratory of Virology, National Reference Laboratory of Gastroenteric Viruses, ÁNTSZ Regional Institute of State Public Health Service, Pécs, Hungary
10. Department of Microbiology, Mater Misericordiae University Hospital, Dublin, Ireland
11. Virology Surveillance and Research Section, Microbiological Diagnostics and Virology, Statens Serum Institut, Denmark
12. Department of Microbiology, Osaka City Institute of Public Health and Environmental Sciences, Tennoji-ku, Osaka, Japan
13. Department of Microbiology, Chinese University of Hong Kong, China
14. Faculty of Veterinary Medicine, Università Aldo Moro di Bari, Valenzano, Italy
15. National Reference Center for Enteric Viruses, Laboratory of Virology, CHU of Dijon, Dijon, France
16. Division of Viral Diseases, National Center for Immunizations and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States
17. School of Biotechnology and Biomolecular Sciences, Faculty of Science, University of New South Wales, Sydney, Australia

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In the winter of 2014/15 a novel GII.P17-GII.17 norovirus strain (GII.17 Kawasaki 2014) emerged, as a major cause of gastroenteritis outbreaks in China and Japan. Since their emergence these novel GII.P17-GII.17 viruses have replaced the previously dominant GII.4 genotype Sydney 2012 variant in some areas in Asia but were only detected in a limited number of cases on other continents. This perspective provides an overview of the available information on GII.17 viruses in order to gain insight in the viral and host characteristics of this norovirus genotype. We further discuss the emergence of this novel GII.P17-GII.17 norovirus in context of current knowledge on the epidemiology of noroviruses. It remains to be seen if the currently dominant norovirus strain GII.4 Sydney 2012 will be replaced in other parts of the world. Nevertheless, the public health community and surveillance systems need to be prepared in case of a potential increase of norovirus activity in the next seasons caused by this novel GII.P17-GII.17 norovirus.

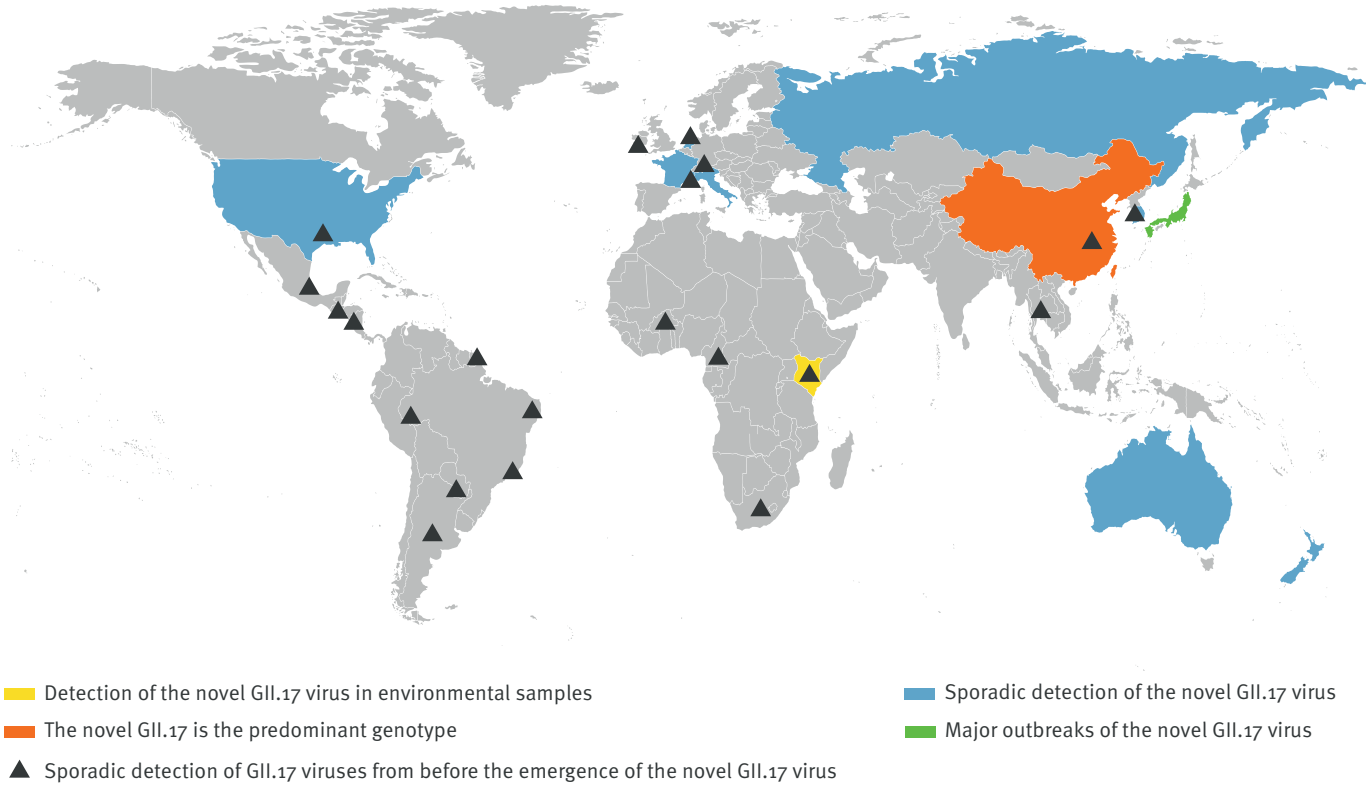
In this issue of *Eurosurveillance*, observations from Japan are reported on an unusual prevalence of a previously rare norovirus genotype, GII.17, in diarrheal disease outbreaks at the end of the 2014/15 winter season [1], similar to what was observed for China [2,3]. Norovirus is a leading cause of gastroenteritis [4]. Although the infection is self-limiting in healthy individuals, clinical symptoms are much more severe and can last longer in immunocompromised individuals, the elderly and young children [5,6].

The *Norovirus* genus comprises seven genogroups (G), which can be subdivided in more than 30 genotypes [7]. Viruses belonging to the GI, GII and GIV genogroups can infect humans, but since the mid-1990s GII.4 viruses have caused the majority (ca 70–80%) of all norovirus-associated gastroenteritis outbreaks worldwide [8-10].

GII.4 viruses can continue to cause widespread disease in the human population because they evolve through accumulations of mutations into so-called drift variants that escape immunity from previous exposures [11]. Contemporary GII.4 noroviruses also demonstrate intra-genotype recombination near the junction of open reading frame (ORF) 1 and ORF2, which is likely to foster the emergence of novel GII.4 variants [12]. In addition, the binding properties of GII.4 viruses have altered over time, resulting in a larger susceptible host population [13].

Figure 1

World map showing areas where GII.17 norovirus strains have been detected, 1978–2015



Emergence and geographical spread of GII.17 genotype noroviruses

Viruses of the GII.17 genotype have been circulating in the human population for at least 37 years; the first GII.17 strain in the National Center for Biotechnology Information (NCBI) databank is from 1978 [14]. Since then viruses with a GII.17 capsid genotype have sporadically been detected in Africa, Asia, Europe, North America and South America (Table, Figure 1). The virus appears to be clinically relevant, as it has been associated with acute gastroenteritis (AGE) in children and adults, and with chronic infection in an immunocompromised renal transplant patient [15] and a leukaemia patient (unpublished data). In the United States (US), only four GII.17 outbreaks were reported between 2009 to 2013 through CaliciNet, with a median of 11.5 people affected by each outbreak [16]. In Noronet, an informal international network of scientists working in public health institutes or universities sharing virological, epidemiological and molecular data on norovirus, GII.17 cases were also sporadically reported in Denmark and South Africa during this period [17].

More widespread circulation of GII.17 was first reported for environmental samples in Korea from 2004 to 2006. This information was published in a report in 2010 by the Korean Food and Drug Administration (KFDA) and was cited by Lee et al. [18], but the original document describing this finding is not publicly available and there are no matching clinical reports. From 2012 to 2013 a novel GII.17 virus accounted for 76% of all detected norovirus strains in rivers in rural and urban areas in Kenya [19]. In the winter of 2014/15, genetically closely related GII.17 viruses were first detected in AGE outbreaks in the Guangdong province in China in schools, colleges, factories and kindergartens [3]. Sequence analyses demonstrated that 24 of the 29 reported outbreaks during that winter were caused by GII.17. A large increase in the incidence of AGE outbreaks was also reported; 29 outbreaks associated with 2,340 cases compared with nine outbreaks and 949 cases in the previous winter when GII.4 Sydney 2012 still was the dominant genotype [3].

During the same winter there was also an increase in outbreak activity in Jiangsu province, which could be attributed to the emergence of this novel GII.17 [2]. This triggered us to investigate the prevalence of GII.17 in other parts of the world by means of a literature study and by inviting researchers collaborating within Noronet to share their data on GII.17. Currently, in Asia, in addition to Guangdong and Jiangsu [2,3], the novel GII.17 is also the predominant genotype in Hong Kong (unpublished data) and Taiwan [20], while in Japan, a sharp increase in the number of cases caused by this novel virus has been observed during the 2014/15 winter season [1]. Related viruses have been detected sporadically in the US [21] (http://www.cdc.gov/norovirus/reporting/calicinet/index.html), Australia, France, Italy, Netherlands, New-Zealand and Russia (unpublished data, www.noronet.nl) (Figure 1). In France the novel GII.17 virus appeared at the beginning of 2013, but since

Table A

Overview of detected GII.17 norovirus strains worldwide, 1978–2015

Country	Geographical spread GII.17[a]	Year[b]	ORF1	ORF2	Study population	Proportions of typed strains or outbreaks[c]	Suspected source of infection	Description of the sequence (size)	Accession number	References
French Guiana	Single location	1978	GII.P4	GII.17	Children with AGE	1 strain	–	Partial genome (7,441 bp)	KC597139, JN699043	[14]
Brazil	Rio de Janeiro	1997 (1994–2008)	–	GII.17	Children with AGE	3/52 strains	–	5'-end ORF2 (300 bp)	JN600531	[31]
Kenya	Nairobi	1999–2000	–	GII.17	HIV positive children with or without AGE	1/11 strains	–	5'-end ORF2 (309 bp)	KF279387	[32]
France	Briançon	2004	GII.P13	GII.17	Child with AGE	1 strain	–	Partial ORF1/2 (1,361 bp)	EF529741	Data not shown
Paraguay	Asuncion	2004–2005	–	GII.17	AGE in children (<5 years)	5/29 strains	–	3'-end ORF2 (255 bp)	KC736582, KC736580, KC736578, KC736569	[33]
Brazil	States of Acre (Brazil)	2005 (2005–2009)	–	GII.17	AGE	2/62 strains	–	3'-end ORF2 (215 bp)	JN587118 JN587117	[34]
United States	Houston	2005	–	GII.17	AGE evacuees hurricane Katrina	Predominant genotype in an outbreak	Sewage	ORF2 and 3 (2,459 bp)	DQ438972	[35]
Argentina	Single location (Argentina)	2005–2006	–	GII.17	River samples	1/33 strains	–	–	–	[36]
Brazil	State of Rio de Janeiro	2005–2006 (2004–2011)	–	GII.17	Outbreaks of AGE	3/112 outbreaks	–	3'-end ORF2 (214 bp)	KJ179752, KJ179753, KJ179754	[37]
Nicaragua	Léon	2005–2006	–	GII.17	AGE	1 strain	–	5'-end ORF2 (244 bp)	EU780764	[26]
France	Sommières	2006	GII.P13	GII.17	AGE	1 strain	Foodborne	Partial ORF1/2 (1,056 bp)	EF529742	Data not shown
Thailand	Lopburi	2006–2007	–	GII.17	AGE	2 strains	–	5'-end ORF2 (209 bp)	GQ325666, GQ325670,	[38]
China	Wuhan	2007 (2007–2010)	GII.P13	GII.17	AGE	1/488 strains	–	Partial ORF1/2 (1,096 bp)	JQ751044	[39]
Mexico	Mexico City	2007	–	GII.17	–	–	Waterborne	5'-end ORF2 (1,337 bp)	JF970609	NCBI[d]
Switzerland	Zürich	2008	–	GII.17	Renal transplant patient	1/9 strains	–	ORF2 (1,599 bp)	GQ266696	[15]
Nicaragua	Léon	2008	–	GII.17	AGE in children (<5 years)	2/38 strains	–	5'-end ORF2 (244 bp)	EU780764	[40]
South Korea	Seoul	2010 (2008–2011)	–	GII.17	AGE	1/710 strains	–	5'-end ORF2 (209)	JQ944348	[41]
Brazil	Quilombola	2009 (2008–2010)	–	GII.17	Children (<10 years)	2/16 strains	–	3'-end ORF2 (215 bp)	JX047021, JX047022	[42]

Table B

Overview of detected GII.17 norovirus strains worldwide, 1978–2015

Country	Geographical spread GII.17[a]	Year[b]	ORF1	ORF2	Study population	Proportions of typed strains or outbreaks[c]	Suspected source of infection	Description of the sequence (size)	Accession number	References
Cameroon	Southwestern region of Cameroon	2009	GII.P13	GII.17	Healthy children and HIV positive adults	4/15 strains	–	Partials ORF1/2 (1,024 bp)	JF802504–JF802507	[43]
Guatemala	Tecpan	2009	–	GII.17	Children after waterborne outbreak	1/18 strains	Waterborne	–	–	[44]
Burkina Faso	Ouagadougou	2009–2010	–	GII.17	AGE in children (<5 years)	1/36 strains	–	5'-end ORF2 (287 bp)	JX416405	[27]
Netherlands	Single location	2002–2007	–	GII.17	Nosocomial	3/264 strains	Nosocomial	–	–	[45]
South Korea	South Korea	2010	–	GII.17	Groundwater samples	2/7 strains	–	5'-end ORF2 (311 bp)	KC915021–KC915022	[18]
Ireland	Ireland	2010	–	GII.17	Influent waste water	4/24 strains	–	5'-end (302 bp)	JQ362530	[46]
South Africa	South Africa	2010–2011	–	GII.17	Waste water	9/69 strains	–	5'-end ORF2 (305 bp)	KC495680, KC495686, KC495672–KC495674, KC495664, KC495657, KC495655, KC495640	[47]
South Korea	Jinhae Bay	2010–2011	–	GII.17	Oysters	1 strain	–	–	–	[48]
Morocco	Oujda (Morocco)	2011	–	GII.17	AGE in children (<5 years)	1/42 strains	–	5'-end (205 bp)	KJ162374	[49]
South Africa	Johannesburg (South Africa)	2011	GII.P16	GII.17	AGE	–		Partial ORF1/2 (1,010 bp)	KC962460	[50]
Cameroon	Limbe	2011–2012	GII.P3	GII.17	Healthy adults and Children	4/100 strains	–	Partial ORF1/2 (653 bp)	KJ946403	[51]
Kenya	Kenya	2012–2013	–	GII.17	Surface water	16/21 strains	–	5'-end ORF2 (306 bp)	KF916584–KF916585, KF808227–KF808254	[19]
South Korea	Gyeonggi	2012	–	GII.17	AGE outbreak	1 strain	Waterborne	5'-end (205 bp)	KC413386 KC413399–KC413403	[22]
China	Guangdong province	2014–2015	–	GII.17	AGE outbreaks	24/29 outbreaks	–	5'-end (249 bp)	KP718638–KP718738	[3]
United States	Gaithersburg	2014	GII.P17	GII.17	AGE in child of 3 years	1 strain	–	Partial genome (7,527 bp)	KR083017	[21]
China	Jiangsu province	2014–2015	GII.P17	GII.17	Outbreaks of AGE	16/23 outbreaks	–	–	KR270442–KR270449	[2]

then, it has not

Table C

Overview of detected GII.17 norovirus strains worldwide, 1978–2015

Country	Geographical spread GII.17[a]	Year[b]	ORF1	ORF2	Study population	Proportions of typed strains or outbreaks[c]	Suspected source of infection	Description of the sequence (size)	Accession number	References
Japan	Japan	2014–2015	GII.P17	GII.17	Outbreaks of AGE	100/2,133 strains	–	Partial genome (7,534–7,555 bp)	AB983218, LC037415, LC043139, LC043167, LC043168, LC043305	[1]

AGE: acute gastroenteritis; HIV: human immunodeficiency virus; NCBI: National Center for Biotechnology Information; ORF: open reading frame.

[a] GII.17 detection location with study location between brackets (when different from GII.17 detection location).

[b] GII.17 detection year(s) with study years between brackets.

[c] Either the proportion of strains that was typed as GII.17 or the proportion of outbreaks that was caused by GII.17 is given.

[d] Information derived from the GenBank entry related to the accession number of the sequence.

resulted in an increase in AGE outbreaks as observed in China, nor replaced the predominant GII.4 in the last seasons (data not shown).

Based on sequence analyses of the ORF1-ORF2 junction region, most diagnostic real-time transcription polymerase chain reactions (PCRs) will be able to detect this novel GII.17 virus, but it is not known whether the same holds true for immunoassays. However, only a small portion of norovirus outbreaks are typed beyond the GI and GII classification, therefore it is possible that GII.17 is more prevalent than we currently suspect.

Phylogenetic analyses and molecular characterisation of the novel GII.17 viruses

Phylogenetic analysis of the viral protein 1 (VP1) of GII.17 strains in the NCBI database demonstrated at least two clusters, with the novel Asian GII.17 strains grouping together with the GII.17 strains detected in the surface water in Kenya (Figure 2,[21]) and in an outbreak in 2012 in Korea [22]. Although the novel GII.17 clusters away from previously identified GII.17 strains, the amino acids changes in VP1 are not sufficient to separate it into a different genotype. For only a limited number of GII.17 strains the full VP1 has been sequenced, which demonstrated three deletions and at least one insertion compared with previous GII.17 strains (comprehensive alignments are given in Fu et al. and Parra et al. [2,21]). The majority of these changes could be mapped in or near major epitopes of the VP1 protein and potentially result in antigenic drift or altered receptor-binding properties [21]. Most publicly available GII.17 sequences only comprise the VP1, and most frequently the 5'-end of VP1 (C region), while most of the observed diversity within the GII.17 genotype is observed in the 3'-end of VP1 (D region) [23].

Previously, viruses with a GII.17 VP1 genotype contained a GII.P13 ORF1 genotype, although recombinants with an ORF1 GII.P16, GII.P3 and GII.P4 genotype have also been identified (Table). Sequence comparison showed that the ORF1 region of the novel GII.17 viruses was not detected before and cluster between GII.P3 and GII.P13 viruses [21]. Since this is the first orphan ORF1 sequence associated with GII.17, it has been designated GII.P17 according to the criteria of the proposal for a unified norovirus nomenclature and genotyping [24]. The novel GII.17 virus was termed Kawasaki 2014 after the first near complete genome sequence (AB983218) submitted to GenBank. Noronet provides a publicly available and widely used tool for the typing of norovirus sequences (http://www.rivm.nl/mpf/norovirus/typingtool). This typing tool was updated to ensure correct classification of both ORF1 and ORF2 sequences of the newly emerged GII.P17-GII.17 viruses.

The acquisition of a novel ORF1 could potentially result in an increase in replication efficiency and may – in part – explain the increase of the AGE outbreak activity. Histo-blood group antigens (HBGAs) function as (co-) receptors for noroviruses. Alpha(1,2)fucosyltransferase

Figure 2

Unrooted maximum likelihood phylogenetic tree based on the 5'-end of virus protein 1 (VP1) sequences (C region) of GII.17 noroviruses, available from the National Center for Biotechnology Information (NCBI)

The tree was estimated under the general time reversible model using PhyML. Bootstrap values above 70% are given. Sequences from Kenya are depicted in red and those from the recent outbreaks (2013–1015) reported in Asia in blue. The scale bar represents nucleotide substitutions per site.

2 (FUT2) adds an alpha-1,2 linked fucose on HBGAs, and individuals lacking the FUT2 gene are referred to as 'non-secretors', while those with a functional FUT2 gene are called 'secretors'. Non-secretors have been shown to be less susceptible to infection with several norovirus genotypes [25]. In studies investigating the genetic susceptibility to norovirus genotypes, a secretor patient with blood type O Lewis phenotype Le^{a-b+} and a secretor patient with blood type B Lewis phenotype Le^{a-b-} were positive for previously identified GII.17 viruses and no non-secretors were found positive [26,27], suggesting that there could be genetic restrictions for GII.17 viruses in infection of humans. How the observed genetic changes have affected the antigenic and binding properties of the novel GII.17 strains, and hereby the susceptible host population, remains to be discovered.

Public health implications

Based on the emergence and spread of new GII.4 variants, we know that noroviruses are able to rapidly spread around the globe [28,29]. The novel GII.17 virus has been detected in sporadic cases throughout the world, but until now it has not resulted in an increase

in outbreak activity or replacement of GII.4 Sydney 2012 viruses outside of Asia. Following the patterns observed in the past years for GII.4 noroviruses and based on the data from China and Japan, an increase in norovirus outbreak activity can be expected if the currently dominant GII.4 is replaced by GII.17. Another possibility – however – would be some restriction to global expansion, as has been observed previously for the norovirus variant GII.4 Asia 2003 [29]. Such restrictions could be due to differences in pre-existing immunity, but could also be the result of differences between populations in the expression of norovirus receptors [29]. Based on current literature on the novel GII.17 virus there is no indication that it will be more virulent compared with GII.4. Nevertheless, the public health community and surveillance systems need to be prepared in case of a potential increase of norovirus activity by this novel GII.17 virus.

Conclusions

Understanding the epidemiology of norovirus genotypes is important given the development of vaccines that are entering clinical trials. Current candidate vaccines have targeted the most common norovirus genotypes, and it remains to be seen if vaccine immunity is cross-reactive with GII.17 viruses [30]. Contemporary norovirus diagnostic assays may not have been developed to detect genotype GII.17 viruses since this genotype was previously only rarely found during routine surveillance. These assays need to be evaluated and updated if necessary to correctly diagnose norovirus outbreaks caused by the emerging GII.17 virus. Norovirus strain typing ideally should include ORF1 sequences and the variable VP1 'D' region as well as metadata on the host, like clinical symptoms, immune status and blood group. This will allow us to better study and monitor the genetic disposition, pathogenesis, evolution and epidemiology of this newly emerged virus.

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Conflict of interest

None declared.

Authors' contributions

MG, JB, HV: compiling the data, drafting the manuscript; AP, FB, KT, MC, JM, JN, GR, ML, LDR, NI JH, VM, KAB, JV, PW: collecting field data, critical review of the manuscript; MK: initiation of study, providing data, critical review of the manuscript.

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An autochthonous case of Zika due to possible sexual transmission, Florence, Italy, 2014

G Venturi [1], L Zammarchi [2 3], C Fortuna [1], ME Remoli [1], E Benedetti [1], C Fiorentini [1], M Trotta [3], C Rizzo [4], A Mantella [2], G Rezza [1], A Bartoloni [2 3]

1. Department of Infectious, Parasitic and Immune-Mediate Diseases, Istituto Superiore di Sanità, Rome, Italy
2. Clinica Malattie Infettive, Dipartimento di Medicina Sperimentale e Clinica, Università degli Studi di Firenze, Florence, Italy
3. SOD Malattie Infettive e Tropicali, Azienda Ospedaliero-Universitaria Caeggi, Florence, Italy
4. National Center for Epidemiology and Health Promotion, Istituto Superiore di Sanità, Rome, Italy.

Correspondence: Alessandro Bartoloni (alessandro.bartoloni@unifi.it)

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We report a case of Zika virus infection imported in Florence, Italy ex-Thailand, leading to a secondary autochthonous case, probably through sexual transmission. The two cases occurred in May 2014 but were retrospectively diagnosed in 2016 on the basis of serological tests (plaque reduction neutralisation) performed on stored serum samples. Our report provides further evidence that sexual transmission of Zika virus is possible.

Case reports

At the beginning of May 2014, an Italian man is his early 30s (patient 1) returned to Florence, Italy, after a 10-day holiday in Thailand. On the day after his arrival, he developed a confluent maculopapular rash, on the face, trunk, arms, and legs, with fever (maximum temperature 38 °C), conjunctivitis, and frontal headache with retroocular pain.

Four days later, patient 1 was admitted to the Infectious and Tropical Diseases Unit of the Florence Careggi University Hospital. Blood tests revealed leucopenia (3,000 cells/µL; reference: 4,000–10,000/µL) while creatinine, platelet count and transaminases were normal. Serological investigation two days after (i.e. 6 days after symptoms onset), showed past exposure to measles and parvovirus, negative results for human immunodeficiency virus (HIV) 1–2 Ab/Ag and chikungunya IgM, a positive result for dengue virus (DENV) IgM, and negative results for DENV IgG, as well as DENV NS1 Ag (Table).

The symptoms subsequently rapidly resolved (total duration of fever and rash: 6 days) and he was discharged nine days after admission with a probable diagnosis of DENV infection.

Perifocal vector control activities (including spraying adult mosquitoes and destruction of larval breeding sites) were implemented the day after the availability of DENV IgM positive results, around the patient's residence and workplace, even though the period of activity of *Aedes albopictus* in Italy is usually considered to start in June and end in October [1]. A second and third blood test using enzyme-linked immunosorbent assay (ELISA), performed 38 and 109 days after symptoms onset, showed DENV IgG seroconversion and IgM negativisation in the third sample.

Nineteen days after the onset of symptoms in patient 1, his girlfriend (patient 2), who was in her late 20s developed diffuse pain, associated to both wrists and oedema on fingers of each hand, maculopapular rash on the trunk, arms, and legs, without fever. Four days later she was evaluated at the outpatient facility of the same hospital. Patient 2 had not travelled to tropical areas during the previous year. Blood tests performed on the next day (5 days after her symptoms started) showed normal white blood cells and platelet count, normal C-reactive protein, creatinine, transaminases, and undetectable beta-human chorionic gonadotropin (HCG). The patient had IgG antibodies against cytomegalovirus, Epstein–Barr virus, parvovirus and rubella, while she was seronegative for coxsackie A, coxsackie B, echovirus and DENV (IgG, IgM and NS1 Ag). Serological tests were repeated 39 and 93 days after symptoms onset, respectively, showing a slight positivity for DENV IgG, with IgM and NS1Ag persistently negative (Table).

Retrospective testing of serum samples in 2015 and 2016

Serum samples of both patients were sent to the Istituto Superiore di Sanità (ISS), Rome, Italy, to perform confirmatory tests (Table) for DENV in June and September 2015, respectively. Plaque reduction neutralisation tests (PRNTs) for DENV gave inconclusive results for both patients: indeed, a 50% of plaque reduction was

Table

Laboratory diagnostic test results for dengue virus and Zika virus in two patients, Italy, 2014–2016

Patient	Days from onset of symptoms	Dengue virus tests						Zika virus tests	
		ELISA IgM[a,b]	ELISA IgG[a,b]	ELISA NS1[a,b]	ELISA IgM[a,c]	PRNT50[a,c] titre	Real-time PCR[a,c]	PRNT80[c,d] titre	Real-time PCR[a,c]
1	6	24.2	5.21	2.23	2.01	Neg	Neg	1:10	Neg
	38	12.3	16.6	NC	2.89	1:10 (b.l.)	NC	≥1:160	NC
	109	3.23	16.4	1.84	0.87	1:10 (b.l.)	NC	≥1:160	NC
2	5	1.34	4.63	3.81	0.46	Neg	Neg	1:10	Neg
	39	3.23	15.5	2.63	0.40	1:10 (b.l.)	NC	≥1:160	NC
	93	2.51	13.2	2.77	0.34	1:10 (b.l.)	NC	≥1:160	NC

b.l.: borderline; ELISA: enzyme-linked immunosorbent assay; NC: not conducted; Neg: negative; PRNT: plaque reduction neutralisation tests; PCR: polymerase chain reaction.

[a] Test performed in 2014.

[b] Tests performed at Azienda Ospedaliero Universitaria Careggi, Florence (Italy). Commercial ELISA (VIRCELL Granada-Spain). Reference values (index): ›11: positive; 9–11: inconclusive; ‹9: negative. Positive results are highlighted in bold.

[c] Tests performed at the Istituto Superiore di Sanità, Rome (Italy). Commercial IgM-capture ELISA system (Focus Diagnostics dengue Virus IgM Capture, DxSelect, California, US). Reference values (index): ›1: positive; ‹1: negative. Positive results are highlighted in bold. Real-time PCRs were conducted on RNA from serum samples, as described in [29] and [30]. Dengue virus for PRNT: serotype 2 dengue virus (New Guinea B strain). PRNT80 titres ≥1:10 are considered positive, while PRNT50 titres ≥1:10 are considered as borderline.

[d] Test retrospectively performed in 2016 on stored samples. Zika virus for PRNT was kindly provided by Dr Isabelle Leparc-Goffart of the French National Reference Center on Arboviruses in Marseille. The test was performed as described in detail for tick-borne encephalitis virus [31], except that Vero cells were used here.

observed at a 1:10 serum dilution in the second and third serum samples of both patients, while we consider the cut-off for a positive result to be at least 80% of plaque reduction. Real-time polymerase chain reaction (PCR) tests for DENV, chikungunya virus (CHIKV), and Zika virus (ZIKV), as well as viral isolation in Vero E6 cell, were also performed on samples collected in the acute phase of the disease, all with negative results.Even though DENV PRNT results were inconclusive, patient 1 was counselled as having had dengue infection, given the history of travel and the classical kinetic of IgG and IgM antibodies measured by ELISA, while we were not able to state a definitive diagnosis for patient 2.After ZIKV for PRNT became available to us, the samples were reanalysed in February 2016 (the patients had given their informed consent for further tests), and showed positive results for ZIKV neutralising antibodies, as reported in the Table, with a clear increase in the antibody titre between the first and the second serum sample for both patients.

Background

ZIKV is an *Aedes*-borne virus (Flaviviridae family), identified in 1947 in monkey rhesus in Uganda [2,3]. Sporadic human cases were reported in Asia and Africa until 2007, when a ZIKV outbreak occurred in Yap, Micronesia [4]. Subsequently, in October 2013, ZIKV reached French Polynesia, causing a large outbreak [5]. In early 2015, autochthonous cases of ZIKV were reported in Brazil [6], and the virus subsequently spread throughout South America, Central America, and the Caribbean [7-9]. An increasing number of imported cases has been observed in Europe and United States (US) [10-13]. The presumed association of ZIKV infection during pregnancy with increased number of babies born with microcephaly in Brazil [14] convinced the World Health Organization to declare ZIKV a 'Global Emergency of Public Health Concern' in February 2016 [15].

Discussion and conclusions

Even if ZIKV transmission is mostly vectorial, transplacental and perinatal transmission have been reported; transmission through blood transfusion may also occur [16-18].

Little evidence supports the possibility of ZIKV sexual transmission to date. In December 2013, ZIKV was isolated from the semen of a patient with haematospermia in Tahiti [19]. Further in 2014, ZIKV RNA was detected 62 days after onset of febrile illness in the semen of a person with ZIKV infection, imported into the United Kingdom from the Cook Islands [20]. Sexual transmission from a man who acquired ZIKV infection in Senegal, to his wife was reported in Colorado, US, in 2007 [21], and more recently from a person who had travelled to Latin America, to his partner in Texas [22].

Possible sexual transmission of ZIKV is of particular concern during pregnancy, and specific guidelines for prevention of ZIKV infection through this route have been published recently [23].

Because patient 2 had not travelled to tropical areas during the previous year and had unprotected sexual intercourse with patient 1 during a 20 day period between his return to Italy and her own onset of symptoms, transmission by semen was suggested. Exact dates of sexual intercourse could not be recalled by the patients, who reported several sexual contact events before patient 2's symptom onset. Other transmission modalities (i.e. direct contact with other bodily fluids)

are unlikely to play a role but may not be completely ruled out.

Transmission through local potentially competent vectors, *Ae. albopictus*, can likely be excluded considering that patient 1 came back to Italy outside the usual period of vector activity and vector control measures were implemented within eight days after his arrival to Italy, possibly before the estimated extrinsic incubation period could be completed [1,24].

Failure to detect viral RNA even in samples collected few days after the onset of symptoms, and an early detection of ZIKV-specific neutralising antibodies, are consistent with previous reports [10,19,25]; however, limits in the sensitivity of the real-time PCR method used in this study cannot be definitively excluded. Serological test results confirm the broad cross-reactivity between DENV and ZIKV. With respect to PRNT results, borderline results for DENV are likely to be due to a low degree of residual cross-reactivity which may not be eliminated even using this test, which is considered highly specific. Another possible limit of our study consists in the fact that only serotype 2 DENV PRNT could be performed; however, this is not likely to affect the interpretation of the results, which clearly show a pattern consistent with ZIKV infection.

Current evidence supports the combined use of PCR and serological tests for the diagnosis of ZIKV infection. PCR can be positive in early serum and saliva samples (‹ 8 days after symptoms onset), with saliva showing higher detection rates, while PCR on urine seems to enlarge the window of detection of ZIKV RNA up to ca 30 days after symptoms onset [26,27]. Five days after disease onset, serological investigations can be conducted by detection of ZIKV-specific IgM antibodies and confirmation by neutralisation [28].

In conclusion, we provide additional evidence for sexual transmission of ZIKV. Further studies are needed to estimate the probability of sexual transmission and its role as a secondary route of transmission of ZIKV in epidemic and non-epidemic areas.

Conflict of interest

None declared.

Authors' contributions

Wrote the manuscript: LZ, GR, GV, AB; performed laboratory investigations: AM, CF, MER, EB, CF, GV; revised the manuscript: GR, MT, CR; managed the patients: LZ, MT.

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Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016

BB Xavier [1,2,3], C Lammens [1,2,3], R Ruhal [1,2,3], S Kumar-Singh [1,3,4], P Butaye [5,6,7], H Goossens [1,2,3], S Malhotra-Kumar [1,2,3]

1. Laboratory of Medical Microbiology, Wilrijk, Belgium
2. Vaccine & Infectious Disease Institute, Wilrijk, Belgium
3. University of Antwerp, Wilrijk, Belgium
4. Molecular Pathology group, Cell Biology and Histology, Wilrijk, Belgium
5. Ghent University, Faculty of Veterinary Medicine, Ghent, Belgium
6. CODA-CERVA, Brussels, Belgium
7. Ross University School of Veterinary Medicine, Basseterre, Saint Kitts and Nevis

Correspondence: Surbhi Malhotra-Kumar (surbhi.malhotra@uantwerpen.be)

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We identified a novel plasmid-mediated colistin-resistance gene in porcine and bovine colistin-resistant *Escherichia coli* that did not contain *mcr-1*. The gene, termed *mcr-2*, a 1,617 bp phosphoethanolamine transferase harboured on an IncX4 plasmid, has 76.7% nucleotide identity to *mcr-1*. Prevalence of *mcr-2* in porcine colistin-resistant *E. coli* (11/53) in Belgium was higher than that of *mcr-1* (7/53). These data call for an immediate introduction of *mcr-2* screening in ongoing molecular epidemiological surveillance of colistin-resistant Gram-negative pathogens.

Following the report of of *mcr-1* detection in China in November 2015 [1], we screened 105 colistin-resistant *Escherichia coli* (colistin minimum inhibitory concentration (MIC) 4–8 mg/L [2]) isolated during 2011–12 from passive surveillance of diarrhoea in 52 calves and 53 piglets in Belgium [3]. *mcr-1* was detected in 12.4% (n = 13) of the *E. coli* isolates, of which six and seven were from calves and piglets, respectively [3,4]. In the present study, we analysed porcine and bovine colistin-resistant *Escherichia coli* isolates that did not show presence of *mcr-1* and identified a novel plasmid-mediated colistin resistance-conferring gene, *mcr-2*.

Identification of *mcr-2* in colistin-resistant *E. coli* isolates not harbouring *mcr-1*

Of 92 porcine and bovine colistin-resistant *Escherichia coli* isolates not harbouring *mcr-1*, 10 were randomly selected for further analysis. Plasmid DNA was isolated (PureLink HiPure Plasmid Miniprep Kit, Invitrogen, Carlsbad, CA, United States), sequenced by Illumina (2 x 250 bp) (Nextera XT sample preparation kit, MiSeq), de novo assembled and annotated using SPAdes (v3.8.1) and RAST [5,6]. Plasmids from three of the 10 *E. coli* isolates showed the presence of a gene for a putative membrane protein, which was identified as a phosphoethanolamine transferase (sulfatase) using pfam and Interproscan protein databases [7,8] The *mcr-2* gene, as we termed it, is 1,617 bp long, nine bases shorter than *mcr-1* (1,626 bp), and shows 76.75% nt identity to *mcr-1* (supplementary material [9]).

The entire *mcr-2* gene was amplified (PCR primers: MCR2-F 5' TGGTACAGCCCCTTTATT 3'; MCR2-R 5'GCTTGAGATTGGGTTATGA 3'), cloned (vector pCR 2.1, TOPO TA Cloning kit, Invitrogen) and electroporated into DH-5 α *E. coli*. Transformants exhibited colistin MICs of 4–8 mg/L (E-test, bioMerieux, Marcy l'Etoile, France), which were reconfirmed by macrobroth dilution (European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [2]).

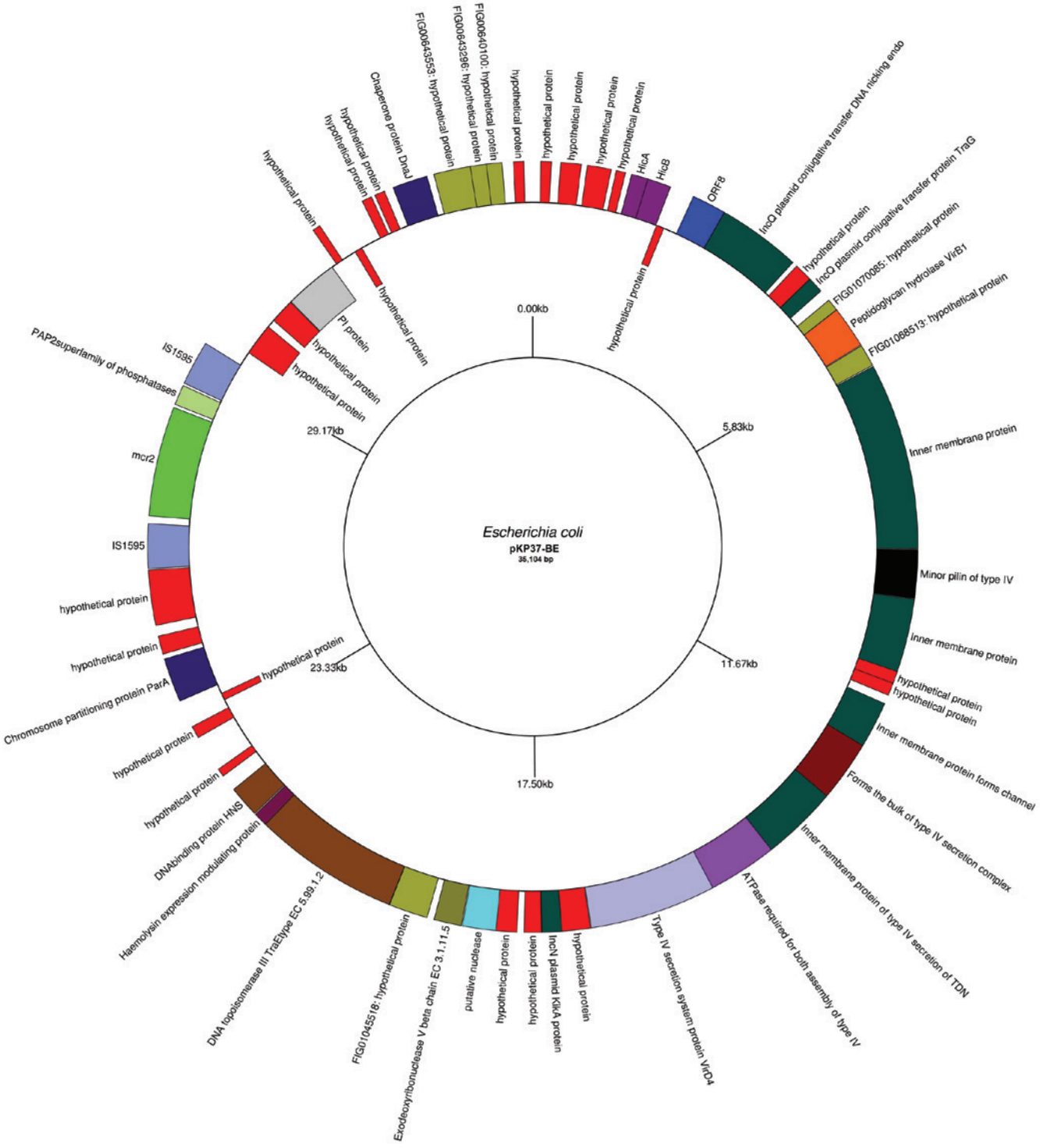
mcr-2 is harboured on IS1595 with likely origins in *Moraxella* spp.

mcr-2-harbouring plasmids from all three *E. coli* isolates were analysed. The mobile element harbouring *mcr-2* was identified as an IS element of the IS1595 superfamily, which are distinguished by the presence of an ISXO2-like transposase domain [10].

We also identified a 297 bp open reading frame downstream of *mcr-2* on this element, which encodes a PAP2 membrane-associated lipid phosphatase with 41% identity to *Moraxella osloensis* phosphatidic acid phosphatase (71% query coverage). Interestingly, a blastn search of the IS1595 backbone, after removal of the *mcr-2* and *pap2* phosphatase gene sequences, identified a single hit to *Moraxella bovoculi* strain 58069 (GenBank accession number CP011374) genomic region

Figure 1

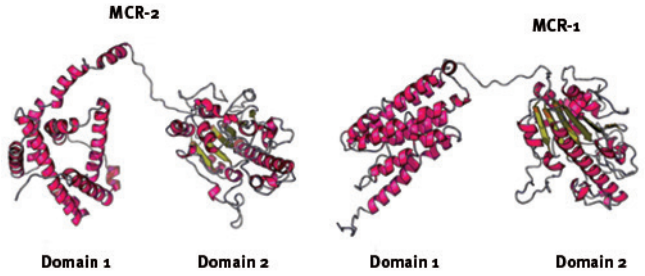
Genetic organisation and structure of the *mcr-2*-harbouring plasmid pKP37-BE from a colistin-resistant *Escherichia coli* isolate not harbouring *mcr-1*, Belgium, June 2016



The plasmid map was generated using GenomeVx [23].

Figure 2

MCR-2 and *MCR-1* predicted tertiary structures



RaptorX [24] was used to generate the structures. For both MCR-2 and MCR-1, domain 1 was predicted to be a transporter and domain 2 a phosphoethanolamine transferase (sulfatase).

(1,531,602 to 1,532,255 bp) with 75% identity and 100% query coverage.

mcr-2 is harboured on an IncX4 incompatibility-type plasmid in *E. coli* ST10

The three *mcr-2* plasmid-harbouring *E. coli* isolates belonged to ST10 (n = 2, porcine) and ST167 (n = 1, bovine). All three plasmids belonged to IncX4 incompatibility type; all three *mcr-2* genes showed 100% homology.

Plasmid pKP37-BE isolated from one of the porcine ST10 *E. coli* isolates was found to have a size of 35,104 bp, 41.3% GC content and 56 protein-encoding gene sequences (RAST) (Figure 1); European Nucleotide Archive accession numbers PRJEB14596 (study) and LT598652 (plasmid sequence).

Apart from IS1595, pKP37-BE did not carry any other resistance genes and the plasmid backbone was highly similar to *Salmonella enterica* subsp. *enterica* serovar Heidelberg plasmid pSH146_32 (GenBank accession number JX258655), with 98% identity and 90% query coverage. Several *Salmonella*-associated virulence genes were found on pKP37-BE, including *virB/D4* that encodes a type 4 secretion system [11].

Conjugation experiments using a rifampicin-resistant *E. coli* recipient (A15) showed an approximately 1,200-fold higher transfer frequency of the *mcr-2*-harbouring pKP37-BE (1.71 × 10−3) compared with the *mcr-1* harbouring IncFII plasmid, pKP81-BE (1.39 × 10−6) [4]. Both *mcr-1* and *mcr-2* transconjugants exhibited colistin MICs of 4–8 mg/L (macrobroth dilution).

Structure predictions and phylogenetic analyses of the MCR-2 protein

MCR-2 protein was predicted to have two domains, with domain 1 (1 to 229 residues) as a transporter and domain 2 (230 to 538 residues) as a transferase domain (Figure 2).

The best template for domain 1 was 4HE8, a secondary membrane transport protein with a role in transferring solutes across membranes [12]. The best-fit template for domain 2 was 4kav (p = 4.13 e-13), a lipooligosaccharide phosphoethanolamine transferase A from *Neisseria meningitides*, also previously shown to be the best-fit template for MCR-1 [1]. 4kav belongs to the YhjW/YjdB/YijP superfamily and its role in conferring polymyxin resistance has been experimentally validated [13]. Overall, the un-normalised global distance test (uGDT) was 318 (GDT: 58) and all 538 residues were modelled (Figure 2).

MCR-1 and MCR-2 proteins showed 80.65% identity (supplementary material [9]). In addition, MCR-2 showed 64% identity to the phosphoethanolamine transferase of *Moraxella osloensis* (WP_062333180) with 99% sequence coverage, and 65%, 65%, and 61% identity to that of *Enhydrobacter aerosaccus* (KND21726), *Paenibacillus sophorae* (WP_063619495) and *Moraxella catarrhalis* (WP_003672704), respectively, all with 97% query coverage.

We also carried out blastp searches of the two domains of MCR-2 separately. The identity level of domain 1 between MCR-1 and MCR-2 was low (72%) compared with that for domain 2 (87.4%). Other blastp hits for the domain 2 transferase were *Enhydrobacter aerosaccus* and *Moraxella osloensis* (69% identity; 100% query coverage) followed by *Paenibacillus sophorae* (68% identity; 100% query coverage) and *Moraxella catarrhalis* (68% identity; 99% query coverage). Phylogenetic analysis showed that MCR-2 might have originated from *Moraxella catarrhalis* (56% bootstrap value) (Figure 3).

PCR-based screening identified a higher prevalence of *mcr-2* than of *mcr-1* in porcine *E. coli* in Belgium

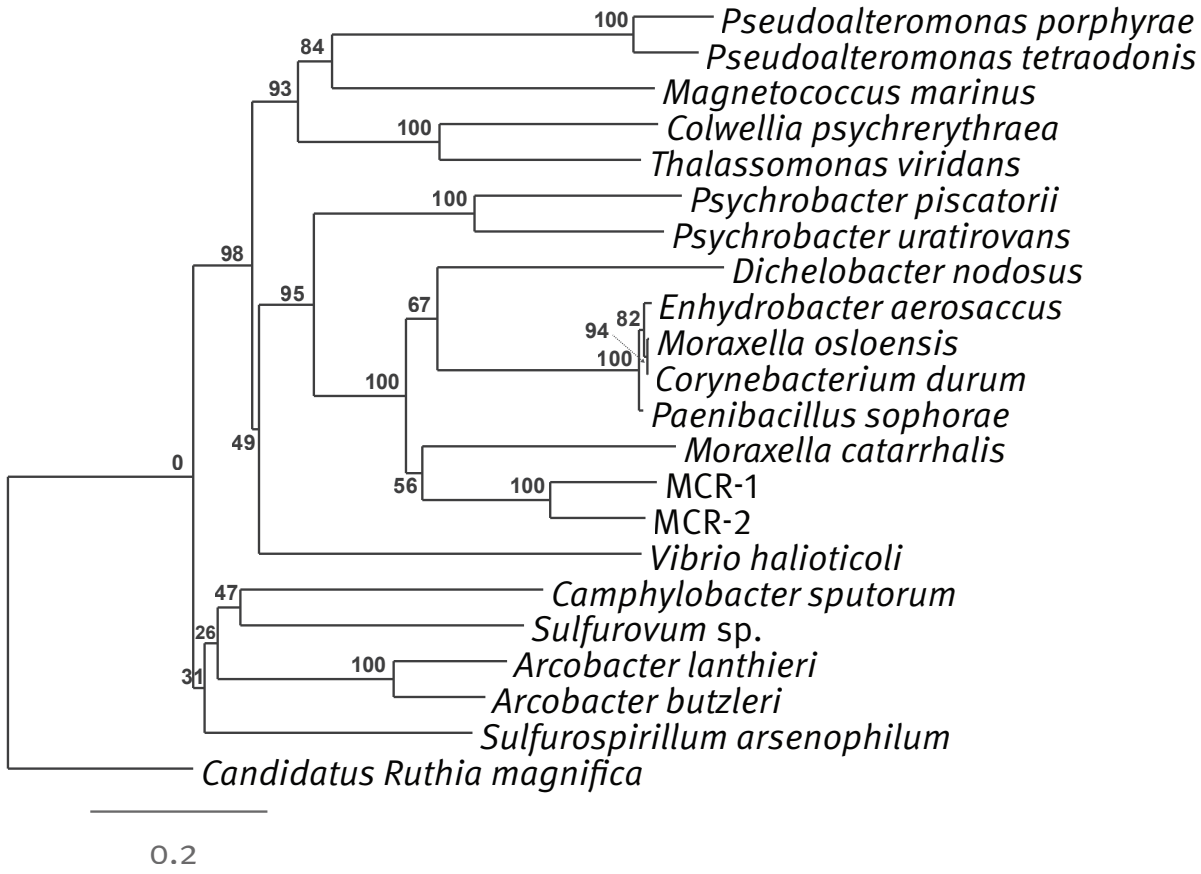
We screened our entire collection of porcine and bovine colistin-resistant *E. coli* isolates (n = 105) using an *mcr-2*-specific PCR approach using primers MCR2-IF 5' TGTTGCTTGTGCCGATTGGA 3' and MCR2-IR 5' AGATGGTATTGTTGGTTGCTG 3', and the following cycling conditions: 33 cycles of 95 °C × 3 min, 65 °C × 30 s, 72 °C × 1 min, followed by 1 cycle of 72 °C × 10 min. We found *mcr-2* in 11/53 porcine and 1/52 bovine colistin-resistant *E. coli* isolates (an overall prevalence of 11.4%).

Discussion

Identification of plasmid-mediated colistin resistance represents a paradigm shift in colistin-resistance mechanisms, which until recently were restricted to chromosomal mutations and vertical transmission. Since *mcr-1* conferring plasmid-mediated colistin resistance was first detected in China, *mcr-1* has been identified in 30 countries across five continents [14-17] (Figure 4).

Figure 3

Phylogenetic analysis of the entire MCR-2 protein sequence



Maximum likelihood tree generated by bootstrap analysis using 1,000 replicates. The analysis was carried out using CLC Genomics workbench v9.0.1 (clcbio, Qiagen) in-built tool for this evolutionary relationship with other related sequences. Branch length is proportional to the number of substitutions per site. Bootstrap values are indicated in the nodes.

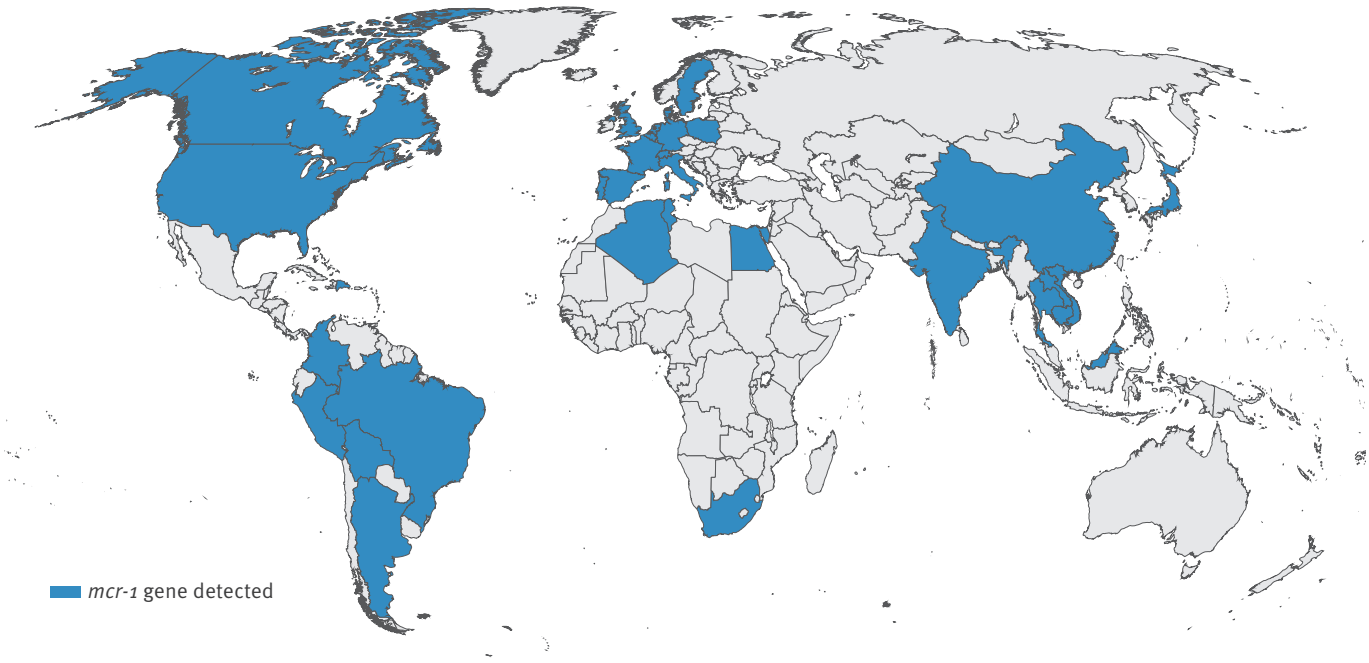
Our analysis identified a novel plasmid-mediated phosphoethanolamine transferase-encoding gene, *mcr-2*, which was detected at an even higher prevalence than that of *mcr-1* among colistin-resistant porcine *E. coli* in our study. We were, however, limited by small sample numbers. It should also be noted that the calves and piglets were from different regions of the country (calves from Wallonia and piglets from Flanders).

Phylogenetic analysis of MCR-2 provided strong evidence that this protein was distinct from MCR-1, and that it might have originated from *Moraxella catarrhalis*. The latter set of data are further strengthened by the fact that *mcr-2* is co-harboured with a lipid phosphatase gene that shows highest homology to a phosphatase from *Moraxella* spp., and that the genetic element IS1595 harbouring these two genes might itself have originated from *Moraxella* spp. While *Moraxella* spp. are not polymyxin producers, this bacterial genus is known to be intrinsically resistant to polymyxins [18] and potential intergeneric transfer of *mcr-2* from co-habiting *Moraxella* spp. of animal, human or environmental origin is therefore highly likely. Phosphoethanolamine transferases are housekeeping enzymes that catalyse the addition of the phosphoethanolamine moiety to the outer 3-deoxy-D-manno-octulosonic acid (Kdo) residue of a Kdo(2)-lipid A [19]. The fact that we did not identify any chromosomal mutations in the known colistin resistance-conferring genes in our *E. coli* isolates (by whole genome sequencing, data not shown) additionally supports the role of the acquired phosphoethanolamine transferase in conferring colistin resistance.

Finally, the high transfer frequency of the *mcr-2*-harbouring IncX4 plasmid might underlie the higher prevalence of *mcr-2* in our porcine isolates. In the three *mcr-2* harbouring isolates analysed, IS1595 showed presence of direct repeats and a complete *tnpA* gene, while inverted repeats were not found (data not shown). However, the carrier plasmid IncX4 is itself highly transmissible, showing 10^2–10^5-fold higher transfer frequencies than, for instance, epidemic IncFII plasmids, as shown previously [20] as well as in our own transconjugation experiments. Importantly, a lack of fitness-burden of IncX4 carriage on bacterial hosts [20]

Figure 4

Countries (n = 30) reporting presence of *mcr-1* in samples of animal, environmental or human origin (data collected till 27 June 2016)



Adapted from [15]; updated using data from [14,16,17,25-27].

makes this plasmid replicon a highly effective vehicle for dissemination of *mcr-2*. IncX4 plasmids have also been previously shown to harbour *mcr-1* [21] as well as extended spectrum beta-lactamase genes, $bla_{CTX\text{-}M}$ [20]. Interestingly, the pKP37-BE backbone, which likely originated from *Salmonella* spp., harboured a battery of virulence genes including the *virB4/D4* genes encoding a type-IV secretion system that has been shown to mediate downregulation of host innate immune response genes and an increased bacterial uptake and survival within macrophages and epithelial cells [11]. Outer membrane modifications leading to colistin resistance have been shown to attenuate virulence [22]: whether these co-harboured virulence genes are able to compensate the pathogenic abilities of colistin-resistant *E. coli* remains to be explored.

Taken together, these data call for immediate inclusion of *mcr-2* screening in ongoing molecular epidemiological surveillance to gauge the worldwide dissemination of *mcr-2* in both human and animal colistin-resistant Gram-negative bacteria of medical importance.

* Authors' correction

The number of countries in which *mcr-1* has been identified was updated to 32 and supporting references were added on 11 July 2016. The references in the article were renumbered accordingly.

Acknowledgements

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The complete plasmid sequence of pKP37-BE was deposited at the European Nucleotide Archive accession numbers PRJEB14596 (study) and LT598652 (plasmid sequence).

Conflict of interest

None declared.

Authors' contributions

This study was designed by SMK. Isolates were collected by PB. Experimental work was done by BBX and CL. Data was analysed and interpreted by BBX, RR, SKS, HG and SMK. The manuscript was drafted by BBX, SKS and SMK, and was reviewed by all authors.

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Estimating HIV incidence and number of undiagnosed individuals living with HIV in the European Union/ European Economic Area, 2015

A Pharris [1], C Quinten [1], T Noori [1], AJ Amato-Gauci [1], A van Sighem [2], the ECDC HIV/AIDS Surveillance and Dublin Declaration Monitoring Networks [3]

1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
2. Stichting HIV Monitoring, Amsterdam, the Netherlands.
3. Members are listed at the end of the article.

Correspondence: Anastasia Pharris (anastasia.pharris@ecdc.europa.eu)

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Since 2011, human immunodeficiency virus (HIV) incidence appears unchanged in the European Union/ European Economic Area with between 29,000 and 33,000 new cases reported annually up to 2015. Despite evidence that HIV diagnosis is occurring earlier post-infection, the estimated number of people living with HIV (PLHIV) who were unaware of being infected in 2015 was 122,000, or 15% of all PLHIV (n=810,000). This is concerning as such individuals cannot benefit from highly effective treatment and may unknowingly sustain transmission.

Although preventable through effective public health measures, human immunodeficiency virus (HIV) persists in the 31 countries of the European Union and European Economic Area (EU/EEA) [1]. In this report an analysis of EU/EEA HIV and acquired immunodeficiency syndrome (AIDS) surveillance data from 2015 as well as from prior years is presented. We estimate that, in 2015, 15% (122,000/810,000) of people living with HIV (PLHIV) in the EU/EEA were unaware of their infection.

Analysis of annual surveillance data

HIV and AIDS surveillance data are reported annually by EU/EEA countries to a joint database for HIV/AIDS within the European Surveillance System (TESSy) coordinated by the European Centre for Disease Prevention and Control (ECDC) and the World Health Organization (WHO) Regional Office for Europe [1].

Annual data on HIV diagnoses from 2003 to 2015 were stratified by the presence of a concurrent AIDS diagnosis, i.e. an AIDS-defining event within 3 months of HIV diagnosis, and, for individuals without AIDS, by CD4 cell count (≥500, 350–499, 200–349, <200 cells/mm3) at the time of diagnosis [2].

The ECDC HIV Modelling Tool version 1.2.2 was used to derive both the estimates of annual HIV incidences, as well as those of the average times from infection to HIV diagnosis each year [3]. These two types of estimates are only presented for the period from 2011 to 2015 due to greater uncertainty of data from the previous years of the study.

The number of PLHIV in 2015 who were not yet diagnosed was obtained by fitting to data on HIV diagnoses from 2003 to 2011, adjusted for reporting delay, using the 'Incidence Method', a CD4 cell count-based back-calculation method [4].

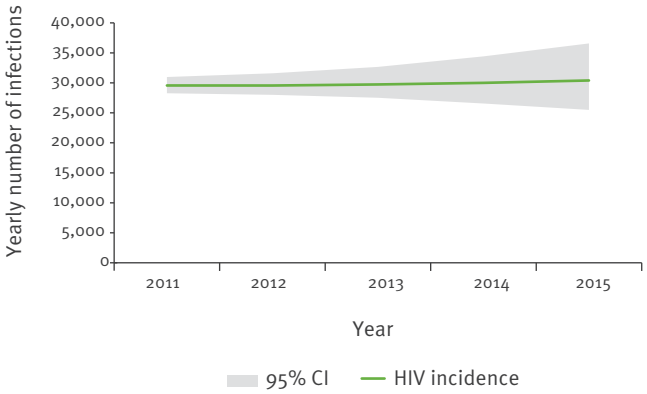
Data on the estimated number of diagnosed PLHIV were reported for 2015 by nominated contact points in EU/EEA countries to ECDC as part of the Dublin Declaration monitoring process in 2016 [5]. In the three countries (Iceland, Liechtenstein, and Norway) not reporting estimates of diagnosed PLHIV, data on cumulative HIV cases reported to TESSy through 2015 minus the number of persons reported to have died, were used as a proxy for diagnosed PLHIV.

The estimated number of diagnosed PLHIV from the Dublin Declaration monitoring reports and the undiagnosed PLHIV estimate from the model were summed to obtain the total number of PLHIV in the EU/EEA for 2015. This was used to derive the proportion undiagnosed PLHIV in that year.

Comparable estimates of the number of diagnosed PLHIV from the Dublin Declaration monitoring are not available for earlier years than 2015, thus the estimates of PLHIV overall and of the proportion of PLHIV unaware of their infection could only be calculated for 2015.

Figure 1

Estimated human immunodeficiency virus incidence by year, European Union/European Economic Area, 2011–2015



CI: confidence interval; HIV: human immunodeficiency virus.

New yearly diagnoses of HIV and estimated annual HIV incidences

In 2015, 29,727 cases of HIV were diagnosed and reported in the EU/EEA, resulting in a rate of 6.3 per 100,000 population when adjusted for reporting delay. The notification rate and the number of new HIV diagnoses reported have remained unchanged since 2011, with between 29,000 and 33,000 new cases reported annually (notification rates of between 6.3 and 6.5 per 100,000 population) [1].

HIV incidence estimates present a stable trend similar to that of HIV cases notified via the surveillance system, with an estimated 30,000 new infections (95% confidence interval (CI): 25,000–37,000) for the year 2015 (Figure 1).

Evolution of CD4 cell count at diagnosis and of the delay between infection and diagnosis in years up to 2015

Late diagnosis is a persistent issue in EU/EEA countries. In the 24 EU/EEA countries reporting data on CD4 cell count at diagnosis among 18,103 persons >15 years-old diagnosed in 2015, nearly half (n=8,490; 47%) of all cases had a CD4 cell count of less than 350 cells/mm3, while 28% (n=5,094) had advanced HIV infection (CD4<200 cells/mm3). In the thirteen countries reporting the CD4 cell count consistently over time, the median CD4 cell count at diagnosis increased significantly from 314 cells/mm3 in 2005 to 377 cells/mm3 in 2015 ($p<0.001$).

Meanwhile, the estimated expected time from HIV infection to diagnosis decreased from 4.2 years (95% CI: 4.1–4.3) on average in 2011 to 3.8 years (95% CI: 3.6–4.0) in 2015 (Figure 2).

Estimated number of persons living with undiagnosed infection

The number of people living with undiagnosed HIV in the EU/EEA in 2015 was estimated at 122,000 (95% CI: 111,000–136,000). The total estimated number of PLHIV in the EU/EEA was 810,000 (0.2% of adult population ≥15 years-old). The resulting estimated proportion of those living with undiagnosed HIV was 15% (95% CI: 14–17%).

Background and discussion

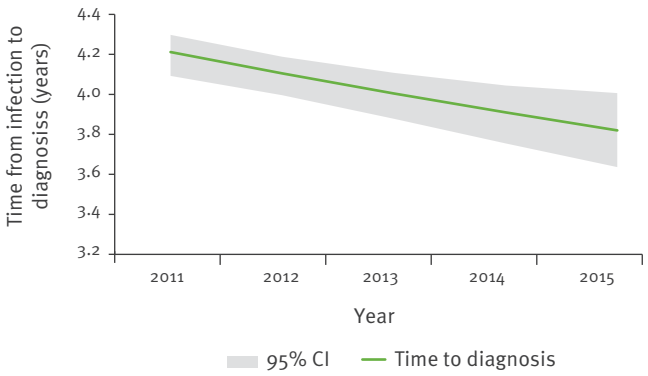
The Joint United Nations Programme on HIV/AIDS (UNAIDS) has set forth ambitious global targets to end AIDS by 2030 and established '90–90–90' targets for 2020 (90% of all people living with HIV will know their status; 90% of people aware of their status will receive sustained antiretroviral treatment; and 90% of those on antiretroviral treatment will have viral suppression) [6]. To better understand HIV trends and estimate the status of the first target (90% of people living with HIV aware of their status) in the EU/EEA, we analysed HIV and AIDS surveillance data through 2015. Despite high treatment coverage [7], earlier diagnosis, and concerted prevention efforts, there is no decline in the number of HIV diagnoses or the number of HIV infections in the EU/EEA in recent years.

This analysis shows that the estimated proportion of all PLHIV in the EU/EEA who are living with undiagnosed HIV is 15%. Using a similar CD4 back-calculation approach on surveillance data, it was estimated that 16% of PLHIV in the United States in 2013 were undiagnosed [8]. The estimate presented here for the EU/EEA is considerably lower than the previous estimate of 30%, which is based on data from 2005 [9]. This could be a result of several factors. First, this might be a reflection of increased or more targeted testing as supported by the observed increase in the CD4 cell counts at diagnosis and decreased time from HIV infection to diagnosis. With treatment guidelines moving towards earlier treatment, and growing awareness of the benefits of early antiretroviral treatment, more persons at higher risk of infection may get tested more frequently. Second, the annual number of new infections is approximately the same as the number of new diagnoses. Thus the number living with undiagnosed HIV remains relatively stable and as people on treatment live longer with HIV, the proportion of undiagnosed persons with HIV will naturally become smaller in relation to the ever-increasing population of diagnosed PLHIV [1]. Third, new methods to estimate the undiagnosed fraction are available and these are informed by improved surveillance data.

While approximately 85% of those living with HIV in the EU/EEA are estimated to be diagnosed, it remains to be seen whether it is possible for the EU/EEA to reach the UNAIDS first '90' target by 2020. A more appropriate measure to gauge progress may be to monitor the reduction in the number of undiagnosed individuals

Figure 2

Average time from infection to diagnosis of human immunodeficiency virus by year, European Union/ European Economic Area, 2011–2015



CI: confidence interval.

living with HIV, rather than monitoring a proportion where the denominator is steadily increasing.

The average time between HIV infection and diagnosis, while improving, is still nearly four years. As starting antiretroviral treatment earlier reduces morbidity and mortality among HIV-positive individuals [10] and reduces HIV transmission to HIV-negative partners [11] it is essential that individuals are diagnosed early. In order to further reduce the time from HIV infection to diagnosis, countries should consider implementing and scaling up innovative approaches to promote greater access to and uptake of HIV testing by those most at risk, including community-based testing, self-testing and home sampling, as well as indicator-condition-guided testing.

This pooled EU/EEA estimate conceals differences between key populations, where the trend over time and proportion undiagnosed is likely to vary. An EU estimate is also more heavily weighted towards the situation of countries with larger populations. The proportion of persons remaining undiagnosed is diverse across countries that have carried out national analyses [12-17] and is likely to be significantly higher than 15% in many countries and among some key population groups. In Europe, further work is needed to carry out key population-specific and country-level estimates of HIV incidence and the undiagnosed number in a standardised manner in order to more accurately monitor progress and inform testing programmes.

This analysis has several important limitations. It was not possible to adjust the data for countries that did not have full coverage of HIV surveillance prior to 2012 (such as Italy and Spain) and this may have resulted in an underestimation of PLHIV. Conversely, PLHIV may have been overestimated due to the inability of many countries to fully link their death, emigration and surveillance registries and, thus, accurately measure the number of those diagnosed still living with HIV. For these reasons, it was not possible to obtain a reliable estimate of PLHIV using only HIV notification data reported to TESSy. Instead, data reported by countries through the Dublin Declaration monitoring process on people diagnosed and living with HIV were used, and these were obtained using different methods, with some countries unable to completely remove all cases who had died or emigrated from the number diagnosed. Until approaches to estimate diagnosed PLHIV can be further standardised, country-reported data provide the best current estimate in the EU/EEA.

Conclusions

Overall, this analysis demonstrates that recent HIV incidence is constant in the EU/EEA, and that a substantial number of people are living with undiagnosed HIV. Efforts to obtain better national and key population-specific estimates and to further increase the offer and uptake of HIV testing among those most at risk remain key to informing HIV prevention efforts and achieving global targets to reduce HIV incidence and the number of persons remaining undiagnosed in the EU/EEA.

Members of the ECDC HIV/AIDS Surveillance and Dublin Declaration Monitoring Networks

ECDC HIV/AIDS Surveillance and Dublin Declaration Monitoring Networks include: Austria: Daniela Schmid, Irene Rueckerl, Robert Zangerle; Belgium: Andre Sasse, Dominique Van Beckhoven, Frédéric Denauw; Bulgaria: Tonka Varleva, Vyara Georgieva; Croatia: Tatjana Nemeth Blazic, Jasmina Pavlic, Josip Begovac; Cyprus: Maria Koliou, Linos Hadjihannas, Anna Demetriou; Czech Republic: Marek Maly, Veronika Šikolová; Denmark: Susan Cowan, Jan Fouchard; Estonia: Kristi Rüütel, Anna-Liisa Pääsukene; Finland: Kirsi Liitsola, Mika Salminen, Henrikki Brummer- Korvenkontio; France: Françoise Cazein, Josiane Pillonel, Florence Lot, Jean-Christophe Comboroure; Germany: Barbara Gunsenheimer-Bartmeyer, Matthias an der Heiden, Gesa Kupfer, Ulrich Marcus; Greece: Georgios Nikolopoulos, Dimitra Paraskeva, Vasileia Konte; Hungary: Maria Dudas, Katalin Szalay; Iceland: Haraldur Briem, Gudrun Sigmundsdottir; Ireland: Derval Igoe, Kate O'Donnell, Caroline Hurley, Fiona Lyons; Italy: Barbara Suligoi, Anna Caraglia; Latvia: Šarlote Konova; Liechtenstein: Sabine Erne; Lithuania: Irma Čaplinskienė; Luxembourg: Aurelie Fischer, Patrick Hoffmann; Malta: Jackie Maistre Melillo, Tanya Melillo; Netherlands: Eline Op de Coul, Silke David; Norway: Hans Blystad, Arild Johan Myrberg; Poland: Magdalena Rosinska, Iwona Wawer; Portugal: Kamal Mansinho, Helena Cortes Martins, Teresa Melo; Romania: Mariana Mardarescu; Slovakia: Peter Truska, Jan Mikas; Slovenia: Irena Klavs; Spain: Asuncion Diaz, Oliver Nuñez, Olivia Castillo; Sweden: Maria Axelsson, Anders Sönnerborg; United Kingdom: Peter Kirwan, Cuong Chau, Sandra Okala, Alison Brown, Valerie Delpech.

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Conflict of interest

None declared.

Authors' contributions

The ECDC HIV/AIDS Surveillance and Dublin Declaration networks supplied the data and provided comments on the manuscript. All co-authors developed the concept of the manuscript. AvS carried out the modelling analysis and AP the remaining analysis. AP wrote the first draft and responded to reviewers comments. All authors have read and approved the final manuscript.

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Blood donor screening for West Nile virus (WNV) revealed acute Usutu virus (USUV) infection, Germany, September 2016

D Cadar [1,2], P Maier [3], S Müller [4], J Kress [4], M Chudy [4], A Bialonski [1], A Schlaphof [1], S Jansen [1,2], H Jöst [1,2], E Tannich [1], S Runkel [5], WE Hitzler [5], G Hutschenreuter [3], M Wessiepe [3,6], J Schmidt-Chanasit [1,2,6]

1. Bernhard Nocht Institute for Tropical Medicine, WHO Collaborating Centre for Arbovirus and Hemorrhagic Fever Reference and Research, Hamburg, Germany
2. German Centre for Infection Research (DZIF), partner site Hamburg-Luebeck-Borstel, Hamburg, Germany
3. Institute for Transfusion Medicine, University Hospital, Aachen, Germany
4. Paul-Ehrlich-Institut, Federal Institute for Vaccines and Biomedicines, Langen, Germany
5. Transfusion Center, University Medical Center of the J.G. University, Mainz, Germany
6. These authors contributed equally to this work

Correspondence: Jonas Schmidt-Chanasit (jonassi@gmx.de)

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Between 1 June and 31 December 2016, 13,023 blood donations from the University Hospital Aachen in Germany were routinely screened for West Nile virus (WNV) RNA using the cobas TaqScreen WNV Test. On 28 September 2016, one blood donor was tested positive. Subsequent analysis revealed an acute Usutu virus (USUV) infection. During the ongoing USUV epizootics in Germany, blood transfusion services, public health authorities and clinicians should be aware of increased human USUV infections.

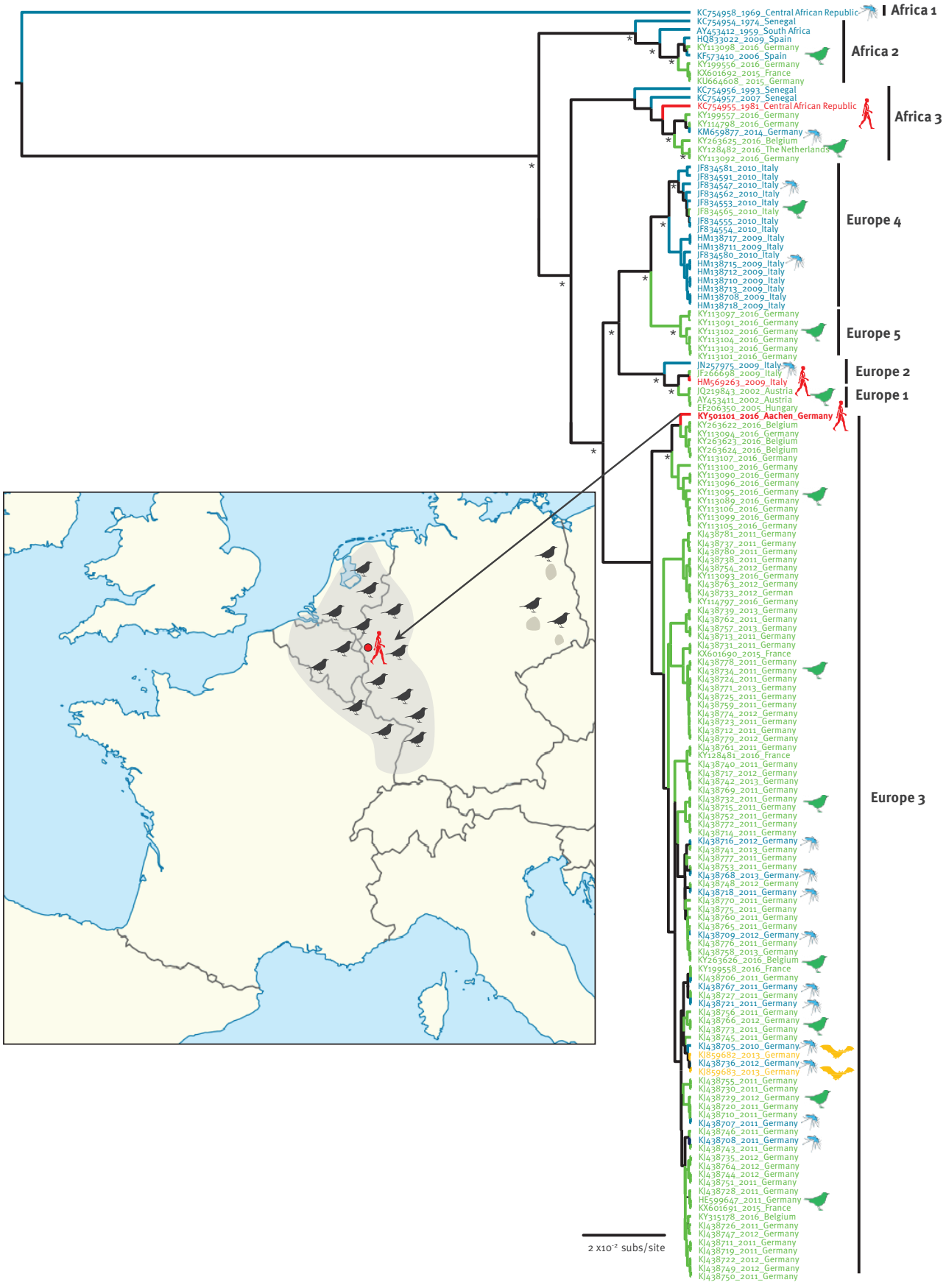
During July–October 2016, several western European countries reported the largest Usutu virus (USUV) epizootic registered so far in Europe causing a massive bird die-off [1]. Blood donor samples collected between 1 June and 31 December in the Institute for Transfusion Medicine, University Hospital, Aachen, are routinely screened for West Nile virus (WNV) RNA. On 17 November 2016, the World Health Organization Collaborating Centre (WHO CC) for Arbovirus and Haemorrhagic Fever Reference and Research in Hamburg was informed about a suspected WNV infection in a blood donor from Aachen. Although the sample was tested positive for the presence of WNV RNA, subsequent sequencing and serological investigations revealed an acute USUV infection of the donor. Here we report the first detection of an acute USUV infection of a blood donor from Germany using a cross-reactive WNV screening test and further successful sequencing of a large portion of the genome using deep-sequencing technology.

Case description

On 26 September 2016, a plasma pool (n = 16) had been detected WNV-positive (Ct: 40.5) using cobas TaqScreen WNV Test (Roche Diagnostics GmbH, Mannheim, Germany) with a sensitivity of 206.4 copies/mL per single donation. In order to detect the positive plasma sample, each sample from the pool was tested individually and the positive sample identified (Ct: 37.5). The blood donor was a German woman in her late 20s, without any travel history outside Germany in the previous 7 months. Furthermore, she had not left the Aachen region at all in the 3 months prior to blood donation. The healthy donor had not experienced any illness or symptoms in the 6 weeks before donation. She reported several mosquito bites before the donation. Blood and urine samples of the donor were sent to the WHO CC in Hamburg for further characterisation. Results of IgG and IgM immunofluorescent assays for WNV, USUV, tick-borne encephalitis virus (TBEV) and Japanese encephalitis virus (JEV) were negative (titres ‹1:20) for the first sample collected on 26 September 2016. In contrast, IgG and IgM seroconversion was demonstrated with the follow up sample collected on 20 November 2016, 55 days later and the results for WNV-IgG (1:160), WNV-IgM (1:160), TBEV-IgG (‹1:20), TBEV-IgM (‹1:20), JEV-IgG (1:640), and JEV-IgM (1:80) and USUV-IgG (1:1280) and USUV-IgM (1:640) suggested a recent USUV infection. The blood donor reported no history of vaccination against YFV and JEV. Extracted RNA of plasma and urine samples were tested for the presence of flavivirus RNA with pan-flavivirus RT-PCR [2]. A positive PCR result was obtained with RNA from the plasma sample and direct Sanger sequencing of the PCR amplicon showed USUV nucleic acid sequence.

Figure 1

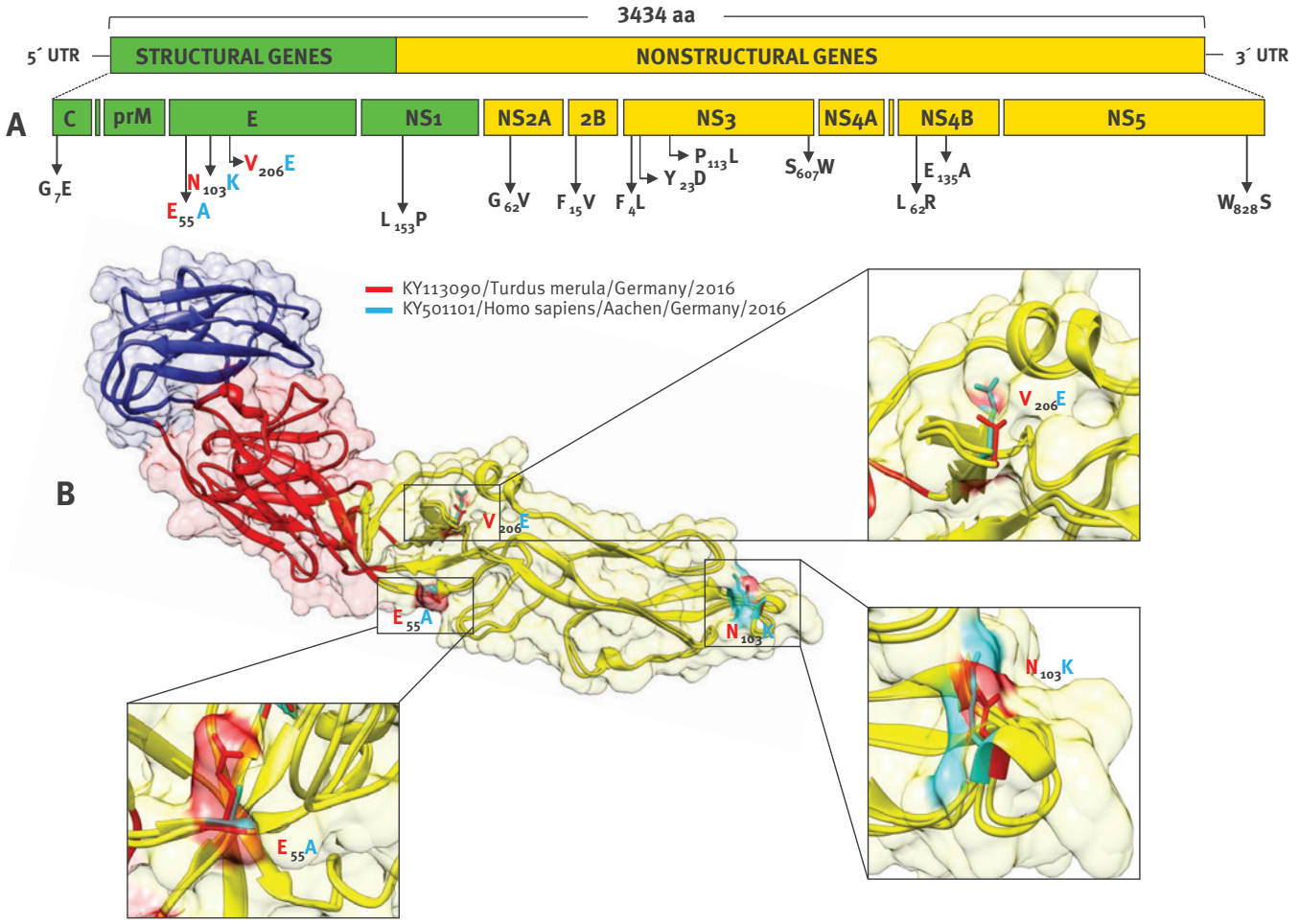
Bayesian maximum clade credibility tree representing the phylogenetic placement of the human Usutu virus (USUV) strain Aachen compared with all available USUV based on partial NS5 gene nt sequences



Phylogenetic analysis was performed by using Bayesian Markov chain Monte Carlo (MCMC) tree-sampling method implemented in BEAST v.1.8.0 (http://beast.bio.ed.ac.uk). Statistical supports of grouping from Bayesian posterior probabilities (clade credibilities≥90%) are indicated at the nodes (asterisks). The map indicates the regions of the European countries which have reported USUV outbreaks in 2016 (grey), and the origin of the human USUV Aachen strain. GenBank accession numbers, years of detection and countries of origin for sequences used to construct the tree are indicated on the branches. Scale bar indicates mean number of nt substitutions per site.

Figure 2

Amino acid mutations in the Usutu virus (USUV) Aachen strain: A. schematic representation of the genome organisation of USUV, B. structural location of the USUV non-synonymous mutations in the Aachen strain depicted on the predicted E glycoprotein structure



In Panel A, the numbers indicate the positions and the single letter the unique non-synonymous amino acid mutations of the Aachen strain. Amino acid substitutions in the envelope glycoprotein are magnified and indicated in red and light blue (Aachen strain) respectively.

The three-dimensional ribbon structure of a single monomer of the USUV envelope glycoprotein is shown with the corresponding three viral domains (domain I in red; domain II in yellow; domain III in blue) and surface exposed variable residues magnified. Homology models for USUV envelope protein was constructed using the initial homology search and template selection method in Chimera [18]. The template sequences used to create the USUV E protein model was the crystal structure of the West Nile virus envelope glycoprotein (PDB 2I69). The final 3D structures were prepared and visualised with Chimera v1.11 [18].

Attempts to isolate USUV in cell culture using the donor plasma were not successful.

Deep sequencing and genetic analysis

The concentrated and purified RNA was further subjected to deep-sequencing using in-house next-generation sequencing pipeline in order to obtain larger fragments of the USUV genome. Thereby, we were able to successfully recover about 60% of the USUV polyprotein gene. USUV from the donor plasma showed 99% homology with those found in the birds during the 2016 epizootics corresponding with the same region from where the donor originated (Figure 1). Phylogenetic analysis demonstrated that USUV 'Aachen' strain clustered together with the 2016 outbreaks strains and formed together with some German and Belgian strains a distinct subclade within the previously assigned European lineage 3 (Figure 1).

The analysis of the polyprotein gene revealed several host-specific unique amino acid mutations from which three were located in domain II of the envelope glycoprotein (Figure 2).

Background

USUV, an Old World flavivirus included in the JEV antigenic complex is transmitted by mosquitoes to birds that act as the main amplifying hosts, while humans are considered incidental or dead-end hosts [3]. Since the first emergence in the mid-1990s in Europe, USUV has been responsible for smaller periodic epizootics in several European countries, the largest one being

registered in 2016 [1,4-6]. USUV can cause Usutu fever in humans with mild to severe symptoms characterised by fever, rash, jaundice, headache, nuchal rigidity, hand tremor, and hyperreflexia [7-10]. So far, humans were considered incidental hosts with very low prevalence, but recent data from Italy indicated that human USUV infection may not be a sporadic event and is more frequent than WNV infections [11]. In 2012, 1 of 4,200 blood donors from south-west Germany was tested positive for USUV-specific IgG and IgM antibodies demonstrating a recent USUV infection of the donor [12]. However, there is no documented case of Usutu fever caused by transfusion of USUV-contaminated blood products.

Discussion and conclusion

The present report, including serological and molecular findings, suggests an acute and asymptomatic USUV infection of a blood donor in Germany in late summer of 2016. The Bayesian phylogenetic analysis revealed that the USUV sequence of the blood donor had a high sequence homology with recent strains responsible for the 2016 USUV epizootics in the western part of Germany from where the donor lived. Since the blood donor had no history of travelling abroad in the 7 months before the end of September 2016, she must have been infected in Germany, which, together with the genetic data obtained, further strengthens an autochthonous USUV infection in the Aachen region.

USUV is considered an emerging arbovirus due to its rising incidence of human infections that are likely to be frequent as WNV infections and the expansion in new, previously known USUV-free areas [1,11]. It is interesting to note the amino acid mutations detected mostly in the envelope protein and NS5 gene. Although the biological consequences of these mutations are not known, similar changes in the related WNV increased the sensitivity to neutralisation by a monoclonal antibody targeting a cryptic epitope in the fusion loop and altered tropism and neuroinvasive capacity [13,14]. The detection of USUV RNA in the blood donor sample using cobas TaqScreen WNV Test, demonstrates the capability of this test to detect other flaviviruses than WNV due to cross-reactivity of the used primer-probe reagents.

To address the emergence of WNV regarding blood safety, the Federal Institute for Vaccines and Biomedicines (Paul-Ehrlich-Institut) as the responsible authority in Germany, implemented a regulation for non-pathogen inactivated blood components in 2003, last updated in 2014 [15]. Since the update in 2014, alternatively to the deferral period of 28 days, donor eligibility is accepted indicating a non-reactive screening result using a nucleic acid amplification technique (NAT)-based test for WNV RNA with a minimum detection sensitivity of 250 copies/mL for each donor sample [15].

Recent molecular and serologic surveillance studies in Germany and neighbouring countries identified epizootic hotspots for USUV that could help to initiate targeted vector control programs to prevent human exposure to the virus [1,3,16,17]. Moreover, the present report highlights the potential risk of transfusion-associated transmission of USUV. However, until now there is no reported case of transfusion-associated Usutu fever in Europe. The demonstrated case should raise awareness of the risk of USUV infection in humans during epizootics, especially in late summer.

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Conflict of interest

None declared.

Authors' contributions

Wrote the manuscript: DC, JSC, ET, PM, MW, GH, WEH; Performed laboratory or epidemiological investigations: DC, JK, JSC, AR, SM, MC, AS, SR, MW, AB, SJ, HJ; Performed data analysis: DC, JSC, SR, MW.

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Research article

Estimating the annual burden of tick-borne encephalitis to inform vaccination policy, Slovenia, 2009 to 2013

M Fafangel [1,2], A Cassini [3,4], E Colzani [3,5], I Klavs [1], M Grgič Vitek [1], V Učakar [1], M Muehlen [3], M Vudrag [1], A Kraigher [1]

1. National Institute of Public Health (NIJZ), Ljubljana, Slovenia
2. European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
3. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
4. Julius Centre for Health Sciences and Primary Care, University Medical Centre Utrecht, Utrecht, The Netherlands
5. Department of Health Science, University of Milano-Bicocca, Monza, Italy

Correspondence: Mario Fafangel (mario.fafangel@nijz.si)

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With an annual incidence between 8 and 15 per 100,000 population in the period from 2009 to 2013, Slovenia has one of the highest notified incidences of tick-borne encephalitis (TBE) in Europe. TBE vaccination coverage remains at about 7.3%. To inform vaccination policy, we used surveillance data from 2009 to 2013 to calculate the overall and age- and sex-specific mean annual TBE incidence. We estimated disability-adjusted life years (DALYs) with 95% uncertainty intervals (UI), using the Burden of Communicable Diseases in Europe approach from the European Centre for Disease Prevention and Control. The mean annual incidence was 11.6 per 100,000 population, peaking in older age groups (50–74 years: 18.5/100,000) while relatively lower among children (5–14 years: 10.2/100,000). We estimated an overall 10.95 DALYs per 100,000 population per year (95% UI: 10.25-11.65). In contrast to the TBE incidence, the disease burden in children aged 5–14 years was higher than in adults aged 50–74 years: 17.31 (95% UI: 14.58–20.08) and 11.58 (95% UI: 10.25–12.91) DALYs per 100,000 stratum-specific population, respectively. In a limited resource setting where prioritisation of TBE vaccination strategies is required, vaccination programmes targeting children may have a higher impact on disease burden.

Introduction

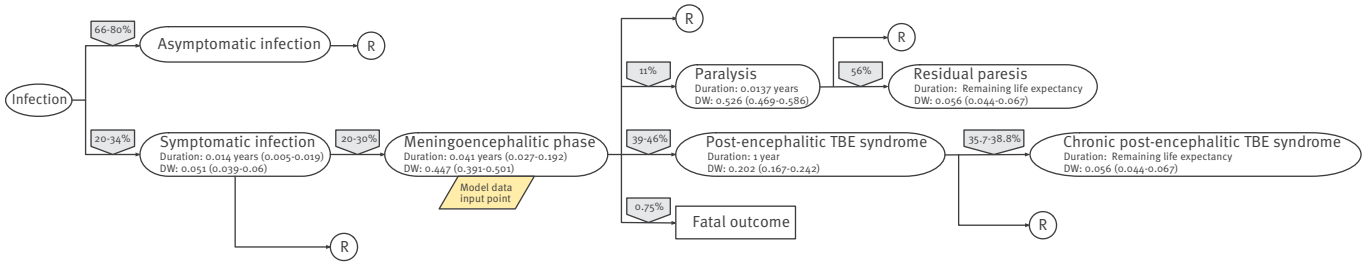
Tick-borne encephalitis (TBE) is a vector-borne disease caused by the TBE virus [1]. It typically presents as a two-phased illness [2-4]. The first phase is associated with symptoms such as fever, fatigue, headache, myalgia and nausea. The second phase involves the nervous system with symptoms related to meningitis and/or encephalitis. Life-long sequelae can have an important impact on the quality of life of those affected [5]. TBE cases notified in Europe have surged in the last three decades with an estimated increase of 193% [6-8].

In Slovenia, notification of TBE is mandatory and based on the European Union (EU) standardised case definition [9]. Only cases with central nervous system involvement (meningoencephalitic TBE) and laboratory confirmation are notified. Slovenia is one of the countries with the highest notified incidence in Europe, ranging from 8 to 15 per 100,000 in the period from 2009 to 2013, with cases occurring throughout the country [10]. Data for the past 20 years show a non-homogenous age distribution with higher incidence in older age groups (› 40 years) [10]. Preventive measures include the use of repellents, appropriate clothing and daily inspection of the skin to remove ticks [11]. The most effective method of preventing TBE is vaccination [11-13]. Mandatory vaccination against TBE was introduced in Slovenia in 1986 for those at risk of occupational exposure, and in 1990 for students at risk of exposure during curricular training, while the rest of the population needs to pay for the vaccination themselves. TBE vaccination coverage in Slovenia remains low: by 2007, the proportion of the general population reporting to ever have been vaccinated against TBE was 12.4% [14].

In a context where limited resources prevent universal TBE vaccination free of charge, data are needed to identify those groups most affected by the disease so that vaccination can be targeted in order to yield the greatest benefit on population health. Countries have used incidence data to guide vaccination strategies towards specific age groups and geographical areas [15-17]. Estimation of the TBE burden in the form of disability-adjusted life years (DALYs), a summary measure of population health, is better suited to express the overall and age group-specific impact of the disease in the population while taking into account the effects of acute illness and its sequelae on mortality and morbidity [18]. The objective of this study was to estimate the

Figure 1

Outcome tree for tick-borne encephalitis virus infection



DW: disability weight; R: resolution of infection; TBE: tick-borne encephalitis.

overall and age- and sex-specific annual burden of TBE in Slovenia in order to inform vaccination policy in a setting with limited resources.

Methods

Model

To estimate the burden of TBE we used the pathogen-based incidence approach developed by the European Centre for Disease Prevention and Control (ECDC) *Burden of communicable diseases in Europe* project (BCoDE) [18-20]. The burden was expressed in DALYs. DALYs have two components: years of life lost due to premature death (YLL) and healthy years of life lost due to disability (YLD) [21].

We used a disease model (outcome tree) based on the current knowledge of the disease progression pathway, linking all health outcomes related to TBE with the initial infection. Starting with the infection a case moved through the outcome tree transitioning into different health outcomes according to different conditional transition probabilities (i.e. probability of occurrence of each health outcome), exiting the tree with a resolved infection, with a life-long disability or with a fatal outcome. In order to measure YLL, life expectancy was based on the standard reference life table developed within the *Global Burden of Disease 2010* project [22]. To measure YLD, each health outcome was characterised by a disease duration and a disability weight. Disability weights quantify health losses to reflect the disability experienced by someone living with a health issue. Based on the severity of the disease, they range from 0 (full health) to 1 (death). The disability weights were generated for BCoDE and the *Global Burden of Disease study* (GBD) 2013 through elicitation methods [23,24]. The outcome tree for TBE used in our model (Figure 1) was based on a thorough review of published studies and on the opinion of ECDC experts [25]. All parameters included in the outcome tree, conditional transition probabilities, durations and disability weights were based on published studies and entailed a certain level of uncertainty. The uncertainty was modelled by incorporating ranges using either uniform or Pert distributions [26] and quantified by performing Monte Carlo simulations with 10,000 iterations to obtain 95% uncertainty intervals (UI). In order to assess age groups of interest for vaccination strategies, we compared the median DALYs and their 95% UIs.

Input data

The ECDC BCoDE toolkit was used for DALY estimation [25]. Input data for the model were the mean annual numbers of meningoencephalitic TBE cases notified to the Slovenian national surveillance system for communicable diseases from 2009 to 2013. They were stratified by 5-year age groups and by sex. For those calculations where a population estimate was required, we used the 2011 population data for Slovenia obtained from Eurostat [27]. The main type of input data for TBE in the BCoDE toolkit was the number of symptomatic infections (first phase of the disease); to obtain this, surveillance data were multiplied by the appropriate transitional probabilities as specified by the TBE outcome tree. No time discounting was applied, thus future and present disabilities were weighted equally.

Results

From 2009 to 2013, a total of 1,190 cases (58% males) of TBE in their meningoencephalitic phase were notified in Slovenia, with a mean of 238 cases/year. The median age at diagnosis was 51 years (range: 1–86 years). The mean annual incidence of meningoencephalitic TBE was 11.6 per 100,000 population (9.6/100,000 for females and 13.6/100,000 for males). Incidence was higher in older individuals (50–74 years: 18.5/100,000) than in children (5–14 years: 10.2/100,000). Data by 5-year age groups and by sex are presented in Figure 2.

The estimated DALYs per year were 224.52 (95% UI: 210.14-238.84), corresponding to 10.95 DALYs per 100,000 per year (95% UI: 10.25-11.65). Each case of TBE accounted for an average of 0.23 DALYs (95% UI: 0.22–0.24) In the Table, DALYs and their components (YLL and YLD) are presented for all health outcomes related to TBE. YLDs per year accounted for 67% of the total disease burden. Late sequelae, following the meningoencephalitic phase of the disease, contributed to 63% of the DALYs per year.

Figure 2

Mean annual incidence per100,000 of tick-borne encephalitis, by age and sex, Slovenia, 2009–2013 (n = 1,190)

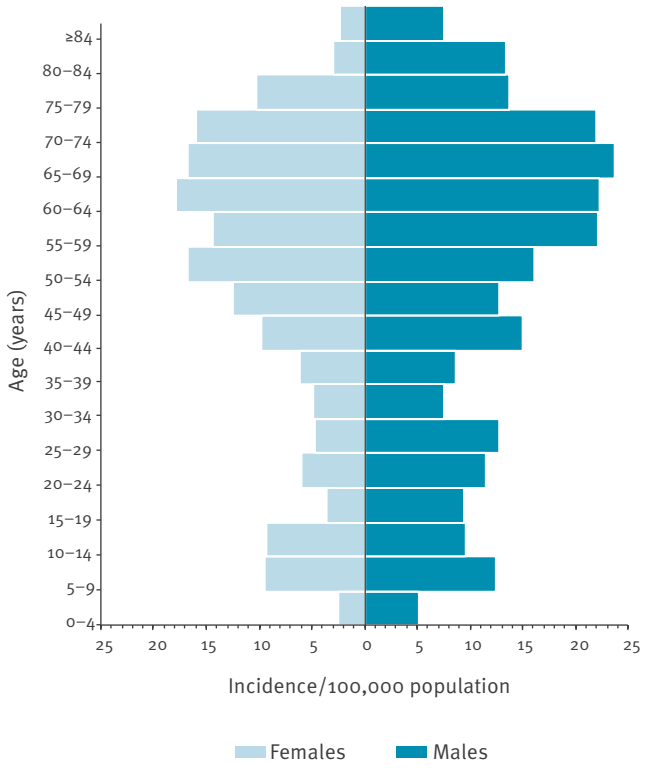
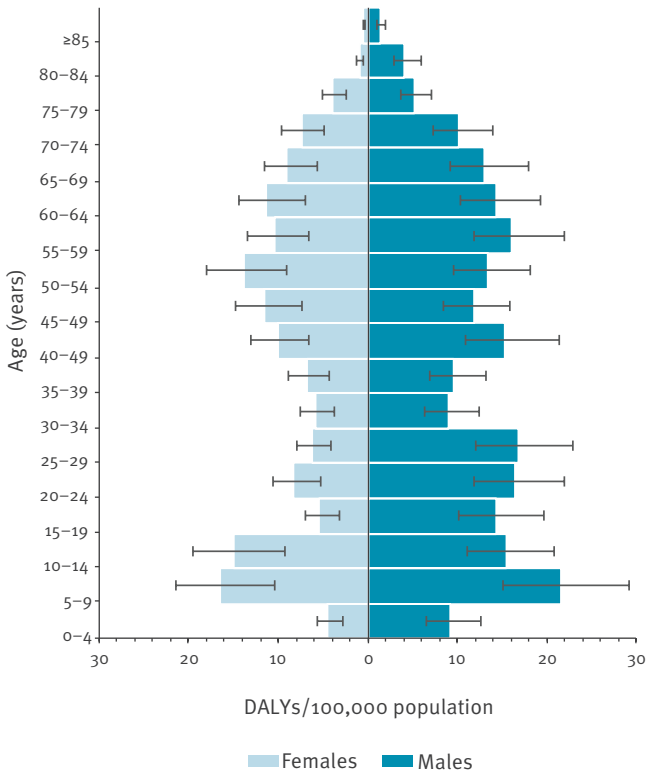


Figure 3

Estimated mean annual disability-adjusted life years per 100,000 stratum-specific population due to tick-borne encephalitis, by age and sex, Slovenia, 2009–2013



DALYs: disability-adjusted life years.

The whiskers represent 95% uncertainty intervals.

The group of 50–54-year-old women and the group of 25–29-year-old men had the highest point estimates of DALYs per year with 10.56 (95% UI: 7.34–14.03) and 13.02 (95% UI: 9.25–17.49) DALYs per year respectively. When looking at both sexes together, the 50–54 and 55–59-year-olds accounted for the highest number of DALYs, 21.08 (95% UI: 14.91–28.40) and 20.48 (95% UI: 14.48–27.70), respectively.

In terms of DALYs per 100,000 stratum-specific population, the highest burden point estimate was among the 5–9-year-olds: 19.29 DALYs per 100,000 stratum-specific population per year (95% UI: 15.41–23.90) with 16.62 DALYs (95% UI: 11.48–22.51) and 21.69 DALYs per 100,000 per year (95% UI: 15.12–29.28) for girls and boys, respectively. Data by 5-year age groups and by sex are presented in Figure 3.

The group of 50–74-year-olds had a lower TBE burden estimate of 11.58 (95% UI: 10.25–12.91) DALYs per 100,000 stratum-specific population per year in comparison to the 5–14-year-olds with a burden of 17.31 (95% UI: 14.58–20.08) DALYs per 100,000 stratum-specific population per year (Figure 4).

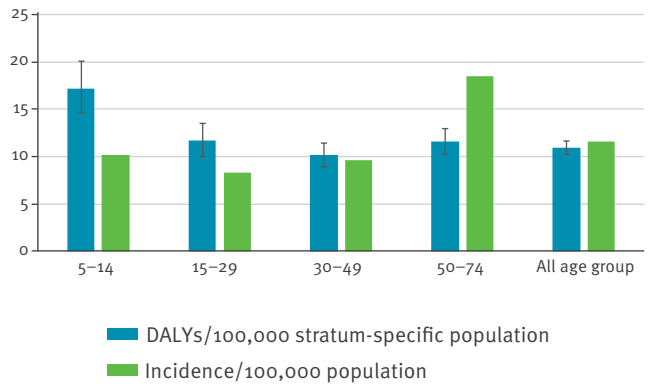
Discussion

In this paper we present the overall and the age- and sex-specific annual burden of TBE in Slovenia expressed in DALYs. The use of DALYs integrates mortality and morbidity from TBE in a single composite health metric, giving a comprehensive estimate of the impact of this disease on population health.

An analysis of notified TBE cases in the 5-year period from 2009 to 2013 confirms Slovenia as one of the countries, together with the Baltic states and the Russian Federation, where reported incidence per 100,000 is the highest in Europe [11,28]. With an estimate of 10.95 DALYs per 100,000 per year (95% UI: 10.25-11.65), TBE has an important impact on the health of the Slovenian population. In accordance with input incidence data, we found consistently higher burden point estimates in male persons across all ages. According to the BCoDE 2009–13 study, the estimated burden of TBE in Slovenia was nine times higher than the corresponding estimated burden of TBE measured in DALYs per 100,000 population per year for the EU and European Economic Area (EEA) for the same time period [29]. Moreover, the impact of TBE on the Slovenian population is comparable to that of healthcare-associated neonatal sepsis (16.8 DALYs/100,000) according to a recent study on healthcare-associated infection in the EU/EEA [30].

Figure 4

Estimated mean annual incidence per 100,000 and mean annual disability-adjusted life years per 100,000 stratum-specific population due to tick-borne encephalitis, by age group, Slovenia, 2009–2013



DALYs: disability-adjusted life years.

The whiskers represent 95% uncertainty intervals.

Looking at incidence data alone, older age groups (50–74-year-olds) appeared most affected by TBE in Slovenia. However, the use of DALYs identified children (5–14-year-olds) as the group with a higher burden. This difference in impact of TBE would not have been detected, if we had limited our assessment to incidence data, ignoring the combined effects of morbidity, short- and long-term sequelae and mortality. Other countries with a similar TBE incidence profile as Slovenia could profit from this approach to identify groups with important burden, particularly when informing decision makers about the allocation of limited resources for targeted public health interventions (i.e. vaccination).

Vaccination is regarded as the most effective preventive measure for TBE [11]. Studies have shown a 96–99% field effectiveness in persons receiving three doses following the recommended schedule [12,13]. In neighbouring Austria, an estimated 88% of the general population are vaccinated with at least one dose, while 58% are vaccinated regularly following the advised schedule [13]. Austria has managed to reduce the number of TBE cases by 90% by increasing its vaccination rate from 6% in 1980 to its current level [13]. Despite the fact that vaccination has been recommended in Slovenia for decades, only 12% of the population was vaccinated with at least one dose by 2007 and only 7.3% get vaccinated regularly following the advised schedule [31].

TBE vaccination remains a self-paid expense for the majority of the population. The costs are covered by the mandatory insurance system or by the employer only in case of occupational exposure or exposure during education or training. Data from 2007 show that only 4.6% of the population paid themselves for TBE vaccination [14]. A recent study from Šmit et al., estimating DALYs of TBE in Slovenia using the GBD project methodological approach, supports the need for a public health strategy aimed at increasing the national vaccination coverage [32]. Multiple factors influencing the decision to get vaccinated against TBE (knowledge, trust, accessibility, cost) should be considered when planning strategies aimed at increasing vaccination coverage [33]. Projections, however, show that the impact of a vaccine subsidy, making the vaccine free of charge, could alone increase coverage by 45%, and even more in low-income households [34].

Increasing TBE vaccination coverage should be considered as an option for intervention to reduce the impact of TBE [10,32]. In the presence of limited resources, the implementation of such a measure could be difficult in the short term. Our results suggest that effective prevention of TBE in children would have the highest impact in terms of DALYs of TBE averted. This novel insight in the distribution of TBE burden should be considered when prioritising access to TBE vaccination and could improve previous recommendations originating from incidence data alone, where the focus was mainly on older age groups [10].

Prioritising vaccination in children could be easier thanks to the well-functioning Slovenian national childhood immunisation programme. It is also important to take into account the need for booster doses of the TBE vaccine. In the age groups of interest, a three-dose primary vaccination schedule with a first booster dose after 3 years and further boosters every 5 years is recommended to maintain seropositivity [35]. A recent study showed that a schedule that includes the first booster dose yields a high and long-lasting (›5 years) immune response, thus suggesting that subsequent TBE booster intervals could be extended beyond the current recommendation [36]. Considering the financial implications of lifelong booster doses (and the different schedules that apply at different ages), age-specific cost-effectiveness studies are needed to inform decisions on the extent to which TBE vaccine can be subsidised in order to achieve the highest level of immunopersistence and impact on TBE burden in a cost-effective manner.

We considered prioritising the most affected areas or regions as an alternative approach. Although some regions in Slovenia are more affected then others, TBE occurs throughout the country. Considering the epidemiological situation of TBE in Slovenia, the country`s relatively small area and population size, as well as the mobility of the population between regions, we consider this approach could be potentially misleading and lead to health inequalities. Other countries where restricted areas or regions are affected could consider a modelling approach stratified by region.

This study has certain limitations. The outcome tree describing the progression pathway of the

Table

Tick-borne encephalitis annual burden estimates, Slovenia, 2009–2013

	DALYs/year (95% UI)	DALYs/100,000 (95% UI)	YLL/yea (95% UI)	YLD/year (95% UI)
Symptomatic infection	0.67 (0.61–0.73)	0.03 (0.03–0.04)	0	0.67 (0.61–0.73)
Meningoencephalitic phase	81.94 (76.77–87.15)	4.00 (3.74–4.25)	74.88 (70.14–79.56)	7.06 (5.92–8.36)
Post-encephalitic TBE syndrome	21.36 (19.87–22.91)	1.04 (0.97–1.12)	0	21.36 (19.87–22.91)
Paralysis	0.20 (0.18–0.21)	< 0.001	0	0.20 (0.18–0.21)
Residual paresis	34.32 (31.98–36.73)	1.67 (1.56–1.79)	0	34.32 (31.98–36.73)
Chronic post-encephalitic TBE syndrome	86.04 (79.87–92.31)	4.20 (3.90–4.50)	0	86.04 (79.87–92.31)
Total	**224.52 (210.14–238.84)**	**10.95 (10.25–11.65)**	**74.88 (70.14–79.56)**	**149.64 (139.67–159.75)**

DALYs: disability-adjusted life years; TBE: tick-borne encephalitis; UI: uncertainty interval; YLD: healthy years of life lost due to disability; YLL: years of life lost.

disease assumes no differences in disease progression between different age groups. Lifelong sequelae make an important contribution to the overall burden, especially in the younger age groups. The disease in children is commonly regarded as mild, but evidence is increasing for the relevance of severe acute disease and long-term sequelae of TBE in children, as well as for the lack of knowledge around the matter [5,37-46]. The uncertainty around the disease progression, overall and for different age groups, can lead to an over- or underestimation of the burden overall and in different age groups. Future study of the disease progression of TBE in different age groups is needed and could improve the accuracy of the model. Another limitation of our study is that the data set used for input in the model was not corrected for underestimation (due to under-reporting and under-ascertainment) of the surveillance system [47]. At the moment of writing, data on underestimation of TBE notification were not available. However, taking into consideration the structure of the morbidity surveillance pyramid [47], we can assume that the notified data were still underestimating the true incidence of disease, thus leading to an underestimation of our burden estimates.

DALYs are a composite health metric highly dependent on the assumptions made; it is commonly used for ranking the relative burden of diseases within the same study, in cost-effectiveness analyses or evaluations of interventions (e.g. DALYs averted). The differences in absolute values between our results and the recent study from Šmit et al. [32] are probably due to differences in underlying assumptions and disease modelling approaches. Šmit et al. used data from a single year that had more cases than the 5-year annual average we used; they used an underestimation coefficient (4.5) for the number of cases of meningoencephalitic TBE, but we did not find enough evidence to make such assumptions; they modelled all neurological sequelae as lifelong. Moreover, Šmit et al. used higher transitional probabilities (in the age groups older than 15 years) and higher disability weights when modelling mild sequelae. Taking this into consideration, a direct comparison is not valid. Our focus on the distribution of the TBE burden across different age groups enabled us to suggest efficient options for vaccination.

Conclusion

We identified a higher burden of TBE among children aged 5–14 years than among adults aged 50–74 years despite a lower TBE incidence. Incidence data alone do not fully reflect the disease impact and should not be the only indicator to inform vaccination policy. In a limited resource setting where prioritisation of TBE vaccination strategies is required, vaccination programmes targeting children should be considered as possibly having a higher impact on disease burden. Our data could be used for future cost-effectiveness studies.

Conflict of interest

None declared.

Authors' contributions

MF and AC were responsible for the conception and design of this study. MF drafted the first study protocol, and AC, EC, IK, MM contributed to further drafts. MF and MG collected and assembled the data. MF undertook the primary data analysis in collaboration with AC. All authors had an opportunity to contribute to the interpretation of the results. MF wrote the first draft of the manuscript, and all other authors contributed to further drafts.

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Challenging measles case definition: three measles outbreaks in three Health Regions of Portugal, February to April 2018

Gonçalo Figueiredo Augusto[1], Diogo Cruz[1], Andreia Silva[1], Natália Pereira[1], Bárbara Aguiar[1], Ana Leça[1], Elisabete Serrada[1], Paula Valente[1], Teresa Fernandes[1], Fernando Guerra[1], Paula Palminha[2], Elsa Vinagre[2], Sílvia Lopo[2], Rita Cordeiro[2], Emma Sáez-López[2,3], Maria Neto[4], Paulo Jorge Nogueira[1], Graça Freitas[1]

1. Directorate-General of Health, Lisbon, Portugal
2. National Institute of Health Dr Ricardo Jorge, Lisbon, Portugal
3. European Public Health Microbiology Training (EUPHEM), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
4. North Region Health Administration (SNS), Porto, Portugal

Correspondence: Paulo Nogueira (paulo.nogueira@dgs.min-saude.pt)

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We report three simultaneous measles outbreaks with 112 confirmed cases in three Health Regions of Portugal, from February to April 2018. The mean age of cases was 30 years, 79% worked in a healthcare setting and 87% were vaccinated. Genotype B3 was identified in 84 cases from the three outbreaks. Primary cases in each outbreak were imported. Several cases presented with modified measles, highlighting the importance of rethinking the measles case definition for vaccinated cases.

We present preliminary findings and implemented control measures of three simultaneous measles outbreaks that occurred in Portugal between February and April 2018. One of the outbreaks took place in a hospital and represented a particular challenge for epidemiological and laboratory investigations as a substantial number of vaccinated healthcare workers (HCWs) developed benign clinical signs and symptoms of measles. We discuss these findings and highlight the need to expand the European Union (EU) measles case definition, in order to increase sensitivity in case capture among vaccinated individuals with modified measles and who do not meet the current European Union (EU) case definition.

Case definition

Measles case definition used for epidemiological surveillance in Portugal is based on the EU case definition [1]. A possible case is any person who meets clinical criteria (i.e. fever, maculopapular rash, and any of cough/coryza/conjunctivitis); a probable case is any person who meets clinical criteria and has an epidemiological link to a confirmed case; a confirmed case is any possible case with laboratory evidence of infection with measles wild virus (i.e. detection of viral RNA in a biological sample and/or a positive IgM result in serum), determined by the World Health Organization (WHO)-certified national reference laboratory for measles and rubella National Institute of Health – Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisbon [2]. Cases are discarded when clinical, epidemiological or laboratory criteria are not met, taking into account vaccination history and risk of measles infection in the community or abroad, following WHO criteria [3].

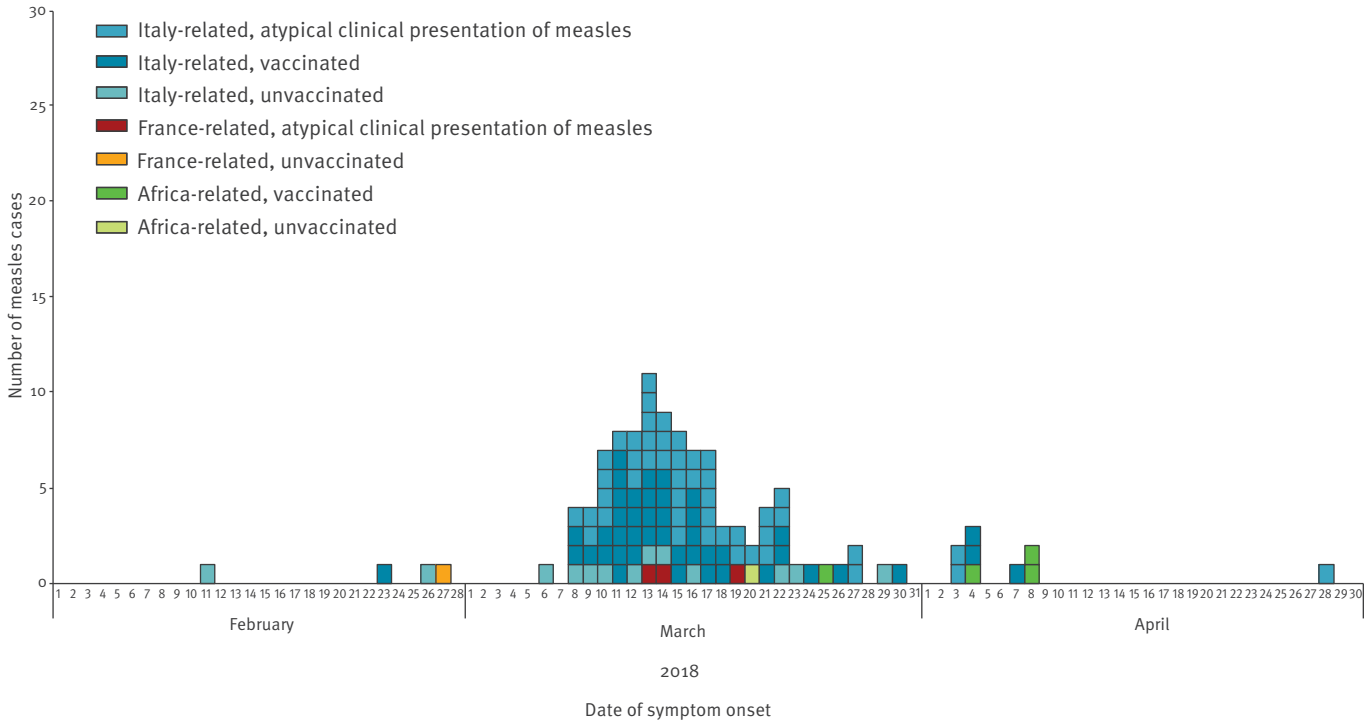
However, symptoms in modified measles cases are masked meaning that cases do not present with the usual signs and symptoms of classic measles, this making a clinical diagnosis more challenging. Modified measles mainly affects young adults who have been vaccinated, suggesting that they could have suboptimal protection against measles whether it be from insufficient number of vaccination doses or that the immunity to disease has waned over time as revealed in the National Serological Survey (2015/2016) [4]. Therefore, the case definition used during this outbreak was expanded to increase sensitivity: clinical criteria included any person with a maculopapular rash, or fever or any of the following three symptoms: cough, coryza, conjunctivitis. Epidemiological criteria included any person with a link to the hospital or with a confirmed measles case.

Outbreak description

On 9 March 2018, a laboratory-confirmed measles case was notified by INSA. It corresponded to an unvaccinated French citizen, recently arrived in the North

Figure 1

Confirmed measles cases by date of symptom onset, chain of transmission and vaccination status, Portugal, 11 February–28 April 2018 (n = 112)



Source: Direção-Geral da Saúde, Sistema Nacional de Vigilância Epidemiológica.

Health region from the Aquitaine Region, where a measles outbreak has been ongoing [5]. Following the laboratory notification, the case was clinically notified on 12 March in the National System for Epidemiological Surveillance (Sistema Nacional de Vigilância Epidemiológica, SINAVE), which is an integrated clinical and laboratory electronic system of mandatory notification. This case was the source of infection for three additional cases in close relatives that either lived with or visited the case; all had been vaccinated with two measles-mumps-rubella (MMR) doses. No further cases were related to this chain of transmission.

On 13 March 2018, the clinical director of a hospital in Oporto reported 24 suspected measles cases among the hospital's HCWs to public health authorities. All suspected cases had a link with the Emergency Department and presented with maculopapular rash, tachycardia, low fever and headache. The following day, INSA confirmed the first two cases along with a third, who was not a HCW and was admitted to another hospital in the city. Epidemiological investigations led to the retrospective identification of the imported primary case, who was an unvaccinated individual from Italy who arrived in Oporto 10 days before the symptom onset and who went to the Emergency Department when they developed a rash. Overall, there were 103 confirmed measles cases associated with this primary case. Most cases were HCWs (n = 87; 84.5%), of which 10 (11.5%) were vaccinated with one dose of a measles-containing vaccine, 66 (75.9%) with two doses, four (4.6%) with three doses, and seven (8.0%) were unvaccinated.

On 26 and 28 March, two cases with history of recent travel to two different African countries and both having a stopover at the same airport on the same day during the incubation period (and evidence of remaining in the same waiting room) were notified in SINAVE. One of the cases was vaccinated with two MMR doses and did not infect further cases. The other case was unvaccinated and infected three additional cases, one at work and two contacts in a hospital Emergency Department. From 12 March until 31 May, a total of 440 suspected measles cases were notified in SINAVE, of which 112 (25.5%) were laboratory-confirmed in INSA, 303 (68.9%) were discarded and 25 (5.7%) were still under investigation. Figure 1shows the distribution of all confirmed measles cases by date of symptom onset and chain of transmission. Overall, 47 (45.6%) cases in this chain of transmission had benign clinical signs and symptoms of measles. History of vaccination was verified from individuals' medical charts or from the national immunisation registry.

Of the 112 confirmed measles cases the mean age was 30 years (SD: 7.7) and 65 cases were female. Preliminary findings show that 111 confirmed measles

Table 1

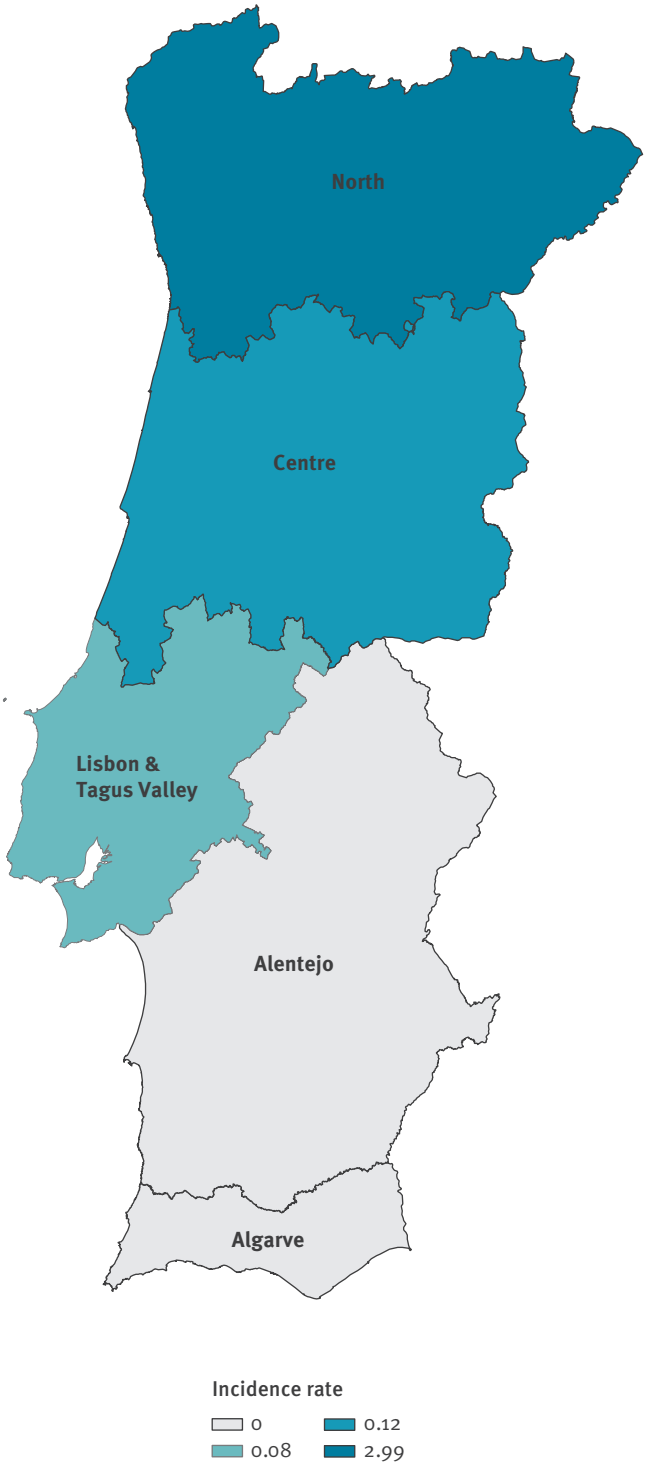
Characteristics of measles cases by chain of transmission Portugal, 11 February – 28 April 2018 (n = 112)

	France-related chain of transmission	Italy-related chain of transmission	Africa-related chain of transmission	Total	
	n	n	n	n	%
Total	**4**	**103**	**5**	**112**	**100.0**
Sex					
Female	2	62	1	**65**	**58.0**
Male	2	41	4	**47**	**42.0**
Age group (years)					
< 1	0	0	0	**0**	**0.0**
1–9	0	1	0	**1**	**0.9**
10–19	0	0	0	**0**	**0.0**
20–29	2	52	1	**55**	**49.1**
30–39	2	40	3	**45**	**40.2**
40–49	0	8	1	**9**	**8.0**
50–59	0	2	0	**2**	**1.8**
≥ 60	0	0	0	**0**	**0.0**
Vaccination status					
Not vaccinated	1	13	1	**15**	**13.4**
1 dose	0	12	2	**14**	**12.5**
2 doses	3	74	2	**79**	**70.5**
3 doses	0	4	0	**4**	**3.6**
Occupation					
Non-Healthcare workers	3	16	4	**23**	**20.5**
Doctors	1	33	0	**34**	**30.4**
Nurses	0	20	0	**20**	**17.9**
Allied professionals	0	15	1	**16**	**14.3**
Medical/Nursing students	0	18	0	**18**	**16.0**
Other Healthcare workers	0	1	0	**1**	**0.9**
Measles symptoms					
Maculopapular rash + Fever + Cough/ Coryza/Conjunctivitis	1	56	5	**62**	**55.4**
Maculopapular rash only	2	11	0	**13**	**11.6**
Fever only	0	2	0	**2**	**1.8**
Maculopapular rash + Fever	1	23	0	**24**	**21.4**
Maculopapular rash + Cough	0	1	0	**1**	**0.9**
Maculopapular rash + Coryza	0	7	0	**7**	**6.2**
Fever + Coryza	0	1	0	**1**	**0.9**
Fever + Cough + Coryza	0	1	0	**1**	**0.9**
Cough + Coryza	0	1	0	**1**	**0.9**
Laboratory results					
Detection of viral RNA	3	80	5	**88**	**78.6**
Positive IgM	0	3	0	**3**	**2.7**
Increase of both IgM and IgG in a pair of samples	1	7	0	**8**	**7.1**
Increase of IgM in a pair of samples	0	3	0	**3**	**2.7**
Increase of IgG in a pair of samples	0	10	0	**10**	**8.9**
Genotype					
B3	2	78	4	**84**	**64.1**

Source: Direção-Geral da Saúde, Sistema Nacional de Vigilância Epidemiológica, Instituto Nacional de Saúde Dr Ricardo Jorge.

Figure 2

Incidence rate of confirmed measles cases per 100,000 population by health region, Portugal, 11 February–28 April 2018 (n = 112)



Source: Direção-Geral da Saúde, Sistema Nacional de Vigilância Epidemiológica. *Note: Map developed by GeoSaúde* (http://www.geosaude.dgs.pt/).

cases occurred in adults (≥ 18 years), with an age range of 20–54 years and one case in a 3-year-old child vaccinated with one MMR dose (Table 1). Among the 112 confirmed cases, 83 (74.1%) were vaccinated with two or more doses of a measles-containing vaccine. Fifty (44.6%) confirmed cases did not meet the clinical criteria from the EU case definition; among them, 24 of 50 had a maculopapular rash and fever as clinical presentation and 13 cases (11.6%) only had a maculopapular rash. Twenty-one cases (18.8%) were confirmed through laboratory results of second samples, where an increase of either IgM, IgG or both was verified (Table 1). Among the 88 cases where viral RNA was detected, 84 cases could be genotyped. Genotype B3 was identified in cases from all the three chains of transmission, although the four cases from the Africa-related chain of transmission had a 5 nucleotide difference from the genotype B3 identified in the other two chains of transmission, which was phylogenetically indistinct.

The measles outbreaks affected three of the seven Portuguese Health Regions (Figure 2), with the majority of cases 107 of 112 (95.5%) reported in the North Health Region.

Control measures

Following the laboratory notification of the first confirmed measles case, DGS issued a warning to healthcare services that was followed by recommendations and guidelines regarding diagnosis, early detection and response to measles cases, within the scope of the National Measles Elimination Programme [6].

In order to control the outbreak on a local level and mitigate transmission outside the healthcare setting, an Emergency Response Team comprising of hospital and public health professionals was created in the affected hospital in Oporto [7]. A vaccination point within the hospital was set-up allowing for rapid vaccination of close contacts and unvaccinated individuals.

During the outbreaks, all suspected measles cases reported were investigated and control measures were promptly implemented to contain further transmission. Local public health teams undertook extensive contact tracing for all suspected measles cases. Surveillance and control measures included immediate isolation of suspected cases, verification of immunisation status of close contacts and administration of prophylactic immunoglobulin or MMR vaccine, whenever necessary. In addition, control measures were complemented with broader public health measures, including the dissemination of key documents to support prevention and control measures [8,9] and raising public awareness about the importance of vaccination through numerous reports in the national media as well as a large media campaign. Daily press releases and epidemiological bulletins were issued by DGS while the outbreaks lasted.

As the primary or index cases did not originate in Portugal or had stayed in another country during their incubation period, the director-general of Health in Portugal notified the Health Authorities from these countries regarding the cases, following the International Health Regulations [10].

Discussion

Following 12 years without endemic measles transmission, Portugal experienced two measles outbreaks in 2017 [11] and, so far, three measles outbreaks in 2018. In two of these transmission mainly occurred in the community setting, whereas one mainly occurred in a healthcare setting. The high coverage of measles vaccination and the timely implementation of control measures allowed for the rapid containment of measles and interruption of all chains of transmission. The outbreaks were declared over on 10 June 2018 and since 29 April 2018 no new cases have been detected [12]. The Immediate isolation of cases, extensive contact tracing and vaccination were crucial to contain the outbreak in the Oporto hospital and avoid its spread to the community.

Vaccination or acquired immunity after illness constitute adequate protection against measles [13]. Since the measles vaccine was introduced in the Portuguese National Immunisation Programme in 1974, the country has achieved a consistent and sustained high immunisation coverage against measles (› 95%) [11,14].

HCWs are at higher risk of measles exposure because the high intensity of the exposure and subsequent transmission to vulnerable patients [15]. According to the National Measles Elimination Programme, HCWs are recommended to receive two doses of measles vaccine (either single measles-containing vaccine or MMR) or to have evidence of previous measles infection [6]. However, measles outbreaks in healthcare settings are becoming more frequent in the European Region [15-18]. Countries, such as Portugal, which maintained a high vaccination coverage for many years and had eliminated measles, are at greater risk of modified measles cases emerging during outbreaks. This is due to suboptimal protection against measles, either from insufficient number of vaccine doses or waning immunity from the vaccine over time (as indicated by the National Serological Survey 2015/2016). Modified measles mainly affect young adults who were adequately vaccinated but with the last dose of the vaccine administered more than 10 years prior.

In one chain of transmission, a hospital cluster was identified and most cases were HCWs vaccinated with two or more doses of MMR vaccine. This was described in other outbreaks [18] and may be related to waning of vaccine-induced immunity in the absence of natural boosting by the wildtype virus [19].

Modified measles cases has been described in vaccinated individuals [20,21]. In the outbreaks reported here, this was the case in 50 of 112 (44.6%) confirmed cases. Early findings of modified measles led us to expand the case definition initially in place in order to increase sensitivity. Interestingly, 5 of 112 (4.5%) confirmed cases did not have a maculopapular rash, and their symptoms would have been easily mistaken for other clinical conditions if they were not investigated in the context of a measles outbreak. Also, laboratory confirmation was only possible due to the collection of second serum samples in 21 of 112 cases, where an increase of IgM or IgG antibodies was verified [22].

The outbreaks described here, which included a number of cases with modified measles and a large number of cases among vaccinated HCWs, highlight the need for further investigation in order to recommend innovative approaches in future outbreaks: Nearly half of these cases would not have been identified using the current EU case definition. Thus, in light of these new findings and in order to increase sensitivity in case capture in the context of an outbreak, it would be important to develop an additional case classification suited for a community with high vaccination coverage with epidemiological criteria, that may lead to the definition of risk levels for public health intervention according to the type of exposure or depending to exposure to cases of reinfection.

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Conflict of interest

None declared.

Authors' contributions

GF, DC, AS and PN coordinated the investigations of the outbreak at the national level. MN coordinated the investigation at the regional level in the North Region. GFA, BA, NP, ES, TF, PV and AL contributed to data collection, case information and data analysis. PP, EV, SL, RC and ESL were involved in the laboratory investigations. GFA drafted the manuscript, with contributions by NP, BA, AL and PP. All authors were involved in revising the manuscript. All authors reviewed and approved the final version.

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Prevalence of healthcare-associated infections, estimated incidence and composite antimicrobial resistance index in acute care hospitals and long-term care facilities: results from two European point prevalence surveys, 2016 to 2017

Carl Suetens[1], Katrien Latour[2], Tommi Kärki[1], Enrico Ricchizzi[3], Pete Kinross[1], Maria Luisa Moro[3], Béatrice Jans[2], Susan Hopkins[4], Sonja Hansen[5], Outi Lyytikäinen[6], Jacqui Reilly[7,8], Aleksander Deptula[9], Walter Zingg[10], Diamantis Plachouras[1], Dominique L Monnet[1], the Healthcare-Associated Infections Prevalence Study Group[11]

1. European Centre for Disease Prevention and Control, Solna, Sweden
2. Sciensano, Brussels, Belgium
3. Agenzia sanitaria e sociale regionale – Regione Emilia Romagna, Bologna, Italy
4. Public Health England, London, United Kingdom
5. Institute of Hygiene and Environmental Medicine, Charité – University Medicine Berlin, Berlin, Germany
6. National Institute for Health and Welfare, Department of Health Security, Helsinki, Finland
7. National Services Scotland, Health Protection Scotland, Glasgow, United Kingdom
8. Glasgow Caledonian University, Glasgow, United Kingdom
9. Department of Propaedeutics of Medicine, Nicolaus Copernicus University, Toruń; Ludwik Rydygier Collegium Medicum; Bydgoszcz, Poland
10. Imperial College London, London, United Kingdom
11. Members of the Healthcare-Associated Infections Prevalence Study Group are listed at the end of this article

Correspondence: Carl Suetens (carl.suetens@ecdc.europa.eu)

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Point prevalence surveys of healthcare-associated infections (HAI) and antimicrobial use in the European Union and European Economic Area (EU/EEA) from 2016 to 2017 included 310,755 patients from 1,209 acute care hospitals (ACH) in 28 countries and 117,138 residents from 2,221 long-term care facilities (LTCF) in 23 countries. After national validation, we estimated that 6.5% (cumulative 95% confidence interval (cCI): 5.4–7.8%) patients in ACH and 3.9% (95% cCI: 2.4–6.0%) residents in LTCF had at least one HAI (country-weighted prevalence). On any given day, 98,166 patients (95% cCI: 81,022–117,484) in ACH and 129,940 (95% cCI: 79,570–197,625) residents in LTCF had an HAI. HAI episodes per year were estimated at 8.9 million (95% cCI: 4.6–15.6 million), including 4.5 million (95% cCI: 2.6–7.6 million) in ACH and 4.4 million (95% cCI: 2.0–8.0 million) in LTCF; 3.8 million (95% cCI: 3.1–4.5 million) patients acquired an HAI each year in ACH. Antimicrobial resistance (AMR) to selected AMR markers was 31.6% in ACH and 28.0% in LTCF. Our study confirmed a high annual number of HAI in healthcare facilities in the EU/EEA and indicated that AMR in HAI in LTCF may have reached the same level as in ACH.

Introduction

In 2016, the European Centre for Disease Prevention and Control (ECDC) estimated that the burden of six main types of healthcare-associated infection (healthcare-associated pneumonia, urinary tract infection, surgical site infection, *Clostridium difficile* infection, neonatal sepsis and primary bloodstream infection) expressed in disability-adjusted life years (DALYs) in the European Union and European Economic Area (EU/EEA) was higher than the combined burden of 31 other infectious diseases under surveillance by ECDC [1,2]. The estimated number of healthcare-associated infections (HAI) used in the study was based on the data of the first ECDC point prevalence survey (PPS) of HAI and antimicrobial use in acute care hospitals (ACH) from 2011 to 2012 [3] and did not take into account HAI occurring in other healthcare facilities. In particular, ECDC had previously estimated that the number of residents with an HAI on any given day in European long-term care facilities (LTCF) was of the same order

Table 1a

Key characteristics of healthcare facilities, patients and residents included in the point prevalence survey (PPS) samples, PPS in acute care hospitals (n = 1,275) and long-term care facilities (n = 2,242), 30 EU/EEA countries, Serbia and the former Yugoslav Republic of Macedonia, 2016–2017

Country	ACH								LTCF								
	Number of hospitals		Type of ACH					Intensive care patients	Number of LTCF		Type of LTCF				Residents in (a) + (b) + (c)		
	Country total	In PPS sample	Primary	Secondary	Tertiary	Specialised	Unknown	(%)	Country total	In PPS sample	General nursing home (a)	Residential home (b)	Mixed LTCF (c)	Other LTCF types	>85 years-old (%)	Urinary catheter (%)	Recent surgery (%) (past 30 days)
Austria	162	49	25	11	2	11	0	4.0	817	14	0	7	5	2	35.8	10.8	1.0
Belgium	197	43	27	9	7	0	0	4.9	1,559	86	79	0	0	7	56.5	3.1	0.9
Bulgaria	241	12	1	4	7	0	0	6.9	33	NP	NA	NA	NA	NA	NA	NA	NA
Croatia	32	34	6	15	9	4	0	6.0	325	8	0	0	8	0	40.9	3.1	1.1
Cyprus	83	8	2	4	2	0	0	9.6	90	13	7	0	4	2	54.8	8.0	4.8
Czech Republic	144	45	2	30	11	2	0	8.1	73	11	0	4	5	2	NA	NA	NA
Denmark	52	NP	NA	NA	NA	NA	NA	NA	827	95	0	0	95	0	51.8	9.0	1.7
Estonia	27	23	10	7	1	4	1	3.3	59	NP	NA	NA	NA	NA	NA	NA	NA
Finland	59	51	18	16	14	2	1	3.8	1,928	157	148	0	1	8	51.4	4.2	0.6
France	1,237	50	32	10	6	2	0	3.8	9,744	91	91	0	0	0	61.6	1.6	0.8
Germany	1,857	49	25	7	4	13	0	5.0	10,389	84	55	15	12	2	49.6	8.6	1.3
Greece	123	42	1	23	16	2	0	7.6	263	13	0	0	13	0	48.8	12.1	0.7
Hungary	94	38	14	10	6	7	1	2.8	1,177	111	65	9	1	36	25.3	1.9	0.7
Iceland	8	2	0	1	1	0	0	5.2	43	NP	NA	NA	NA	NA	NA	NA	NA
Ireland	60	60	9	17	7	27	0	3.0	578	185	75	0	34	76	47.7	7.0	1.5

ACH: acute care hospital; EU/EEA: European Union/European Economic Area; LTCF: long-term care facility; NA: not applicable; ND: no data collected in national protocol; NP: did not participate; PPS: point prevalence survey; UK: United Kingdom.

Country data representativeness was poor in Bulgaria and the Netherlands for the PPS in ACH and poor in Austria, Croatia, Cyprus, Greece, Luxembourg, Malta and Poland for the PPS in LTCF. The Czech Republic only submitted data on institutional indicators.

Table 1b

Key characteristics of healthcare facilities, patients and residents included in the point prevalence survey (PPS) samples, PPS in acute care hospitals (n = 1,275) and long-term care facilities (n = 2,242), 30 EU/EEA countries, Serbia and the former Yugoslav Republic of Macedonia, 2016–2017

Country	ACH								LTCF								
	Number of hospitals		Type of ACH					Intensive care patients	Number of LTCF		Type of LTCF				Residents in (a)+(b)+(c)		
	Country total	In PPS sample	Primary	Secondary	Tertiary	Specialised	Unknown	(%)	Country total	In PPS sample	General nursing home (a)	Residential home (b)	Mixed LTCF (c)	Other LTCF types	>85 years-old (%)	Urinary catheter (%)	Recent surgery (%) (past 30 days)
Italy	1,134	56	13	14	25	4	0	6.0	3219	215	61	85	50	19	54.0	12.1	1.3
Latvia	24	14	0	9	3	2	0	3.5	82	NP	NA	NA	NA	NA	NA	NA	NA
Lithuania	64	62	25	26	8	3	0	2.8	154	26	0	0	26	0	12.4	0.8	0.3
Luxembourg	12	12	2	5	1	3	1	5.9	62	16	15	1	0	0	58.4	5.3	1.5
Malta	4	4	1	1	1	1	0	4.8	41	11	0	8	3	0	51.1	5.0	0.6
The Netherlands	79	19	10	8	1	0	0	6.0	700	57	0	0	57	0	43.0	6.6	3.5
Norway	53	43	11	9	4	0	19	6.3	907	62	62	0	0	0	NA	10.0	3.4
Poland	936	80	22	20	23	15	0	3.8	373	25	12	12	0	1	30.5	19.4	0.9
Portugal	225	93	24	40	18	9	2	4.2	360	268	0	0	132	136	29.6	15.1	0.9
Romania	311	40	16	10	3	11	0	6.4	628	NP	NA	NA	NA	NA	NA	NA	NA
Slovakia	107	50	20	11	7	12	0	5.2	677	69	27	0	32	10	28.3	3.1	1.1
Slovenia	21	20	0	11	3	6	0	5.8	90	NP	NA	NA	NA	NA	NA	NA	NA
Spain	576	96	17	39	32	5	3	5.0	5,387	46	0	0	46	0	48.1	5.1	5.1
Sweden	144	NP	NA	NA	NA	NA	NA	NA	2,300	417	285	0	0	132	57.9	9.9	2.1
UK–England	158	32	0	19	10	3	0	3.4	17,473	NP	NA	NA	NA	NA	NA	NA	NA
UK–Northern Ireland	16	16	6	4	2	4	0	3.2	445	70	0	15	55	0	44.8	5.0	0.6
UK–Scotland	46	45	12	14	7	12	0	2.8	873	52	34	17	1	0	43.9	8.5	0.3
UK–Wales	21	21	6	10	4	1	0	3.7	795	30	9	7	12	2	49.7	7.8	1.7
EU/EEA	8,307	1,209	357	414	245	165	28	4.6%	62,471	2,232	1025	180	592	435	45.6%	6.7%	1.5%
EU/EEA (n, %, mean of countries)	252	100%	29.5%	34.2%	20.3%	13.6%	2.3%	4.9%	1,893	100%	45.9%	12.3%	22.3%	19.5%	44.8%	7.3%	1.5%
Former Yugoslav Republic of Macedonia	ND	NP	NA	NA	NA	NA	NA	NA	21	4	3	0	1	0	15.3	8.8	0.7
Serbia	66	66	1	45	14	6	0	6.5	90	6	0	0	6	0	28.1	6.1	0.6

ACH: acute care hospital; EU/EEA: European Union/European Economic Area; LTCF: long-term care facility; NA: not applicable; ND: no data collected in national protocol; NP: did not participate; PPS: point prevalence survey; UK: United Kingdom.

Country data representativeness was poor in Bulgaria and the Netherlands for the PPS in ACH and poor in Austria, Croatia, Cyprus, Greece, Luxembourg, Malta and Poland for the PPS in LTCF. The Czech Republic only submitted data on institutional indicators.

of magnitude as the number of patients with an HAI on any given day in ACH [4-6].

In the period from 2016 to 2017, ECDC organised two PPS of HAI and antimicrobial use: the second PPS in ACH and the third PPS in LTCF in the EU/EEA. The objective of the current study was to report on the HAI and antimicrobial resistance results of both surveys and to estimate the combined total number of HAI on any given day and the number of HAI per year in 2016 and 2017 in the EU/EEA.

Methods

Participation of countries

All EU/EEA countries and EU candidate and potential candidate countries were invited to organise a national PPS in ACH and LTCF in their country in any of four periods (April to June or September to November of 2016 or 2017). For reasons of feasibility at national level, the PPS in ACH and LTCF could be organised during different periods. Data were collected according to two specific standardised ECDC protocols [7,8]. All countries used the ECDC protocols and included all HAI types except for one country (Norway) for ACH and four countries (France, the Netherlands, Norway and Sweden) for LTCF Norway used national protocols with the same case definitions as in the ECDC protocols, but provided fewer details and did not require the inclusion of all types of HAI. LTCF data from France and the Netherlands were also collected using national protocols not including all types of HAI. LTCF protocols in France, the Netherlands and Norway all included urinary tract infections, lower respiratory tract infections and skin infections, in addition to other HAI types varying by country. Surveys in separate healthcare administrations in the United Kingdom (UK), i.e. England, Northern Ireland, Scotland and Wales, were organised independently and results were reported separately.

Selection of participating facilities and patients

It was recommended that countries selected the participating ACH and LTCF by systematic random sampling from national lists ranked by type and size to ensure optimal country representativeness. For each country, the required sample size was calculated for an estimated prevalence of 6% for ACH and 4% for LTCF, based on the results of the previous PPS [3,6], with an absolute precision of 1%. Representativeness was categorised as optimal, good, poor or very poor, depending on the sampling method of the facilities, the number of included patients/residents and the number of included facilities [7,8]. For example, 'optimal representativeness' meant that the country performed systematic sampling of at least 25 healthcare facilities or included at least 75% of all facilities or beds at national level, and achieved the recommended sample size.

For ACH, the protocol recommended that data from a single ward should be collected on one single day and that the time frame for data collection for all wards of a single hospital would not exceed 3 weeks. For LTCF, it was recommended to collect data on a single day, except for larger LTCF.

We included all patients/residents present on the hospital ward or LTCF at 8:00 on the day of the PPS and still present at the time of day when the PPS was performed. In addition, LTCF residents needed to be full-time residents (i.e. living 24 hours a day in the LTCF). Patients/residents who were temporarily absent from their room, e.g. for diagnostic procedures, had to be included.

Case definitions

Case definitions for HAI differed for ACH and for LTCF, reflecting differences in access to diagnostic methods between the two settings, as well as the specific signs and symptoms of infection in elderly LTCF residents [7,8]. For both PPS, an HAI was defined as active on the day of the PPS when signs and symptoms were present on the date of the PPS, or when signs and symptoms were no longer present but the patient/resident was still receiving treatment for that infection on the date of the PPS. HAI present on admission were included in both protocols. In the LTCF protocol, HAI associated with a stay in any other healthcare facility – another LTCF or a hospital – were included. In the ACH protocol, however, only HAI imported from other ACH were included, excluding HAI present on admission associated with a previous LTCF stay. LTCF data in France and Sweden did not include HAI imported from other healthcare facilities.

Data analysis

Data were analysed with Stata, version 14.1 (StataCorp, Texas, United States). The prevalence of HAI was expressed as the percentage of patients/residents with at least one HAI on the day of the PPS. To account for clustering within ACH or LTCF, 95% confidence intervals (CI) were calculated using the svy proportion command in Stata. Overall weighted prevalence percentages were calculated by applying the country-specific prevalence on the number of occupied beds in each country and summing up the total number of patients with at least one HAI for EU/EEA countries. National denominator data were obtained by questionnaire from national survey coordinators, from Eurostat data if national denominator data were not submitted [9-11] or from the previous PPS if Eurostat data were missing or incomplete [3,4,6]. To estimate the total number of HAI or patients with at least one HAI for the whole EU/EEA, the average results from participating EU/EEA countries were applied to the national denominator data from non-participating EU/EEA countries. For data collected using national protocols which did not include all types of HAI, imputation of non-included types of HAI was done based on EU/EEA averages to make prevalence percentages comparable. In ACH, imputation resulted in adding 7.3% (36/495) of patients with HAI in Norway. In LTCF, imputation resulted in adding 5.8% (12/206) of residents with HAI in France, 6.9% (11/160)

Table 2a

Prevalence and estimated incidence of healthcare-associated infections in European acute care hospitals, 28 EU/EEA countries and Serbia, 2016–2017 (n = 325,737 patients)

Country	Patients in PPS sample	Patients with at least one HAI in PPS sample (HAI prevalence)[a]			Validation-corrected HAI prevalence[b]	Occupied beds in the country (average per day)	Patients with at least one HAI on a given day, estimated		Hospital discharges annually in the country	HAI incidence, estimated		Patients with at least one HAI, annually, estimated	
	n	n	%	95% CI	%	n	n	95% CI	n	%	95% CI	n	95% CI
Austria	13,461	541	4.0	3.4–4.7	NR	36,351	1,461	1,243–1,716	2,707,753	2.3	1.5–3.3	62,306	40,978–89,762
Belgium	11,800	856	7.3	6.4–8.3	NR	37,651	2,731	2,397–3,109	1,858,726	5.4	3.7–7.6	101,110	68,186–141,713
Bulgaria[c]	2,200	76	3.5	1.7–6.8	NR	25,324	875	434–1,733	1,632,089	1.8	0.9–3.8	29,572	13,909–61,597
Croatia	10,466	551	5.3	4.5–6.2	NR	11,047	581	495–683	667,849	4.1	2.8–5.6	27,129	18,937–37,561
Cyprus	1,036	85	8.2	5.4–12.4	ND	1,437	118	77–178	166,295	4.8	2.5–8.7	8,010	4,158–14,541
Czech Republic	15,117	1,015	6.7	5.9–7.6	NR	40,691	2,732	2,413–3,090	2,260,239	5.4	3.9–7.3	122,313	87,039–165,208
Estonia	4,220	178	4.2	2.4–7.3	NR	4,582	193	111–332	222,363	3.3	1.6–6.6	7,393	3,558–14,761
Finland	9,079	803	8.8	7.5–10.4	NR	15,894	1,406	1,187–1,660	915,892	5.1	3.3–7.5	46,735	30,053–68,350
France	16,522	965	5.8	4.9–7.0	NR	159,810	9,334	7,823–11,116	11,330, 996	4.1	2.7–5.9	467,961	311,830–671,498
Germany	11,324	409	3.6	2.8–4.7	NR	400,132	14,452	11,087–18,789	19,480,504	3.1	1.9–4.8	604,495	373,766–938,383
Greece	9,401	938	10.0	8.5–11.6	NR	18,252	1,821	1,559–2,121	1,562,761	4.3	3.1–5.7	66,487	48,386–89,068
Hungary	20,588	818	4.0	3.3–4.8	NR	46,134	1,833	1,516–2,212	2,226,485	3.5	2.1–5.4	78,095	46,906–120,082
Iceland	633	40	6.3	0.8–36.8	5.7	642	41	5–237	39,198	6.7	0.6–48.6	2,609	239–19,038
Ireland	10,333	633	6.1	5.0–7.5	NR	10,932	670	546–820	705,000	4.2	2.7–6.3	29,671	18,846–44,323
Italy	14,773	1,186	8.0	6.8–9.5	NR	167,619	13,457	11,362–15,899	8,930,979	6.0	4.2–8.3	534,709	373,705–740,544

CI: confidence interval; EU/EEA: European Union/European Economic Area; HAI: healthcare-associated infection; NA: not applicable; ND: validation study not done NR: validation study not representative of country PPS sample; PPS: point prevalence survey; UK: United Kingdom.

[a] Country-weighted HAI prevalence for the EU/EEA= estimated number of patients with at least one HAI a single day / occupied beds.

[b] Validation-corrected prevalence of patients with at least one HAI: only given for countries that reached national representativeness for their national validation study (at least 75% of recommended sample size of 750 validated patients and/or validation of at least 75% of included hospitals).

[c] Poor country representativeness in Bulgaria and the Netherlands.

Table 2b

Prevalence and estimated incidence of healthcare-associated infections in European acute care hospitals, 28 EU/EEA countries and Serbia, 2016–2017 (n = 325,737 patients)

Country	Patients in PPS sample	Patients with at least one HAI in PPS sample (HAI prevalence)[a]			Validation-corrected HAI prevalence[b]	Occupied beds in the country (average per day)	Patients with at least one HAI on a given day, estimated		Hospital discharges annually in the country	HAI incidence, estimated		Patients with at least one HAI, annually, estimated	
	n	n	%	95% CI	%	n	n	95% CI	n	%	95% CI	n	95% CI
Latvia	3,807	140	3.7	2.6–5.2	4.9	5,127	189	132–268	300,575	2.5	1.4–4.1	7,447	4,322–12,399
Lithuania	12,415	359	2.9	2.1–4.0	3.2	14,613	423	301–590	705,224	2.6	1.3–4.6	18,046	9,322–32,167
Luxembourg	2,018	103	5.1	4.0–6.5	8.5	1,860	95	75–120	74,782	3.4	2.1–5.3	2,569	1,560–3,995
Malta	961	60	6.2	5.2–7.4	7.9	972	61	51–72	72,909	2.6	1.9–3.4	1,877	1,380–2,507
The Netherlands[c]	4,441	170	3.8	3.4–4.3	NR	24,167	925	826–1,036	1,700,000	2.3	1.6–3.2	39,585	27,525–54,115
Norway[d]	9,628	495	5.1	4.1–6.4	ND	10,505	540	430–677	776,203	2.4	1.5–3.6	18,767	11,873–28,340
Poland	21,712	1,249	5.8	4.8–6.9	4.7	120,492	6,931	5,764–8,317	8,254,611	3.5	2.3–5.0	289,602	193,881–415,274
Portugal	16,982	1,544	9.1	8.1–10.2	7.8	27,907	2,537	2,236–2,841	1,128,245	5.9	4.4–7.8	66,860	49,568–87,500
Romania	11,443	417	3.6	2.8–4.7	5.9	57,091	2,080	1,610–2,682	3,674,275	2.6	1.7–4.0	97,257	62,340–146,893
Slovakia	9,145	370	4.1	3.1–5.3	NR	20,279	820	630–1,066	1,005,003	3.1	2.1–4.6	31,519	20,848–46,607
Slovenia	5,720	373	6.5	5.8–7.3	ND	5,581	363	322–409	380,077	4.4	3.3–5.6	16,635	12,630–21,441
Spain	19,546	1,516	7.8	7.1–8.5	NR	84,908	6,586	5,983–7,243	5,247,215	4.9	3.6–6.4	255,169	186,398–335,644
UK–England	20,148	1,297	6.4	5.4–7.6	NR	96,774	6,230	5,264–7,358	9,450,142	2.2	1.4–3.2	205,722	130,191–303,990
UK–Northern Ireland	3,813	234	6.1	4.8–7.9	5.8	4,965	305	236–392	302,008	3.5	1.8–5.9	10,527	5,559–17,841
UK–Scotland	11,623	504	4.3	3.5–5.3	NR	11,448	496	406–606	1,156,473	2.2	1.5–3.2	25,539	16,992–36,977
UK–Wales	6,400	362	5.7	4.7–6.7	6.0	6,715	380	318–453	827,634	2.2	1.3–3.3	17,880	10,595–27,545
Participating EU/EEA countries[a,e]	**310,755**	**18,287**	**5.5**	**4.5–6.7**	**6.5**	**1,469,903**	**80,665**	**66,864–97,824**	**89,762,505**	**3.7**	**2.4–5.3**	**3,293,595**	**2,185,484–4,789,661**
Serbia	14,982	650	4.3	3.5–5.4	NR	18,920	821	656–1,024	988,383	3.3	2.3–4.6	32,337	22,714–45
EU/EEA, corrected[e,f]	NA	NA	5.5	4.5–6.7	6.5	1,503,881	82,713	67,674–99,256	91,885,503	3.7	2.4–5.3	3,372,146	2,220,554–4,854,535
EU/EEA, corrected after validation	**NA**	**NA**	**6.5**	**5.4–7.8**	**NA**	**1,503,881**	**98,166**	**81,022–117,484**	**91,885,503**	**4.1**	**3.4–4.9**	**3,758,014**	**3,122,024–4,509,617**

CI: confidence interval; EU/EEA: European Union/European Economic Area; HAI: healthcare-associated infection; NA: not applicable; ND: validation study not done NR: validation study not representative of country PPS sample; PPS: point prevalence survey; UK: United Kingdom.

[a] Country-weighted HAI prevalence for the EU/EEA= estimated number of patients with at least one HAI a single day / occupied beds.

[b] Validation-corrected prevalence of patients with at least one HAI: only given for countries that reached national representativeness for their national validation study (at least 75% of recommended sample size of 750 validated patients and/or validation of at least 75% of included hospitals).

[c] Poor country representativeness in Bulgaria and the Netherlands.

[d] Norway used a national PPS protocol requiring imputation of non-included types of HAI for 24 hospitals.

[e] Cumulative 95% CI for the EU/EEA. Cumulative sums are rounded and may differ from the sum of the individual rounded country estimates.

[f] Corrected for non-participating EU countries with estimation for Denmark and Sweden combined.

Table 3

Country-weighted prevalence and estimated incidence of healthcare-associated infections (HAI) by type of HAI in European acute care hospitals (n = 19,626) and long-term care facilities (n = 3,858), 30 EU/EEA countries, 2016–2017

Type of HAI	Acute care hospitals								Long-term care facilities							
	HAI in PPS sample		Country-weighted HAI prevalence		Estimated HAI on a given day, EU/EEA[a]		Estimated annual HAI, EU/EEA[a]		HAI in PPS sample		Country-weighted HAI prevalence		Estimated HAI on a given day, EU/EEA[a]		Estimated annual HAI, EU/EEA[a]	
	N	% total	n	95% cCI	N	95% cCI	n	95% cCI	n	% total	%	95% cCI	n	95% cCI	n	95% cCI
Pneumonia	4,200	21.4	1.26	0.96–1.68	18,935	14,398–25,265	862,084	567,728–1 283,203	143	3.7	0.15	0.06–0.32	4,948	1,946–10 658	112,868	44,390–243,134
Other lower respiratory tract infection[b]	838	4.3	0.24	0.15–0.41	3,568	2,208–6,192	183,232	91,731–376,990	847	22.0	0.88	0.59–1.14	29,010	19,412–37,826	1,058,853	708,542–1 380,653
Common cold/ influenza	NI	NA	NA	NA	NA	NA	NA	NA	290	7.5	0.29	0.13–0.51	9,678	4,368–16,782	441,543	199,312–765,693
Urinary tract infection	3,710	18.9	1.10	0.85–1.43	16,491	12,822–21,455	869,941	572,105–1,278,951	1,233	32.0	1.29	0.87–1.66	42,687	28,898–54,825	1,298,388	878,983–1,667,596
Surgical site infection	3,601	18.3	1.08	0.81–1.44	16,130	12,185–21,715	518,182	293,036–858,222	66	1.7	0.09	0.03–0.20	2,829	944–6,500	57,366	19,133–131,803
Bloodstream infection	2,116	10.8	0.69	0.48–1.00	10,294	7,241–15,097	375,050	227,552–613,624	19	0.5	0.04	0.01–0.07	1,168	193–2,389	23,692	3,908–48,442
Clostridium difficile infection	951	4.8	0.32	0.21–0.51	4,786	3,105–7,721	189,526	105,154–340,978	37	1.0	0.05	0.01–0.14	1,787	424–4,755	18,118	4,296–48,206
Other gastrointestinal infection	792	4.0	0.24	0.14–0.41)	3,549	2,108–6,166	144,926	64,880–312,212	75	1.9	0.1	0.03–0.20	3,187	1,012–6,473	145,409	46,184–295,333
Skin and soft tissue infection	823	4.2	0.21	0.13–0.36	3,146	1,900–5,451	108,269	45,149–242,816	828	21.5	0.83	0.51–1.19	27,459	17,021–39,307	626,415	388,293–896,687
Eye, ear, nose or mouth infection	557	2.8	0.16	0.09–0.35	2,400	1,278–5 194	123,091	54,155–303,206	183	4.7	0.17	0.08–0.31	5,712	2,707–10,369	173,733	82,323–315,390
Systemic infection	1,069	5.4	0.29	0.17–0.52	4,388	2,586–7,799	251,237	110,732–549,877	35	0.9	0.04	0.01–0.08	1,223	286–2,534	37,201	8,691–77,061
Other infection	969	4.9	0.30	0.19–0.50	4,518	2,867–7,574	154,138	65,647–332,357	102	2.6	0.12	0.04–0.24	3,878	1,366–8,077	117,958	41,556–245,683
All types of HAI, EU/EEA[a]	19,626	100	NA	NA	88,204	62,697–129,630	3,779,677	2,197,869–6,492,437	3,858	100	NA	NA	133,565	78,576–200,494	4,111,544	2,425,610–6,115,682
All types of HAI, EU/EEA, corrected after validation	**NA**	**NA**	**NA**	**NA**	**104,177**	**74,743–152,575**	**4,464,159**	**2,620,139–7,641,606**	**NA**	**NA**	**NA**	**NA**	**143,565**	**64,736–260,655**	**4,422,629**	**1,998,384–7,950,784**

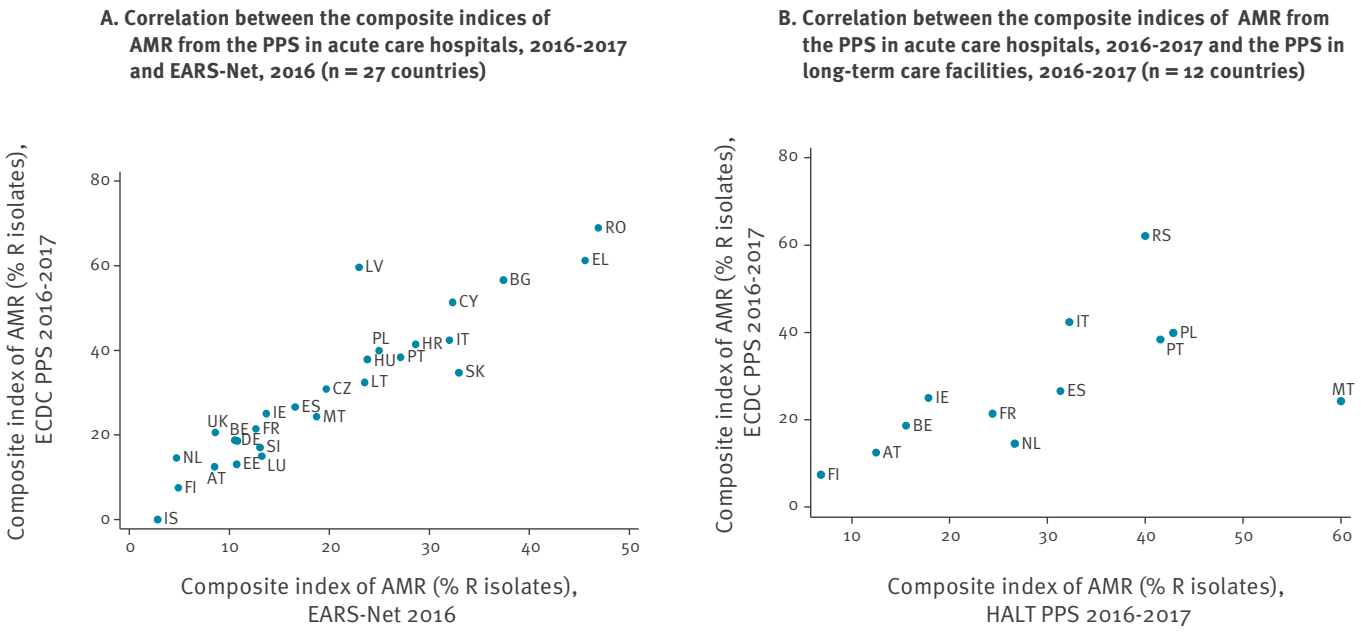
cCI: cumulative 95% confidence interval (sum of country-specific lower respectively upper country interval limits); EU/EEA: European Union/European Economic Area; HAI: healthcare-associated infection; NA: not applicable; NI: not included in protocol; PPS: point prevalence survey.

[a] After correction for non-participating countries. Cumulative sums are rounded and may differ from the sum of the individual rounded country estimates.

[b] Other lower respiratory tract infections included bronchitis, tracheobronchitis, bronchiolitis, tracheitis, lung abcess or empyema, without evidence of pneumonia.

Figure

Correlations of composite index of antimicrobial resistance, EU/EEA countries and Serbia, 2016–2017



ACH: acute care hospital; AMR: antimicrobial resistance; AT: Austria; BE: Belgium; BG: Bulgaria; CY: Cyprus; CZ: Czech Republic; DE: Germany; EARS-Net: European Antimicrobial Resistance Surveillance Network; ECDC: European Centre for Disease Prevention and Control; EE: Estonia; EL: Greece; ES: Spain; FI: Finland; FR: France; HALT: Healthcare-associated infections in LTCF project; HR: Croatia; HU: Hungary; IE: Ireland; IS: Iceland; IT: Italy; LT: Lithuania; LTCF: long-term care facility; LU: Luxembourg; LV: Latvia; MT: Malta; NL: the Netherlands; NO: Norway; PL: Poland; PPS: point prevalence survey; PT: Portugal; RO: Romania; RS: Serbia; SI: Slovenia; SK: Slovakia; UK: United Kingdom.

Composite index of AMR: *Staphylococcus aureus* resistant to meticillin, *Enterococcus faecium* and *Enterococcus faecalis* resistant to vancomycin, Enterobacteriaceae resistant to third-generation cephalosporins, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* resistant to carbapenems; EARS-Net: Enterobacteriaceae other than *Escherichia coli* and *Klebsiella pneumoniae* not included. Other species represented 32.5% of tested Enterobacteriaceae in ACH. France: percentage non-susceptible (resistant + intermediate) isolates instead of percentage resistant isolates. In addition to poor representativeness of participating LTCF in Malta, specimens in these LTCF were known to be taken predominantly in cases of treatment failure (panel B).

in the Netherlands and 7.6% (9/119) in Norway, or 0.8% (32/3,780) overall. As these imputations were done for the aggregated national results, correction of CI for clustering within LTCF could not be applied for these countries and binomial exact CI were used instead.

Antimicrobial resistance

Antimicrobial resistance (AMR) in HAI was evaluated using two indicators: a composite index of AMR and the percentage of carbapenem-resistant Enterobacteriaceae. The composite index of AMR was calculated as the percentage of resistant isolates for the 'first level' AMR markers in the PPS protocols divided by the sum of the isolates for which results from antimicrobial susceptibility testing (AST) were reported. These first level markers were *Staphylococcus aureus* resistant to meticillin (MRSA), *Enterococcus faecium* and *Enterococcus faecalis* resistant to vancomycin, Enterobacteriaceae resistant to third-generation cephalosporins, and *Pseudomonas aeruginosa* and *Acinetobacter baumannii* resistant to carbapenems. The percentage of resistant isolates was not calculated when less than 10 isolates with known AST results were reported. The composite index of AMR at country level was validated by examining the correlation with the composite AMR index calculated from EARS-Net data from 2016, including all components of the index except AST results for Enterobacteriaceae other than *Escherichia coli* and *Klebsiella pneumoniae* because they are not included in EARS-Net [12,13]. Correlations were analysed using the Spearman correlation coefficient rho and the R-squared (R^2) and regression coefficient from linear regression.

Prevalence to incidence conversion

Estimates of the total number of HAI and patients acquiring at least one HAI per year in ACH were based on prevalence to incidence conversion using the Rhame and Sudderth formula [14]. Details of the method are reported in the ECDC PPS report for 2011 and 2012 [3]. In addition, sensitivity analyses of the conversion were carried out using a method developed by Willrich et al. (personal communication: Niklas Willrich, 24 May 2018), in which the estimates of the length of stay were based on a Grenander estimator for discrete monotonously decreasing distributions [15].

Table 4a

Composite index of antimicrobial resistance in bacteria from healthcare-associated infections in acute care hospitals (n = 8,413) and long-term care facilities (n = 565), 30 EU/EEA countries, Serbia and the former Yugoslav Republic of Macedonia[a], 2016–2017

Country	Acute care hospitals[a]								Long-term care facilities[a]			
	Composite index of AMR				Carbapenem-resistant Enterobacteriaceae				Composite index of AMR		Carbapenem-resistant Enterobacteriaceae	
	Tested isolates	Resistant isolates	Estimated annual HAI		Tested isolates	Resistant isolates	Estimated annual HAI		Tested isolates	Resistant isolates	Tested isolates	Resistant isolates
	n	%	n	95% CI	n	%	n	95% CI	n	%	n	%
Austria[b]	217	12.4	1,759	713–3,984	124	0.8	55	8–387	16	12.5	12	0.0
Belgium	495	18.6	8,458	4,422–14,621	318	1.3	261	104–654	45	15.6	34	0.0
Bulgaria[b]	53	56.6	8,687	3,189–23,328	30	10.0	2,014	479–8,291	NP	NA	NA	NA
Croatia[b]	280	41.4	3,823	2,491–5,808	114	5.3	300	80–1,053	6	NA	4	NA
Cyprus[a,b]	37	51.4	1,070	431–2,380	15	6.7	19	3–119	0	NA	NA	NA
Czech Republic[a]	627	30.8	16,348	9,726–25,665	393	0.8	87	30–261	NP[a]	NA	NA	NA
Denmark[a]	NP	NA	UNK	NA	NA	NA	UNK	NA	0	NA	0	NA
Estonia	107	13.1	462	138–1,398	58	0.0	0	NA	NP	NA	NA	NA
Finland	188	7.4	298	139–619	92	0.0	0	NA	44	6.8	36	0.0
France[a]	738	21.4	44,953	21,316–86,180	413	0.5	785	129–4,943	41	24.4	35	14.3
Germany	197	18.8	27,228	13,378–52,651	95	2.1	1,769	420–7,444	2	NA	1	NA
Greece[b]	456	61.2	10,605	7,809–14,193	197	43.7	4,157	2,467–6,831	2	NA	1	NA
Hungary	256	37.9	5,383	2,578–9,837	126	0.8	41	6–289	7	NA	6	NA
Iceland	15	0.0	0	NA	10	0.0	0	NA	NP	NA	NA	NA
Ireland	192	25.0	1,206	454–2,704	107	0.9	45	6–306	28	17.9	12	8.3
Italy	555	42.3	63,930	39,969–98,909	306	16.7	11,660	6,489–20,554	93	32.3	67	5.6
Latvia	47	59.6	804	309–2,043	19	5.3	38	4–356	NP	NA	NA	NA
Lithuania	108	32.4	1,509	680–3,224	35	0.0	0	NA	2	.	3	NA
Luxembourg[b]	67	14.9	79	26–228	38	2.6	4	0–46	3	.	2	NA

AMR: antimicrobial resistance; CI: confidence interval; EU/EEA: European Union/European Economic Area; HAI: healthcare-associated infection; NA: not applicable; ND: no data collected in national PPS; NP: did not participate; PPS: point prevalence survey; UNK: unknown; UK: United Kingdom.

[a]Antimicrobial resistance data were not reported by Norway and UK–Scotland in the PPS in acute care hospitals and by Denmark, Norway and UK–Scotland in the PPS in long-term care facilities. Cyprus did not submit case-based HAI data for long-term care facilities. The Czech Republic only collected institutional indicators for the PPS in long-term care facilities. For France, the percentage of non-susceptible (resistant+intermediate) isolates is given instead of the percentage resistant isolates.

[b]Country data representativeness was poor in Bulgaria and the Netherlands for the PPS in acute care hospitals and poor in Austria, Croatia, Cyprus, Greece, Luxembourg, Malta and Poland for the PPS in long-term care facilities.

[c]Cumulative 95% confidence intervals for the EU/EEA. Cumulative sums are rounded and may differ from the sum of the individual rounded country estimates.

Composite index of AMR: *Staphylococcus aureus* resistant to meticillin, *Enterococcus faecium* and *Enterococcus faecalis* resistant to vancomycin, *Enterobacteriaceae* resistant to third-generation cephalosporins, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* resistant to carbapenems. Enterobacteriaceae selected for the AMR markers: *Escherichia coli, Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp., *Serratia* spp. and *Morganella* spp. The percentage of resistance was not calculated if less than 10 isolates were reported.

Table 4b

Composite index of antimicrobial resistance in bacteria from healthcare-associated infections in acute care hospitals (n = 8,413) and long-term care facilities (n = 565), 30 EU/EEA countries, Serbia and the former Yugoslav Republic of Macedoniaa, 2016–2017

Country	Acute care hospitals[a]								Long-term care facilities[a]			
	Composite index of AMR				Carbapenem-resistant Enterobacteriaceae				Composite index of AMR		Carbapenem-resistant Enterobacteriaceae	
	Tested isolates	Resistant isolates	Estimated annual HAI		Tested isolates	Resistant isolates	Estimated annual HAI		Tested isolates	Resistant isolates	Tested isolates	Resistant isolates
	n	%	n	95% CI	n	%	n	95% CI	n	%	n	%
Malta[b]	33	24.2	195	69–544	25	4.0	23	0–2,216	15	60.0	7	NA
The Netherlands[b]	110	14.5	2,755	1,201–6,052	73	2.7	167	40–688	15	26.7	13	0.0
Norway[a]	ND	NA	UNK	NA	ND	NA	UNK	NA	ND	NA	ND	NA
Poland[b]	531	39.9	30,356	18,445–47,719	262	6.9	2,535	976–6,569	21	42.9	13	0.0
Portugal	829	38.4	9,177	5,431–14,287	462	6.9	1,062	347–2,643	65	41.5	47	10.6
Romania	164	68.9	13,913	7,377–25,458	80	33.8	3,475	1,726–6,923	NP	NA	NA	NA
Slovakia	164	34.8	3,061	1,543–5,848	101	2.0	247	60–1,022	8	NA	4	NA
Slovenia	194	17.0	969	397–2,087	117	1.0	3	1–17	NP	NA	NA	NA
Spain	926	26.6	25,722	15,842–38,973	512	4.1	2,632	1,136–5,609	134	31.3	82	0.0
Sweden	NP	NA	UNK	NA	NA	NA	UNK	NA	3	NA	1	NA
UK–England	370	20.5	7,634	3,950–13,560	205	1.5	316	101–986	NP	NA	NA	NA
UK–Northern Ireland	40	25.0	333	145–758	17	0.0	0	NA	2	NA	0	NA
UK–Scotland[a]	ND	NA	UNK	NA	ND	NA	UNK	NA	ND	NA	ND	NA
UK–Wales	35	37.1	351	67–1,213	8	NA	0	NA	1	NA	0	NA
EU/EEA[c]	8,031	31.6	291,067	162,417–504,270	4,352	6.2	31,696	14,611–78,205	553	28.0	380	4.2
Former Yugoslav Republic of Macedonia	NP	NA	UNK	NA	ND	NA	UNK	NA	2	NA	1	NA
Serbia	382	62.0	7,555	4,516–12,230	201	25.4	1,435	801–2,481	10	40.0	8	NA

AMR: antimicrobial resistance; CI: confidence interval; EU/EEA: European Union/European Economic Area; HAI: healthcare-associated infection; NA: not applicable; ND: no data collected in national PPS; NP: did not participate; PPS: point prevalence survey; UNK: unknown; UK: United Kingdom.

[a]Antimicrobial resistance data were not reported by Norway and UK–Scotland in the PPS in acute care hospitals and by Denmark, Norway and UK–Scotland in the PPS in long-term care facilities. Cyprus did not submit case-based HAI data for long-term care facilities. The Czech Republic only collected institutional indicators for the PPS in long-term care facilities. For France, the percentage of non-susceptible (resistant + intermediate) isolates is given instead of the percentage resistant isolates.

[b]Country data representativeness was poor in Bulgaria and the Netherlands for the PPS in acute care hospitals and poor in Austria, Croatia, Cyprus, Greece, Luxembourg, Malta and Poland for the PPS in long-term care facilities.

[c]Cumulative 95% confidence intervals for the EU/EEA. Cumulative sums are rounded and may differ from the sum of the individual rounded country estimates.

Composite index of AMR: *Staphylococcus aureus* resistant to meticillin, *Enterococcus faecium* and *Enterococcus faecalis* resistant to vancomycin, *Enterobacteriaceae* resistant to third-generation cephalosporins, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* resistant to carbapenems. Enterobacteriaceae selected for the AMR markers: *Escherichia coli, Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp., *Serratia* spp. and *Morganella* spp. The percentage of resistance was not calculated if less than 10 isolates were reported.

Table 5

Prevalence of healthcare-associated infections in long-term care facilities, 23 EU/EEA countriesa, Serbia and the former Yugoslav Republic of Macedonia, 2016–2017 (n = 103,763 residents)

Country	LTCF included in analysis	Residents included in analysis	Residents with at least one HAI in PPS sample (HAI prevalence)[b]			HAI from other facility[c]	HAI prevalence origin own LTCF[d]	LTCF beds in the country	Residents with at least one HAI on a given day, estimated	
	n	n	n	%	95%CI	%	n	n	n	(95% CI)
Austria[e]	12	2,065	105	5.1	2.8–8.9	6.5	4.6	72,602	3,504	1,966–6,145
Belgium	79	8,206	354	4.3	3.6–5.1	4.9	3.6	146,462	5,997	5,037–7,152
Croatia[e]	8	1,607	15	0.9	0.4–1.9	13.3	0.7	37,249	329	159–679
Cyprus[e]	11	312	15	4.8	2.7–7.8	ND	ND	3,436	157	89–255
Denmark	95	3,346	175	5.2	4.5–6.1	5.0	4.8	42,668	2,120	1,808–2,481
Finland	149	5,914	208	3.5	3.0–4.1	5.1	3.2	50,373	1,685	1,436–1,967
France[f]	91	6,957	206	3.0	2.6–3.4	ND	3.0	687,936	19,352	16,831–22,134
Germany	82	6,705	115	1.7	1.3–2.3	13.0	1.3	852,849	13,936	10,209–18,878
Greece[e]	13	812	51	6.3	3.7–10.5	3.8	5.9	10,849	647	381–1,079
Hungary	75	7,670	73	1.0	0.7–1.4	4.1	0.9	57,929	523	369–743
Ireland	109	5,613	276	4.9	4.2–5.8	6.0	4.5	30,531	1,427	1,207–1,682
Italy	196	11,417	442	3.9	3.3–4.6	13.6	3.1	186,872	6,870	5,787–8,149
Lithuania	26	3,438	32	0.9	0.4–1.9	15.6	0.6	11,722	104	50–212
Luxembourg[e]	16	1,616	30	1.9	1.1–3.0	0.0	1.8	6,966	123	75–199
Malta[e]	11	2,485	76	3.1	1.6–5.9	12.3	2.3	5,035	146	75–281
The Netherlands[f]	57	4,547	160	3.5	3.0–4.1	5.0	3.2	92,000	3,075	2,624–3,580
Norway[f]	62	2,447	119	4.9	4.0–5.8	2.5	4.6	39,583	1,829	1,521–2,178
Poland[e]	24	2,281	90	3.9	2.1–7.3	7.6	3.5	17,291	649	345–1,198
Portugal	132	3,633	214	5.9	4.5–7.6	15.9	4.3	8,400	470	362–608
Slovakia	59	5,091	108	2.1	1.5–3.0	4.5	2.0	27,497	554	392–778
Spain	46	6,808	579	8.5	7.0–10.3	18.9	6.2	372,306	30,064	24,688–36,501
Sweden	285	3,604	57	1.6	1.2–2.1	ND	1.6	93,000	1,396	1,051–1,864
UK–Northern Ireland	70	2,614	97	3.7	2.9–4.7	7.1	3.4	15,924	561	443–710
UK–Scotland	52	2,147	125	5.8	4.5–7.5	2.4	5.3	37,746	2,087	1,610–2,697
UK–Wales	28	966	58	6.0	4.4–8.2	0.0	6.0	24,646	1,405	1,026–1,915
Participating EU/EEA countries[b,g]	**1,788**	**102,301**	**3,780**	**3.6**	**2.9–4.5**	**8.9**	**3.1**	**2,931,872**	**99,008**	**79,539–124,064**
Former Yugoslav Republic of Macedonia	4	294	10	3.4	2.3–4.9	0.0	2.7	1,166	38	26–55
Serbia	6	1,168	37	3.2	1.9–5.1	7.3	2.8	19,654	592	362–960
EU/EEA, corrected[g,h]	NA	NA	NA	3.6	2.9–4.5	NA	NA	3,486,999	117,754	94,599–147,553
EU/EEA, corrected after validation	**NA**	**NA**	**NA**	**3.9**	**2.4–6.0**	**NA**	**NA**	**3,486,999**	**129,940**	**79,570–197,625**

EU/EEA: European Union/European Economic Area; HAI: healthcare-associated infection; LTCF: long-term care facility; PPS: point prevalence survey; ND: no data collected in national protocol; UK: United Kingdom.

[a] The Czech Republic only submitted data on institutional indicators from 11 LTCF and was not included in the current analysis.

[b] Country-weighted HAI prevalence for the EU/EEA= estimated number of residents with at least one HAI on a single day / occupied beds; occupied beds = number of LTCF beds × average occupancy of 0.95.

[c] Percentage of HAI imported from a hospital or another LTCF; not included in France and Sweden, and unknown for Cyprus (aggregated data).

[d] HAI prevalence for HAI with the own LTCF as origin, i.e. excluding HAI imported from other healthcare facilities and HAI with unknown origin (Supplement).

[e] Country data representativeness was poor in Austria, Croatia, Cyprus, Greece, Luxembourg, Malta and Poland.

[f] France, the Netherlands and Norway used a national protocol which required imputation of non-included types of HAI.

[g] Cumulative 95% confidence intervals for the EU/EEA.

[h] Corrected for non-participating EU/EAA countries with estimation for Bulgaria, Czech Republic, Estonia, Iceland, Latvia, Romania, Slovenia and UK–England combined.

In LTCF, only the number of HAI could be estimated. As LTCF usually are permanent residences, HAI do not prolong the length of stay of a resident as they do in ACH. Therefore, the incidence of HAI in LTCF per year was estimated by multiplying the prevalence by 365 days and dividing it by the duration of infection (in days), with a correction for an average occupancy of LTCF beds of 95%, calculated from institutional denominator data. The duration of infection was estimated, by type of HAI, from the date of onset to the date of the PPS, using the median duration of HAI until the day of the PPS multiplied by 2.

Validation studies

It was strongly recommended that all participating EU/EEA countries perform validation studies of their national PPSs. For the PPS in ACH, ECDC also offered financial support to national institutions coordinating PPS so that they could organise validation studies with a minimum requirement to re-examine 250 patient charts in five ACH. For both the PPS in ACH and that in LTCF, the objective was to estimate representative validity parameters at the EU/EEA level rather than at country level ([16]; ACH validation protocol available from the authors on request). Validation studies were performed by national validation teams composed of members of the national coordination teams, using the ECDC HAI case definitions as gold standard. Validation results were calculated for each country, by matching patients included in the validation sample with their corresponding data collected in the primary PPS. The percentage of false positives (FP) and false negatives (FN) was calculated from the matched analysis and applied to the total national database to calculate the sensitivity and specificity for each country, as several countries selected high prevalence wards for validation to improve precision as recommended by the validation study protocol. For correction of the EU/EEA prevalence of HAI, the EU/EEA mean FN and FP were applied to the total number of patients. The validation-corrected HAI prevalence was converted using the Rhame and Sudderth formula to estimate the corrected HAI incidence and total number of patients in ACH with at least one HAI per year in the period 2016 to 2017.

To calculate CI around EU/EEA estimates, the number of patients with at least one HAI obtained from the lower and upper limits of the country-specific 95% CIs were summed up and divided by the total number of occupied beds (for prevalence) or the total number of discharges (for estimated incidence) in the EU/EEA. These 'cumulative 95% CI' (95% cCI) therefore reflect a larger, more conservative uncertainty than would be obtained by calculating 95% CI on the EU/EEA totals, which is in accordance with the limitations of the prevalence measurement and the uncertainty inherent to the conversion of prevalence to incidence.

Results

Point prevalence survey in acute care hospitals

Participation

In total, 1,735 hospitals from 28 EU/EEA countries and one EU candidate country (Serbia) participated in the second PPS of HAI and antimicrobial use in European ACH in the period 2016 to 2017. Counting UK administrations separately, the country representativeness of the sample was optimal in 20 countries, good in 10, and poor in two countries. After adjustment for over-representation of countries contributing more than 20,000 patients to the PPS, 325,737 patients from 1,275 ACH remained in the final sample. Aggregated results were only reported for the EU/EEA, corresponding to 310,755 patients from 1,209 ACH. The distribution of the type of ACH and the percentage of patients requiring intensive care by country is shown in Table 1.

Prevalence and estimated incidence of healthcare-associated infections

A total of 19,626 HAI were reported in 18,287 patients with HAI (1.07 HAI per infected patient). The prevalence of patients with at least one HAI in the EU/EEA sample was 5.9% (country range: 2.9–10.0%; Table 2). The prevalence varied between 4.4% (2,177/49,381 patients) in primary care hospitals (n = 333) to 7.1% (7,591/104,562 patients) in tertiary care hospitals (n = 222) and was highest in patients admitted to intensive care units, where 19.2% (2,751/14,258) patients had at least one HAI compared with 5.2% (15,536/296,397) on average for all other specialties combined (Supplement).

When extrapolated to the average daily number of occupied beds per country, the weighted HAI prevalence was 5.5% (95% cCI: 4.5–6.6%). The weighted annual incidence of patients acquiring at least one HAI per year in the period 2016 to 2017, estimated using prevalence to incidence conversion, was 3.7 (95% cCI: 2.4–5.3) patients per 100 admissions. National PPS validation studies were carried out by 28 countries (UK administrations counted separately) in a total of 236 ACH in the EU/EEA. National validation teams re-examined 12,228 patient charts independently from the primary PPS surveyors. These studies showed that on average, 2.3% (country range: 0.3–5.6%) of patients who were reported as not having a HAI actually had an HAI (false negatives) while one in five (mean: 20.3%, country range: 0–46.2%) patients reported as having an HAI did not have an HAI (false positives), resulting in a mean sensitivity of HAI detection of 69.4% (country range: 40.1–94.4%) and a mean specificity of 98.8% (country range: 96.1–100%). When correcting for these results, the adjusted prevalence of patients with at least one HAI was estimated at 6.5% (95% cCI: 5.4–7.8%). Using the Rhame and Sudderth formula to convert the latter percentage, the corrected annual incidence was estimated at 4.1 (95% cCI: 3.4–4.9) patients per 100 admissions. Applying the EU/EEA averages to denominator data from non-participating EU/EEA

countries (Denmark and Sweden), this resulted in an estimated total of 98,166 (95% cCI: 81,022–117,484) patients with at least one HAI on any given day and 3,758,014 (95% cCI: 3,122,024–4,509,617) patients with at least one HAI per year in the period 2016 to 2017 in ACH in the EU/EEA.

Types of HAI and isolated microorganisms

The most frequently reported types of HAI were respiratory tract infections (21.4% pneumonia and 4.3% other lower respiratory tract infections), urinary tract infections (18.9%), surgical site infections (18.4%), bloodstream infections (10.8%) and gastro-intestinal infections (8.9%), with *C. difficile* infections accounting for 44.6% of the latter or 4.9% of all HAI. Twenty-three per cent of HAI were present on admission. One third of HAI on admission were surgical site infections. Country-weighted prevalence percentages and estimated numbers of HAI per year are shown in Table 3. After correction for non-participating countries and validation, a total of 4.5 million (95% cCI: 2.6–7.6 million) HAI were estimated to occur per year in the period 2016 to 2017 in ACH in the EU/EEA.

A total of 13,085 microorganisms were reported in 10,340 (52.7%) HAI. The 10 most frequently isolated microorganisms were *E. coli* (16.1%), *S. aureus* (11.6%), *Klebsiella* spp. (10.4%), *Enterococcus* spp. (9.7%), *P. aeruginosa* (8.0%), *C. difficile* (7.3%), coagulase-negative staphylococci (7.1%), *Candida* spp. (5.2%), *Enterobacter* spp. (4.4%) and *Proteus* spp. (3.8%).

Antimicrobial resistance in healthcare-associated infections and correlation with EARS-Net data

AST data were available for 8,031 (88.9%) of 9,034 microorganisms included in the composite index of AMR. The index was 31.6% overall (mean of countries: 30.8%) and varied from 0% in Iceland to 68.9% in Romania. The index by country was strongly correlated with the index calculated from 2016 EARS-Net data on invasive isolates (Spearman's correlation coefficient *rho*: 0.93; $p<0.001$; R^2: 0.86. Figure) and was on average 36% higher for HAI in ACH from the PPS than in the EARS-Net data (mean of countries in EARS-Net: 20.3%). Carbapenem resistance in Enterobacteriaceae was 6.2% overall (mean of countries: 5.9%) and ranged from 0% in Estonia, Finland, Iceland, Lithuania and UK–Northern Ireland to 43.7% in Greece (Table 4). This indicator also correlated well with carbapenem resistance in *E. coli*and *K. pneumoniae* in EARS-Net data (Spearman's *rho*: 0.76; $p<0.001$) and was on average 45% higher in HAI in ACH from the PPS than in EARS-Net data (mean of countries in EARS-Net: 2.6%). The total number of patients acquiring an HAI with at least one resistant microorganism was estimated at 291,067 (95% cCI: 162,417–504,270) patients for the composite index of AMR and 31,696 (95% cCI: 14,611–78,205) patients for carbapenem-resistant Enterobacteriaceae.

Point prevalence survey in long-term care facilities

Participation

In total, 3,062 LTCF from 24 EU/EEA countries and two EU candidate countries (Serbia and the former Yugoslav Republic of Macedonia) participated in the third PPS of HAI and antimicrobial use in European LTCF in the period 2016 to 2017. Counting UK administrations separately, good or optimal representativeness of the national sample was obtained in 18 of 24 EU/EEA countries. After adjustment for over-representation, 117,138 residents from 2,221 LTCF were included for analysis. The main aggregated results were reported for 80.5% of participating LTCF, i.e. general nursing homes (n = 1,025), residential homes (n = 176) and mixed LTCF (n = 587), corresponding to 102,301 residents and 1,788 LTCF in EU/EEA countries. The characteristics of LTCF and residents by country are shown in Table 1.

Prevalence of healthcare-associated infections

A total of 3,858 HAI were reported in 3,780 residents with HAI (1.02 HAI per infected resident). The prevalence of residents with at least one HAI was 3.7% (country range: 0.9–8.5%). When extrapolated to the average number of occupied LTCF beds per country, the weighted HAI prevalence in LTCF was 3.6% (95% cCI: 2.9–4.5%). Validation of the PPS in LTCF was performed for 953 residents in 17 LTCF in 10 countries. National validation teams found 1.1% (95% CI: 0.5–2.0%) false-negative residents and 19.6% (95% CI: 9.4–33.9%) false-positive residents, yielding a sensitivity of 73.7% and a specificity of 99.2% when applied on the total EU/EEA database. The country-weighted, validation-corrected HAI prevalence was 3.9% (95% cCI: 2.4–6.0%). Applying the EU/EEA prevalence to denominator data from non-participating EU/EEA countries, the total number of residents with at least one HAI on any given day in EU/EEA LTCF was estimated at 129,940 (95% cCI: 79,570–197,625) residents (Table 5).

Types of healthcare-associated infections and isolated microorganisms

The most frequently reported types of HAI in LTCF were respiratory tract infections (33.2% overall, 3.7% pneumonia, 22.0% other lower respiratory tract infections, 7.2% common cold/pharyngitis, 0.3% influenza), urinary tract infections (32.0%) and skin infections (21.5%). The majority of the reported HAI (84.7%) were associated with the LTCF where the PPS was performed, while 7.5% and 1.4% were associated with a hospital or another LTCF, respectively. The origin was unknown for 6.4% of HAI in LTCF. Country-weighted prevalence percentages and estimated number of infections per year are given by type of HAI in Table 3. The total number of HAI in LTCF in the EU/EEA, after applying EU averages for non-participating EU/EEA countries and correcting for validation, was estimated at 4.4 million (95% cCI: 2.0–8.0 million). Microbiological data in LTCF were available for 742 (19.2%) HAI. The 10 most frequently isolated bacteria were *E. coli* (30.7%), *S.*

aureus (12.3%), *Klebsiella* spp. (11.4%), *Proteus* spp. (10.6%), *P. aeruginosa* (7.1%), *Enterococcus* spp. (4.8%), *C. difficile* (4.4%), *Streptococcus* spp. (2.8%) *Enterobacter* spp. (2.1%) and coagulase-negative staphylococci (1.9%).

Antimicrobial resistance in healthcare-associated infections and correlation with data from the hospital point prevalence survey

AST results were available for 553 (77.6%) of 713 microorganisms included in the composite index of AMR. The index could be calculated for 11 countries with at least 10 isolates, and was 28.0% overall, ranging from 6.8% in Finland to 60.0% in Malta (Table 4). The composite index of AMR correlated well between ACH and LTCF, although Malta was an outlier (Figure, Spearman's *rho* excluding Malta: 0.86; $p<0.001$; $R^2 = 0.69$). On average, the percentage of resistant microorganisms was similar in both settings (regression coefficient excluding Malta: 1.08). Carbapenem resistance in Enterobacteriaceae in LTCF was 4.2% overall and did not correlate significantly with the percentage in ACH (Table 4).

Discussion

Because both the PPS in ACH and that in LTCF were performed during 2016 and 2017, this provided the first opportunity to estimate the prevalence, incidence and annual number of HAI for ACH and for LTCF in the EU/EEA for the same time period. As expected, the overall prevalence of HAI was higher in ACH than in LTCF, also after correction based on validation study results. However, when estimating the total number of HAI, both settings were shown to have similarly high numbers of HAI annually. In total, 8.9 million distinct HAI episodes were estimated to occur annually in ACH and LTCF in the EU/EEA. In ACH, where the incidence per patient could be calculated, the number of patients with at least one HAI was estimated at 3.8 (95% cCI: 3.1–4.6) million patients per year in the period 2016 to 2017.

The country-weighted HAI prevalence before validation correction in ACH of 5.5% (95% cCI: 4.5–6.7%) was similar to the HAI prevalence of 5.7% (95% cCI: 4.5–7.4%) in the ECDC PPS in ACH in the period 2011 to 2012 [3]. The unweighted HAI prevalence in LTCF of 3.7% before correction was only slightly higher than the prevalence of 3.4% found in the ECDC PPS in LTCF in 2013 [6], although imported HAI were included in the period 2016 to 2017. The final corrected country-weighted HAI prevalence estimates of 6.5% in ACH and 3.9% in LTCF were higher because they were corrected for the results of the validation studies, which made the current estimates more robust than the previous estimates. Similarly, the estimated incidence and number of HAI in ACH presented in this study were higher than the number estimated in the ECDC PPS from 2011 to 2012 [3] because of the correction for the results of the validation study and should therefore not be interpreted as an increase for ACH compared with the period 2011 to 2012.

The strong correlation of the composite index of AMR in the ECDC PPS in ACH with the EARS-Net data supports the validity of AMR data collected in the PPSs. The 36% higher percentage of resistant isolates in HAI in the ECDC PPS was expected given that EARS-Net only includes data from invasive isolates, i.e. from bloodstream infections and meningitides, and that a large proportion of isolates reported to EARS-Net are from community-associated bloodstream infections, especially for MRSA and *E. coli* resistant to third-generation cephalosporins. However, the fact that the composite index of AMR in LTCF was at the same level as in ACH, at least in countries where both indicators could be calculated, is of concern. Even though the low testing frequency in LTCF is probably biased towards HAI which are non-responsive to empiric treatment, this finding emphasises the urgent need to reinforce measures to improve infection prevention and control, antimicrobial stewardship as well as microbiological laboratory support for LTCF.

Our study has several limitations. Firstly, the small number of countries and LTCF that performed validation studies in the PPS in LTCF resulted in less robust prevalence estimates for LTCF than for ACH, even though the LTCF validation results could be used at the EU/EEA level. Secondly, the conversion from prevalence to incidence using the Rhame and Sudderth formula has been shown to have several limitations in itself, especially for smaller samples [17,18]. The estimates depend on the estimators used, as not all data can be acquired from a cross-sectional prevalence study. Nevertheless, sensitivity analyses that we performed with more recent estimator methodology (personal communication: Niklas Willrich, 24 May 2018) [15] yielded EU/EEA estimates which were close to those reported here, with few exceptions at individual country level. Especially considering the wide CI, this gave more weight to our estimates (Supplement). Thirdly, the estimates also strongly depended on the quality of the national denominator data of the number of beds, and, for ACH, discharges and patient days. Providing reliable national denominator data has been shown to be difficult for many countries that sometimes provided estimates rather than precise numbers, especially for LTCF. In addition, as national denominator data for specialised LTCF were only available in two countries, a specific incidence for these types of LTCF could not be estimated. In several countries, however, the number of beds for these LTCF are included in the total number of LTCF beds for the country. We only reported results for the main types of LTCF, as these types were consistently included in all countries. Fourthly, the number of residents with at least one HAI each year could not be estimated for LTCF in the EU/EEA. Longitudinal HAI incidence data would be required to produce such estimates. Fifthly, three countries preferred using their national PPS protocols for LTCF and one country for

ACH, resulting in less robust estimates. Sixthly, the total number of HAI with resistant pathogens could only be estimated for ACH because of the poor availability of microbiological results in LTCF Moreover, the annual incidence estimates of HAI with resistant pathogens in ACH are underestimated because: (i) in almost half of the HAI in ACH, a microorganism was not reported, (ii) for 11% of the reported microorganisms, AST results were not yet available on the day of the PPS and (iii) correction for countries without data and correction for validation was not performed. Despite these limitations, the estimated number of HAI with carbapenem-resistant Enterobacteriaceae using Rhame and Sudderth conversion in our study (31,696 infections, of which 27,393 were HAI with carbapenem-resistant *E. coli* or *K. pneumoniae*) was close to the number of 33,172 infections with carbapenem-resistant *E. coli* or *K. pneumoniae* recently estimated by Cassini et al. using a different methodology [19].

The main strengths of this study are its large sample size and the use of standardised protocols for data collection and validation across participating ACH and LTCF. Despite some countries providing less representative samples, these PPSs as a whole offer a representative picture of HAI in the EU/EEA, with benchmarks to help direct future action in ACH and LTCF in participating countries.

Conclusion

This study reports, to our knowledge, the most accurate and robust estimates of the total number of HAI in healthcare facilities in the EU/EEA to date, and confirms that HAI, and AMR in bacteria responsible for HAI, represent a significant healthcare issue and public health challenge for the EU/EEA. Considering that previous studies have shown that HAI in ACH alone are responsible for more deaths in the EU/EEA than all other infectious diseases under surveillance at European level [1,2], and that our study showed that there are as many HAI in LTCF as there are in ACH, more focus needs to be dedicated to the prevention of HAI and AMR, through the application of available recommendations and guidelines [20-25], in both ACH and LTCF.

*Erratum

The list of members of the Healthcare-Associated Infections Prevalence Study Group was left out in the original publication and was added on 16 November 2018.

Members of the Healthcare-Associated Infections Prevalence Study Group*

Elisabeth Presterl (Medical University Vienna, Vienna, Austria); Reinhild Strauss (Federal Ministry for Labour, Social Security, Health and Consumer Protection (BMAGSK) Vienna); Eline Vandael (Sciensano, Brussels, Belgium); Boudewijn Catry (Sciensano, Brussels, Belgium; Elina Dobreva (National Center of Infectious and Parasitic Diseases (NCIPD), Sofia, Bulgaria); Nadezhda Vladimirova (National Center of Infectious and Parasitic Diseases (NCIPD), Sofia, Bulgaria); Zrinka Bošnjak (University of Zagreb School of Medicine, University Hospital Centre Zagreb, Zagreb, Croatia); Ana Budimir (University of Zagreb School of Medicine, University Hospital Centre Zagreb, Zagreb, Croatia); Elena Gabriel (Ministry of Health, Nicosia, Cyprus); Linos Hadjihannas (Ministry of Health, Nicosia, Cyprus); Vlastimil Jindrák (National Institute of Public Health, Prague, Czech Republic); Dana Hedlová (National Institute of Public Health, Prague, Czech Republic); Christian Stab Jensen (Statens Serum Institut (SSI), Copenhagen, Denmark); Pille Märtin (West Tallinn Central Hospital, Health Board), Tallinn, Estonia); Piret Mitt (Tartu University Hospital, Tartu, Estonia); Emmi Sarvikivi (National Institute for Health and Welfare, Helsinki, Finland); Dinah Arifulla (National Institute for Health and Welfare, Helsinki, Finland); Saija Toura (National Institute for Health and Welfare, Helsinki, Finland); Anne Berger-Carbonne (National Public Health Agency (Santé publique France), Saint Maurice, France); Anne Savey (CPias Auvergne-Rhône-Alpes, University Hospital (HCL), Lyon, France); Côme Daniau (National Public Health Agency (Santé publique France), Saint Maurice, France); Claudia Ruscher (Robert Koch Institute, Berlin, Germany); Petra Gastmeier (Institute of Hygiene and Environmental Medicine, Charité – University Medicine Berlin, Berlin, Germany); Seven Aghdassi (Institute of Hygiene and Environmental Medicine, Charité – University Medicine Berlin, Berlin, Germany); Nicole Schmidt (Robert Koch-Institute, Berlin, Germany); Achilleas Gikas (Department of Internal Medicine, University Hospital of Heraklion, Heraklion, Crete, Greece); Meropi Gkika (Faculty of Medicine, University of Crete, Heraklion, Greece); Symeon H. Panagiotakis (University Hospital of Heraklion, Heraklion, Crete, Greece); Andrea Kurcz (Ministry of Human Capacities, Budapest, Hungary); Ágnes Hajdu (Ministry of Human Capacities, Budapest, Hungary); István Veress (Ministry of Human Capacities, Budapest, Hungary); Ólafur Guðlaugsson (Landspitali University Hospital, Reykjavik, Iceland); Karen Burns (Health Protection Surveillance Centre, Beaumont Hospital & Royal College of Surgeons in Ireland, Dublin, Ireland); Helen Murphy (Health Protection Surveillance Centre, Dublin, Ireland); Carla M. Zotti (Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy); Francesca Quattrocolo (Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy); Angelo D'Ambrosio (Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy); Elina Dimiņa (Centre for Disease Prevention and Control, Riga, Latvia); Aija Vilde (Pauls Stradins Clinical University Hospital, Riga, Latvia); Ieva Kisielienė (Institute of Hygiene, Vilnius, Lithuania); Rolanda Valintėlienė (Institute of Hygiene, Vilnius, Lithuania); Martine Debacker (Ministère de la santé - Direction de la santé, Luxembourg, Luxembourg); Murielle Weydert (Ministère de la Famille, de l'Intégration et à la Grande Région, Luxembourg, Luxembourg); Branka Petrovska Basovska (National

Institute of Public Health, Skopje, former Yugoslav Republic of Macedonia); Dragan Kochinski (National Institute of Public Health, Skopje, former Yugoslav Republic of Macedonia); Michael A Borg (Mater Dei Hospital & University of Malta, Msida, Malta); Elizabeth Scicluna (Mater Dei Hospital, Msida, Malta); Mark Bonanno (St Vincent De Paul Long Term Care Facility, Luqa, Malta); Titia EM Hopmans (National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands); Mayke BG Koek (National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands); Linda Verhoef (National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands); Kati Halonen (National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands); Hanne-M. Eriksen (Norwegian Institute of Public Health, Oslo, Norway); Horst Bentele (Norwegian Institute of Public Health, Oslo, Norway); Nina Sorknes (Norwegian Institute of Public Health, Oslo, Norway); Anna Różańska (Chair of Microbiology, Faculty of Medicine Jagiellonian University Medical School, Krakow, Poland); Jadwiga Wojkowska-Mach (Chair of Microbiology, Faculty of Medicine Jagiellonian University Medical School, Krakow, Poland); Isabel Neves, (Direção-Geral da Saúde (DGS), Lisbon, Portugal); Margarida Valente, (Direção-Geral da Saúde (DGS), Lisbon, Portugal); Pedro Pacheco (Direção-Geral da Saúde (DGS), Lisbon, Portugal); Roxana Ioana Serban (National Institute of Public Health (NIPH), Bucharest, Romania); Andreea Sorina Niculcea (National Institute of Public Health (NIPH), Bucharest, Romania); Ljiljana Markovic-Denic (University of Belgrade, Faculty of Medicine, Belgrade, Serbia); Ivana Cirkovic (University of Belgrade, Faculty of Medicine, Belgrade, Serbia); Mária Štefkovičová (Alexander Dubcek University in Trenčín and Regional Public Health Authority in Trenčín, Slovakia); Slavka Litvová (Regional Public Health Authority in Trenčín, Slovakia); Irena Klavs (National Institute of Public Health, Ljubljana, Slovenia); Mojca Serdt (National Institute of Public Health, Ljubljana, Slovenia); Angel Asensio (Hospital Universitario Puerta de Hierro-Majadahonda, Madrid, Spain); Mireia Cantero (Hospital Universitario Puerta de Hierro-Majadahonda, Madrid, Spain); Pilar Gallego Berciano (National Centre for Epidemiology, Instituto de Salud Carlos III, Madrid, Spain. Biomedical Research Centre Network for Epidemiology and Public Health (CIBERESP)); Jenny Hellman (The Public Health Agency of Sweden, Solna, Sweden); Tomas Söderblom (The Public Health Agency of Sweden, Solna Sweden); Alan Johnson (Public Health England, London, UK); Muhammad Sartaj (HSC Public health Agency, Belfast, Northern Ireland, UK); Mark McConaghy (Public Health Agency, Belfast, Northern Ireland, UK); Shona Cairns (Health Protection Scotland National Services Scotland, Glasgow, UK); Wendy Harrison (Public Health Wales, Cardiff, Wales, UK); Liselotte Diaz Högberg (European Centre for Disease Prevention and Control); Alessandro Cassini (European Centre for Disease Prevention and Control); Olivia Aya Nakitanda (European Centre for Disease Prevention and Control); Ole Heuer (European Centre for Disease Prevention and Control)

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Conflict of interest

None declared.

Authors' contributions

Carl Suetens performed the analysis and wrote the original draft; Katrien Latour, Tommi Kärki, Enrico Ricchizi and Pete Kinross performed analyses, contributed to the development of the study design and the coordination of the execution of the study; Katrien Latour, Enrico Ricchizi, Béatrice Jans and Maria Luisa Moro were the contractor team that supported ECDC for the coordination of the third PPS in long-term care facilities (ECDC-funded HALT-3 project). Sonja Hansen, Susan Hopkins, Outi Lyytikäinen, Jacqui Reilly, Alexander Deptula and Walter Zingg were members of the HAI-Net PPS expert group that developed the methodology of the survey in acute care hospitals; Pete Kinross contributed to the coordination of the execution of the study; Diamantis Plachouras and Dominique L Monnet contributed to the analysis plan and the methodology of the survey; the members of the Healthcare-Associated Infections study group members contributed to the development of the study design, approved the design of the survey, contributed to the coordination of the execution of the study in their respective countries, and provided national interpretations on the analysis. All authors critically reviewed and edited the manuscript.

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Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR

Victor M Corman[1], Olfert Landt[2], Marco Kaiser[3], Richard Molenkamp[4], Adam Meijer[5], Daniel KW Chu[6], Tobias Bleicker[1], Sebastian Brünink[1], Julia Schneider[1], Marie Luisa Schmidt[1], Daphne GJC Mulders[4], Bart L Haagmans[4], Bas van der Veer[5], Sharon van den Brink[5], Lisa Wijsman[5], Gabriel Goderski[5], Jean-Louis Romette[7], Joanna Ellis[8], Maria Zambon[8], Malik Peiris[6], Herman Goossens[9], Chantal Reusken[5], Marion PG Koopmans[4], Christian Drosten[1]

1. Charité – Universitätsmedizin Berlin Institute of Virology, Berlin, Germany and German Centre for Infection Research (DZIF), Berlin, Germany
2. Tib-Molbiol, Berlin, Germany
3. GenExpress GmbH, Berlin, Germany*
4. Department of Viroscience, Erasmus MC, Rotterdam, the Netherlands
5. National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands
6. University of Hong Kong, Hong Kong, China
7. Universite d Aix-Marseille, Marseille, France
8. Public Health England, London, United Kingdom
9. Department of Medical Microbiology, Vaccine and Infectious Diseases Institute, University of Antwerp, Antwerp, Belgium

Correspondence: Christian Drosten (christian.drosten@charite.de)

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Background: The ongoing outbreak of the recently emerged novel coronavirus (2019-nCoV) poses a challenge for public health laboratories as virus isolates are unavailable while there is growing evidence that the outbreak is more widespread than initially thought, and international spread through travellers does already occur. **Aim:** We aimed to develop and deploy robust diagnostic methodology for use in public health laboratory settings without having virus material available. **Methods:** Here we present a validated diagnostic workflow for 2019-nCoV, its design relying on close genetic relatedness of 2019-nCoV with SARS coronavirus, making use of synthetic nucleic acid technology. **Results:** The workflow reliably detects 2019-nCoV, and further discriminates 2019-nCoV from SARS-CoV. Through coordination between academic and public laboratories, we confirmed assay exclusivity based on 297 original clinical specimens containing a full spectrum of human respiratory viruses. Control material is made available through European Virus Archive – Global (EVAg), a European Union infrastructure project. **Conclusion:** The present study demonstrates the enormous response capacity achieved through coordination of academic and public laboratories in national and European research networks.

Introduction

According to the World Health Organization (WHO), the WHO China Country Office was informed of cases of pneumonia of unknown aetiology in Wuhan City, Hubei Province, on 31 December 2019 [1]. A novel coronavirus currently termed 2019-nCoV was officially announced as the causative agent by Chinese authorities on 7 January. A viral genome sequence was released for immediate public health support via the community online resource virological.org on 10 January (Wuhan-Hu-1, GenBank accession number MN908947 [2]), followed by four other genomes deposited on 12 January in the viral sequence database curated by the Global Initiative on Sharing All Influenza Data (GISAID). The genome sequences suggest presence of a virus closely related to the members of a viral species termed severe acute respiratory syndrome (SARS)-related CoV, a species defined by the agent of the 2002/03 outbreak of SARS in humans [3,4]. The species also comprises a large number of viruses mostly detected in rhinolophid bats in Asia and Europe.

As at 20 January 2020*, 282 laboratory-confirmed human cases have been notified to WHO [5]. Confirmed cases in travellers from Wuhan were announced on 13 and 17 January in Thailand as well as on 15 January in Japan and 19 January in Korea. The extent of human-to-human transmission of 2019-nCoV is unclear at the time of writing of this report but there is evidence of some human-to-human transmission.

Among the foremost priorities to facilitate public health interventions is reliable laboratory diagnosis. In acute respiratory infection, RT-PCR is routinely used to detect causative viruses from respiratory secretions. We have previously demonstrated the feasibility of introducing robust detection technology based on real-time RT-PCR in public health laboratories during international

Table 1***

Primers and probes, real-time RT-PCR for 2019 novel coronavirus

Assay/use	Oligonucleotide	Sequence[a]	Concentration[b]
RdRP gene	RdRp_SARSr-F	GTGARATGGTCATGTGTGGCGG	Use 600 nM per reaction
	RdRp_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ	Specific for 2019-nCoV, will not detect SARS-CoV. Use 100 nM per reaction and mix with P1
	RdRP_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ	Pan Sarbeco-Probe will detect 2019-nCoV, SARS-CoV and bat-SARS-related CoVs. Use 100 nM per reaction and mix with P2
	RdRp_SARSr-R	CARATGTTAAASACACTATTAGCATA	Use 800 nM per reaction
E gene	E_Sarbeco_F	ACAGGTACGTTAATAGTTAATAGCGT	Use 400 nM per reaction
	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ	Use 200 nM per reaction
	E_Sarbeco_R	ATATTGCAGCAGTACGCACACA	Use 400 nM per reaction
N gene	N_Sarbeco_F	CACATTGGCACCCGCAATC	Use 600 nM per reaction
	N_Sarbeco_P	FAM-ACTTCCTCAAGGAACAACATTGCCA-BBQ	Use 200 nM per reaction
	N_Sarbeco_R	GAGGAACGAGAAGAGGCTTG	Use 800 nM per reaction

[a] W is A/T; R is G/A; M is A/C; S is G/C. FAM: 6-carboxyfluorescein; BBQ: blackberry quencher.

[b] Optimised concentrations are given in nanomol per litre (nM) based on the final reaction mix, e.g. 1.5 µL of a 10 µM primer stock solution per 25 µL total reaction volume yields a final concentration of 600 nM as indicated in the table.

health emergencies by coordination between public and academic laboratories [6-12]. In all of these situations, virus isolates were available as the primary substrate for establishing and controlling assays and assay performance.

In the present case of 2019-nCoV, virus isolates or samples from infected patients have so far not become available to the international public health community. We report here on the establishment and validation of a diagnostic workflow for 2019-nCoV screening and specific confirmation, designed in absence of available virus isolates or original patient specimens. Design and validation were enabled by the close genetic relatedness to the 2003 SARS-CoV, and aided by the use of synthetic nucleic acid technology.

Methods

Clinical samples and coronavirus cell culture supernatants for initial assay evaluation

Cell culture supernatants containing typed coronaviruses and other respiratory viruses were provided by Charité and University of Hong Kong research laboratories. Respiratory samples were obtained during 2019 from patients hospitalised at Charité medical centre and tested by the NxTAG respiratory pathogen panel (Luminex, S´Hertogenbosch, The Netherlands) or in cases of MERS-CoV by the MERS-CoV upE assay as published before [10]. Additional samples were selected from biobanks at the Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Bilthoven, at Erasmus University Medical Center, Rotterdam, at Public Health England (PHE), London, and at the University of Hong Kong. Samples from all collections comprised sputum as well as nose and throat swabs with or without viral transport medium.

Faecal samples containing bat-derived SARS-related CoV samples (identified by GenBank accession numbers) were tested: KC633203, Betacoronavirus BtCoV/Rhi_eur/BB98–98/BGR/2008; KC633204, Betacoronavirus BtCoV/Rhi_eur/BB98–92/BGR/2008; KC633201, Betacoronavirus BtCoV/Rhi_bla/BB98–22/BGR/2008; GU190221 Betacoronavirus Bat coronavirus BR98–19/BGR/2008; GU190222 Betacoronavirus Bat coronavirus BM98–01/BGR/2008; GU190223, Betacoronavirus Bat coronavirus BM98–13/BGR/2008. All synthetic RNA used in this study was photometrically quantified.

RNA extraction

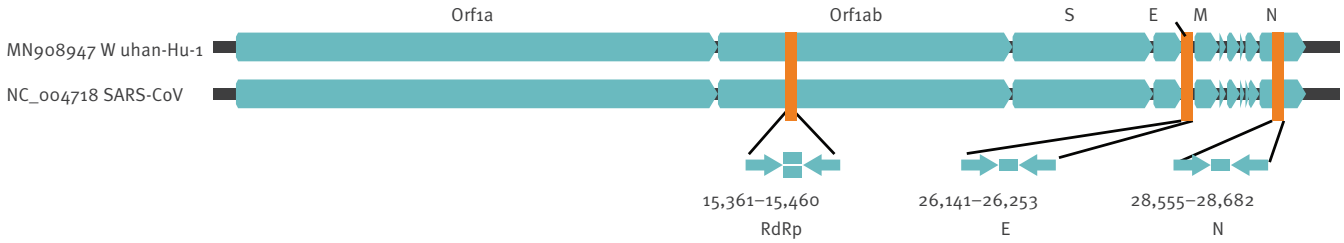
RNA was extracted from clinical samples with the MagNA Pure 96 system (Roche, Penzberg, Germany) and from cell culture supernatants with the viral RNA mini kit (QIAGEN, Hilden, Germany).

Real-time reverse-transcription PCR

A 25 µL reaction contained 5 µL of RNA, 12.5 µL of 2 × reaction buffer provided with the Superscript III one step RT-PCR system with Platinum Taq Polymerase (Invitrogen, Darmstadt, Germany; containing 0.4 mM of each deoxyribont triphosphates (dNTP) and 3.2 mM magnesium sulphate), 1 µL of reverse transcriptase/Taq mixture from the kit, 0.4 µL of a 50 mM magnesium sulphate solution (Invitrogen), and 1 µg of nonacetylated bovine serum albumin (Roche). Primer and probe sequences, as well as optimised concentrations are shown in Table 1. All oligonucleotides were synthesised and provided by Tib-Molbiol (Berlin,

Figure 1

Relative positions of amplicon targets on the SARS coronavirus and the 2019 novel coronavirus genome



E: envelope protein gene; M: membrane protein gene; N: nucleocapsid protein gene; ORF: open reading frame; RdRp: RNA-dependent RNA polymerase gene; S: spike protein gene.

Numbers below amplicons are genome positions according to SARS-CoV, GenBank NC_004718.

Germany). Thermal cycling was performed at 55 °C for 10 min for reverse transcription, followed by 95 °C for 3 min and then 45 cycles of 95 °C for 15 s, 58 °C for 30 s. Participating laboratories used either Roche Light Cycler 480II or Applied Biosystems ViiA7 instruments (Applied Biosystems, Hong Kong, China).

Protocol options and application notes

Laboratories participating in the evaluation used the TaqMan Fast Virus 1-Step Master Mix (Thermo Fisher) with the same oligonucleotide concentrations and cycling conditions. The QIAGEN One-Step RT-PCR Kit was also tested and found to be compatible.

The intended cross-reactivity of all assays with viral RNA of SARS-CoV allows us to use the assays without having to rely on external sources of specific 2019-nCoV RNA.

For a routine workflow, we recommend the E gene assay as the first-line screening tool, followed by confirmatory testing with the RdRp gene assay. Application of the RdRp gene assay with dual colour technology can discriminate 2019-nCoV (both probes positive) from SARS-CoV RNA if the latter is used as positive control. Alternatively, laboratories may choose to run the RdRp assay with only the 2019-nCoV-specific probe.

Ethical statement

The internal use of samples for diagnostic workflow optimisation was agreed under the medical ethical rules of each of the participating partners.

Results

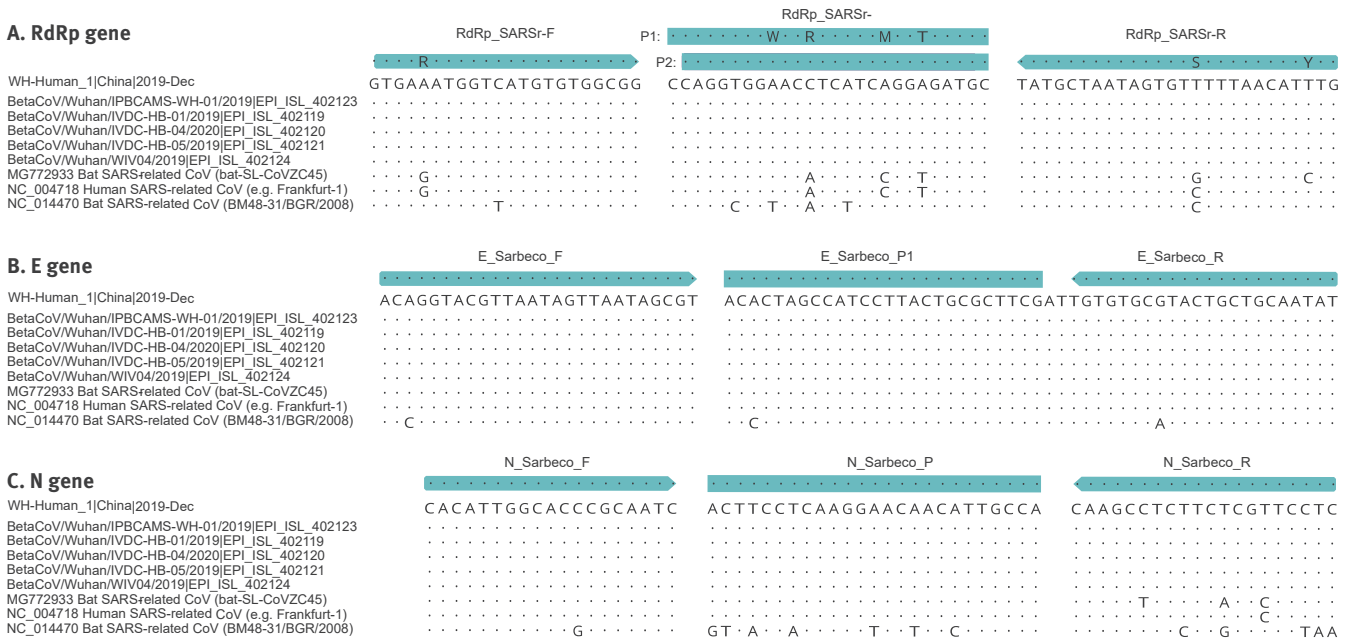
Before public release of virus sequences from cases of 2019-nCoV, we relied on social media reports announcing detection of a SARS-like virus. We thus assumed that a SARS-related CoV is involved in the outbreak. We downloaded all complete and partial (if > 400 nt) SARS-related virus sequences available in GenBank by 1 January 2020. The list (n = 729 entries) was manually checked and artificial sequences (laboratory-derived, synthetic, etc), as well as sequence duplicates were removed, resulting in a final list of 375 sequences. These sequences were aligned and the alignment was used for assay design (Supplementary Figure S1). Upon release of the first 2019-nCoV sequence at virological.org, three assays were selected based on how well they matched to the 2019-nCoV genome (Figure 1). The alignment was complemented by additional sequences released independently on GISAID (https://www.gisaid.org), confirming the good matching of selected primers to all sequences. Alignments of primer binding domains with 2019-nCoV, SARS-CoV as well as selected bat-associated SARS-related CoV are shown in Figure 2.

Assay sensitivity based on SARS coronavirus virions

To obtain a preliminary assessment of analytical sensitivity, we used purified cell culture supernatant containing SARS-CoV strain Frankfurt-1 virions grown on Vero cells. The supernatant was ultrafiltered and thereby concentrated from a ca 20-fold volume of cell culture supernatant. The concentration step simultaneously reduces the relative concentration of background nucleic acids such as not virion-packaged viral RNA. The virion preparation was quantified by real-time RT-PCR using a specific in vitro-transcribed RNA quantification standard as described in Drosten et al. [8]. All assays were subjected to replicate testing in order to determine stochastic detection frequencies at each assay's sensitivity end point (Figure 3A and B). All assays were highly sensitive, with best results obtained for the E gene and RdRp gene assays (5.2 and 3.8 copies per reaction at 95% detection probability, respectively). These two assays were chosen for further evaluation. One of the laboratories participating in the external evaluation used other basic RT-PCR reagents (TaqMan Fast Virus 1-Step Master Mix) and repeated the sensitivity study, with equivalent results (E gene: 3.2 RNA copies/reaction (95% CI: 2.2–6.8); RdRP: 3.7 RNA copies/reaction (95% CI: 2.8–8.0). Of note, the N gene assay also performed well but was not subjected

Figure 2

Partial alignments of oligonucleotide binding regions, SARS-related coronaviruses (n = 9)



The panels show six available sequences of 2019-nCoV, aligned to the corresponding partial sequences of SARS-CoV strain Frankfurt 1, which can be used as a positive control for all three RT-PCR assays. The alignment also contains a closely related bat virus (Bat SARS-related CoV isolate bat-SL-CoVZC45, GenBank accession number MG772933) as well as the most distant member within the SARS-related bat CoV clade, detected in Bulgaria (GenBank accession number NC_014470). Dots represent identical nucleotides compared with the WH_Human_1 sequence. Nucleotide substitutions are specified. Blue arrows: oligonucleotides as specified in Table 1. More comprehensive alignments can be found in the Supplement.

to intensive further validation because it was slightly less sensitive (Supplementary Figure S2)

Sensitivity based on in vitro-transcribed RNA identical to 2019 novel coronavirus target sequences

Although both assays detected 2019-nCoV without polymorphisms at oligonucleotide binding sites (Figure 2), we additionally generated in vitro-transcribed RNA standards that exactly matched the sequence of 2019-nCoV for absolute quantification and studying the limit of detection (LOD). Replicate reactions were done at concentrations around the detection end point determined in preliminary dilution experiments. The resulting LOD from replicate tests was 3.9 copies per reaction for the E gene assay and 3.6 copies per reaction for the RdRp assay (Figure 3C and D). These figures were close to the 95% hit rate of 2.9 copies per reaction, according to the Poisson distribution, expected when one RNA molecule is detected.

Discrimination of 2019 novel coronavirus from SARS coronavirus by RdRp assay

Following the rationale that SARS-CoV RNA can be used as a positive control for the entire laboratory procedure, thus obviating the need to handle 2019-nCoV RNA, we formulated the RdRp assay so that it contains two probes: a broad-range probe reacting with SARS-CoV and 2019-nCoV and an additional probe that reacts only with 2019-nCoV. By limiting dilution experiments, we confirmed that both probes, whether used individually or in combination, provided the same LOD for each target virus. The specific probe RdRP_SARSr-P2 detected only the 2019-nCoV RNA transcript but not the SARS-CoV RNA.

Detection range for SARS-related coronaviruses from bats

At present, the potential exposure to a common environmental source in early reported cases implicates the possibility of independent zoonotic infections with increased sequence variability [5]. To show that the assays can detect other bat-associated SARS-related viruses, we used the E gene assay to test six bat-derived faecal samples available from Drexler et al. [13] und Muth et al. [14]. These virus-positive samples stemmed from European rhinolophid bats. Detection of these phylogenetic outliers within the SARS-related CoV clade suggests that all Asian viruses are likely to be detected. This would, theoretically, ensure broad sensitivity even in case of multiple independent acquisitions of variant viruses from an animal reservoir.

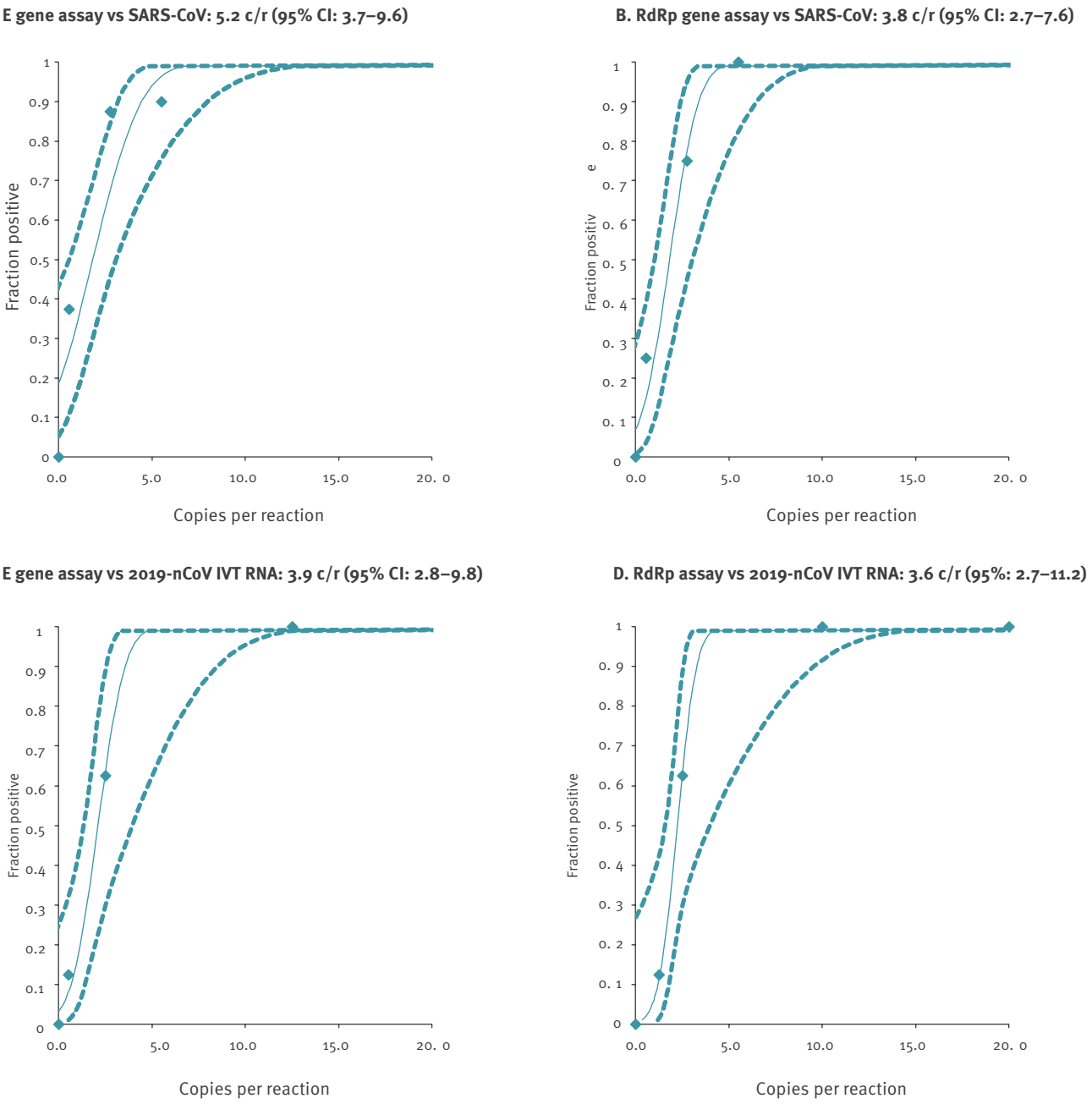
Specificity testing

Chemical stability

To exclude non-specific reactivity of oligonucleotides among each other, causing artificial fluorescent

Figure 3

Determination of limits of detection based on SARS coronavirus genomic RNA and 2019 novel coronavirus-specific in vitro transcribed RNA



CI: confidence intervals; c/r: copies per reaction; IVT: in vitro-transcribed RNA.

A: E gene assay, evaluated with SARS-CoV genomic RNA. B: RdRp gene assay evaluated with SARS-CoV genomic RNA. C: E-gene assay, evaluated with 2019-nCoV-specific in vitro-transcribed RNA standard. D: RdRp gene assay evaluated with 2019-nCoV-specific in vitro-transcribed RNA standard.

The x-axis shows input RNA copies per reaction. The y-axis shows positive results in all parallel reactions performed, squares are experimental data points resulting from replicate testing of given concentrations (x-axis) in parallels assays (eight replicate reactions per point).

Technical limits of detection are given in the panels headings. The inner line is a probit curve (dose-response rule). The outer dotted lines are 95% CI.

Table 2

Tests of known respiratory viruses and bacteria in clinical samples and cell culture preparations for cross-reactivity in 2019 novel coronavirus E and RdRp gene assays (n = 310)

Clinical samples with known viruses	Clinical samples[a]	Virus isolates[b]
HCoV-HKU1	14	1[c]
HCoV-OC43	16	2[d]
HCoV-NL63	14	1[e]
HCoV-229E	18	2[f]
MERS-CoV	5	1[g]
Influenza A(H1N1)pdm09	17	1
Influenza A(H3N2)	16	1
Influenza A (untyped)	11	NA
Influenza A(H5N1)	1	1
Influenza A(H7N9)	0	1
Influenza B (Victoria or Yamagata)	31	1
Rhinovirus/enterovirus	31	NA
Respiratory syncytial virus (A/B)	33	NA
Parainfluenza 1 virus	12	NA
Parainfluenza 2 virus	11	NA
Parainfluenza 3 virus	14	NA
Parainfluenza 4 virus	11	NA
Human metapneumovirus	16	NA
Adenovirus	13	1
Human bocavirus	6	NA
Legionella spp.	3	NA
Mycoplasma spp.	4	NA
Total clinical samples	**297**	**NA**

[a] For samples with multiple viruses detected, the virus with highest concentration is listed, as indicated by real-time PCR Ct value.

[b] Directly quantified or spiked in human negative-testing sputum.

[c] 1×10^5 RNA copies/mL, determined by specific real-time RT-PCR. Isolated from human airway epithelial culture.

[d] 1×10^{10} RNA copies/mL, determined by specific real-time RT-PCR of one isolate. The other isolate was not quantified but spiked in human negative-testing sputum.

[e] 4×10^9 RNA copies/mL, determined by specific real-time RT-PCR.

[f] 3×10^9 RNA copies/mL, determined by specific real-time RT-PCR of one isolate. The other isolate was not quantified spiked in human negative-testing sputum.

[g] 1×10^8 RNA copies/mL, determined by specific real-time RT-PCR.

signals, all assays were tested 120 times in parallel with water and no other nucleic acid except the provided oligonucleotides. In none of these reactions was any positive signal detected.

Cross-reactivity with other coronaviruses

Cell culture supernatants containing all endemic human coronaviruses (HCoV)229E, NL63, OC43 and HKU1 as well as MERS-CoV were tested in duplicate in all three assays (Table 2). For the non-cultivable HCoV-HKU1, supernatant from human airway culture was used. Viral RNA concentration in all samples was determined by specific real-time RT-PCRs and in vitro-transcribed RNA standards designed for absolute quantification of viral load. Additional undiluted (but not quantified) cell culture supernatants were tested as summarised in Table 2. These were additionally mixed into negative human sputum samples. None of the tested viruses or virus preparations showed reactivity with any assay.

Exclusivity of 2019 novel coronavirus based on clinical samples pre-tested positive for other respiratory viruses

Using the E and RdRp gene assays, we tested a total of 297 clinical samples from patients with respiratory disease from the biobanks of five laboratories that provide diagnostic services (one in Germany, two in the Netherlands, one in Hong Kong, one in the UK). We selected 198 samples from three university medical centres where patients from general and intensive care wards as well as mainly paediatric outpatient departments are seen (Germany, the Netherlands, Hong Kong). The remaining samples were contributed by national public health services performing surveillance studies (RIVM, PHE), with samples mainly submitted by practitioners. The samples contained the broadest range of respiratory agents possible and reflected the general spectrum of virus concentrations encountered in diagnostic laboratories in these countries (Table 2). In total, this testing yielded no false positive outcomes. In four individual test reactions, weak initial reactivity was seen but they were negative upon retesting with the same assay. These signals were not associated with any particular virus, and for each virus with which initial positive reactivity occurred, there were other samples that contained the same virus at a higher concentration but did not test positive. Given the results from the extensive technical qualification described above, it was concluded that this initial reactivity was not due to chemical instability of real-time PCR probes but most probably to handling issues caused by the rapid introduction of new diagnostic tests and controls during this evaluation study.

Discussion

The present report describes the establishment of a diagnostic workflow for detection of an emerging virus in the absence of physical sources of viral genomic nucleic acid. Effective assay design was enabled by the willingness of scientists from China to share genome information before formal publication, as well as the availability of broad sequence knowledge from ca 15 years of investigation of SARS-related viruses in animal reservoirs. The relative ease with which assays could be designed for this virus, in contrast to SARS-CoV in 2003, proves the huge collective value of descriptive studies of disease ecology and viral genome diversity [8,15-17].

Real-time RT-PCR is widely deployed in diagnostic virology. In the case of a public health emergency, proficient diagnostic laboratories can rely on this robust technology to establish new diagnostic tests within their routine services before pre-formulated assays become available. In addition to information on

reagents, oligonucleotides and positive controls, laboratories working under quality control programmes need to rely on documentation of technical qualification of the assay formulation as well as data from external clinical evaluation tests. The provision of control RNA templates has been effectively implemented by the EVAg project that provides virus-related reagents from academic research collections [18]. SARS-CoV RNA was retrievable from EVAg before the present outbreak; specific products such as RNA transcripts for the here-described assays were first retrievable from the EVAg online catalogue on 14 January 2020 (https://www.european-virus-archive.com). Technical qualification data based on cell culture materials and synthetic constructs, as well as results from exclusivity testing on 75 clinical samples, were included in the first version of the diagnostic protocol provided to the WHO on 13 January 2020. Based on efficient collaboration in an informal network of laboratories, these data were augmented within 1 week comprise testing results based on a wide range of respiratory pathogens in clinical samples from natural infections. Comparable evaluation studies during regulatory qualification of in vitro diagnostic assays can take months for organisation, legal implementation and logistics and typically come after the peak of an outbreak has waned. The speed and effectiveness of the present deployment and evaluation effort were enabled by national and European research networks established in response to international health crises in recent years, demonstrating the enormous response capacity that can be released through coordinated action of academic and public laboratories [18-22]. This laboratory capacity not only supports immediate public health interventions but enables sites to enrol patients during rapid clinical research responses.

*Author's correction

The sentence As at 20 January 2020, 282 laboratory-confirmed human cases have been notified to WHO was originally published with a wrong date (As at 20 January 2019...). This mistake was corrected on 8 April 2020.

On 29 July 2020 the correct affiliation of Marco Kaiser was added and the remaining affiliations were renumbered.

**Addendum

The Conflict of interest section was updated on 29 July 2020.

***Erratum

In the second half of Table 1, nM (nanomolar) was misspelled as nm when this article was published. This mistake was corrected on 4 February 2021. We apologise for any inconvenience this typo may have caused.

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Conflict of interest **

Olfert Landt is CEO of Tib-Molbiol; Marco Kaiser is senior researcher at GenExpress and serves as scientific advisor for Tib-Molbiol.

Authors' contributions

VMC: Planned and conducted experiments, conceptualised the laboratory work

OL: Planned and conducted experiments, conceptualised the laboratory work

MK: Planned and conducted experiments

RM: Planned and conducted experiments, conceptualised the laboratory work

AM: Planned and conducted experiments, conceptualised the laboratory work

DKWC: Planned and conducted experiments

TB: Planned and conducted experiments

SB: Planned and conducted experiments

JS: Planned and conducted experiments

MLS: Planned and conducted experiments

DGJCM: Planned and conducted experiments

BLH: Planned and conducted experiments

BvdV: Planned and conducted experiments

SvdB: Planned and conducted experiments

LW: Planned and conducted experiments

GG: Planned and conducted experiments

JLR: Contributed to overall study conceptualization

JE: Planned and conducted experiments, conceptualised the laboratory work

MZ: Planned laboratory work, contributed to overall study conceptualization

MP: Planned laboratory work, contributed to overall study conceptualization

HG: Contributed to overall study conceptualization

CR: Planned experiments, conceptualised the laboratory work

MPGK: Planned experiments, conceptualised the laboratory work

CD: Planned experiments, conceptualised the laboratory work, conceptualised the overall study, wrote the manuscript draft.

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Excess all-cause mortality during the COVID-19 pandemic in Europe – preliminary pooled estimates from the EuroMOMO network, March to April 2020

Lasse S Vestergaard[1] , Jens Nielsen[1] , Lukas Richter[2] , Daniela Schmid[2] , Natalia Bustos[3] , Toon Braeye[3] , Gleb Denissov[4] , Tatjana Veideman[4] , Oskari Luomala[5] , Teemu Möttönen[5] , Anne Fouillet[6] , Céline Caserio-Schönemann[6] , Matthias an der Heiden[7] , Helmut Uphoff[8] , Theodore Lytras[9] , Kassiani Gkolfinopoulou[9] , Anna Paldy[10] , Lisa Domegan[11,12] , Joan O'Donnell[11] , Francesca de' Donato[13] , Fiammetta Noccioli[13] , Patrick Hoffmann[14] , Telma Velez[14] , Kathleen England[15] , Liselotte van Asten[16] , Richard A White[17] , Ragnhild Tønnessen[17] , Susana P da Silva[18] , Ana P Rodrigues[18] , Amparo Larrauri[19] , Concepción Delgado-Sanz[19] , Ahmed Farah[20] , Ilias Galanis[20] , Christoph Junker[21] , Damir Perisa[22] , Mary Sinnathamby[23] , Nick Andrews[23] , Mark O'Doherty[24] , Diogo FP Marquess[25] , Sharon Kennedy[25] , Sonja J Olsen[26] , Richard Pebody[26] , ECDC Public Health Emergency Team for COVID-19[27] , Tyra G Krause[1] , Kåre Mølbak[1,28]

1. Statens Serum Institut, Copenhagen, Denmark
2. Austrian Agency for Health and Food Safety, Vienna, Austria
3. Sciensano, Brussels, Belgium
4. National Institute for Health Development, Tallinn, Estonia
5. Finnish National Institute for Health and Welfare, Helsinki, Finland
6. French Public Health Agency (Santé Publique France), Saint-Maurice, France
7. Robert Koch Institute, Berlin, Germany
8. Hessisches Landesprüfungs- und Untersuchungsamt im Gesundheitswesen, Dillenburg, Germany
9. National Public Health Organization, Athens, Greece
10. National Public Health Institute, Budapest, Hungary
11. Health Service Executive - Health Protection Surveillance Centre, Dublin, Ireland
12. European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden.
13. Dipartimento Epidemiologia del S.S.R., Lazio - ASL Roma 1, Rome, Italy
14. Health Directorate Luxembourg – Division de l'inspection sanitaire, Luxembourg
15. Directorate for Health Information and Research, Ministry for Health, Malta
16. National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands
17. Norwegian Institute of Public Health, Oslo, Norway
18. Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisbon, Portugal
19. National Centre of Epidemiology, CIBER Epidemiología y Salud Pública (CIBERESP), Carlos III Health Institute, Madrid, Spain
20. Public Health Agency of Sweden, Stockholm, Sweden
21. Federal Statistical Office, Neuchâtel, Switzerland
22. Federal Office of Public Health, Bern, Switzerland
23. Public Health England, Colindale, United Kingdom
24. Public Health Agency, Northern Ireland, United Kingdom
25. Public Health Scotland, Glasgow, United Kingdom
26. World Health Organization, Regional Office for Europe, Copenhagen, Denmark
27. The members of the ECDC Public Health Emergency Team for COVID-19 are listed below
28. Department of Veterinary and Animal Science, Faculty of Health and Medical Science, University of Copenhagen, Copenhagen, Denmark

Correspondence: Lasse S. Vestergaard (lav@ssi.dk)

ECDC Public Health Emergency Team for COVID-19: Pasi Penttinen, Nick Bundle, Cornelia Adlhoch

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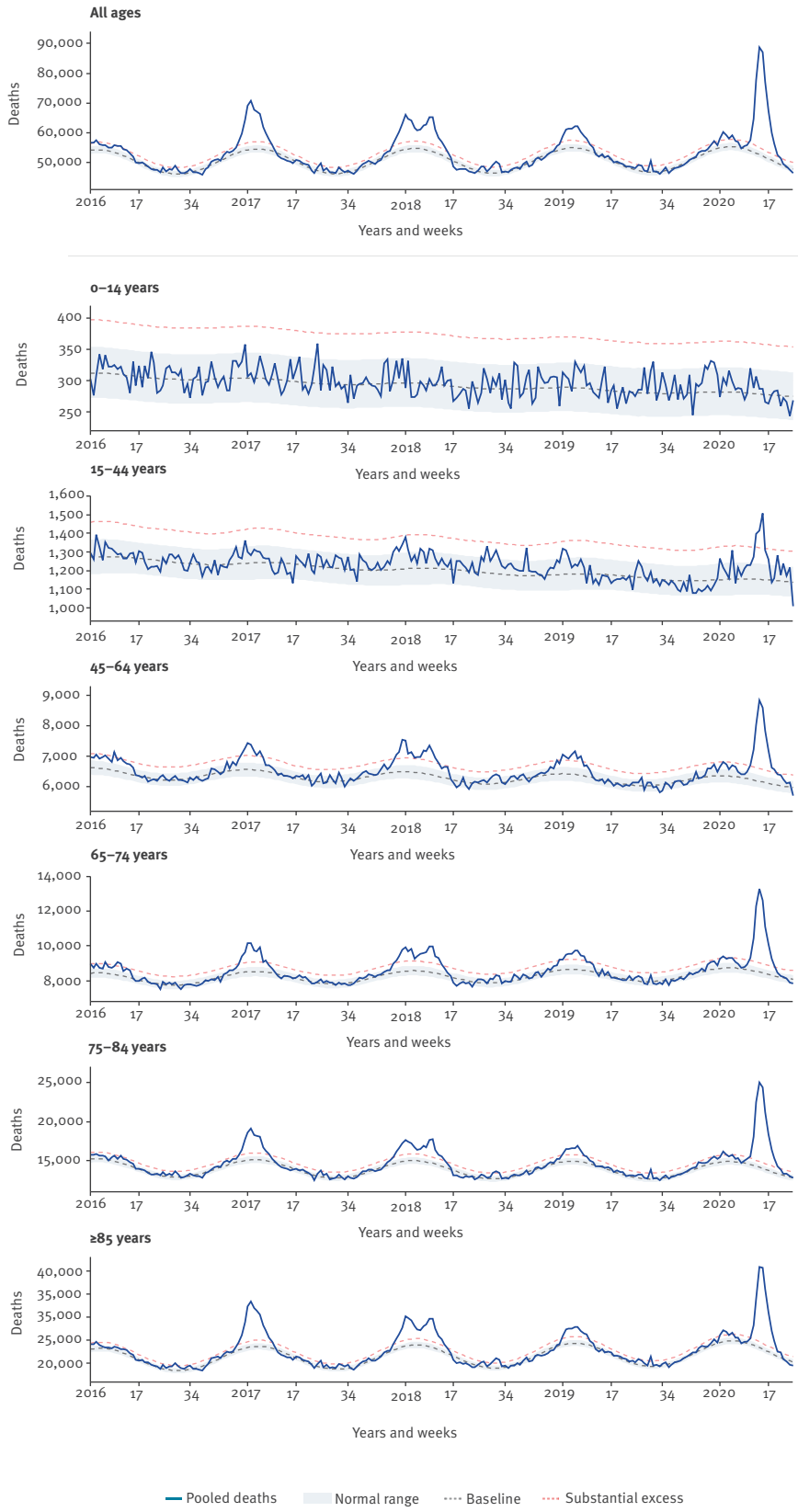
A remarkable excess mortality has coincided with the COVID-19 pandemic in Europe. We present preliminary pooled estimates of all-cause mortality for 24 European countries/federal states participating in the European monitoring of excess mortality for public health action (EuroMOMO) network, for the period March–April 2020. Excess mortality particularly affected ≥65 year olds (91% of all excess deaths), but also 45–64 (8%) and 15–44 year olds (1%). No excess mortality was observed in 0–14 year olds.

We present pooled European-wide weekly mortality estimates from the European monitoring of excess mortality for public health action (EuroMOMO) network from the beginning of 2020 until week 18 (23 April–3 May) of this year. This period includes the initial 2 months of the coronavirus disease (COVID-19) pandemic in Europe, March and April, a time frame characterised by the end of the influenza season but widespread COVID-19 community transmission. We also calculate the weekly and cumulative excess all-cause mortality from week 1 to week 18/2020, and

Figure 1

EuroMOMO pooled estimates of all-cause mortality shown for all ages combined and by age group, week 1/2016–week 18/2020

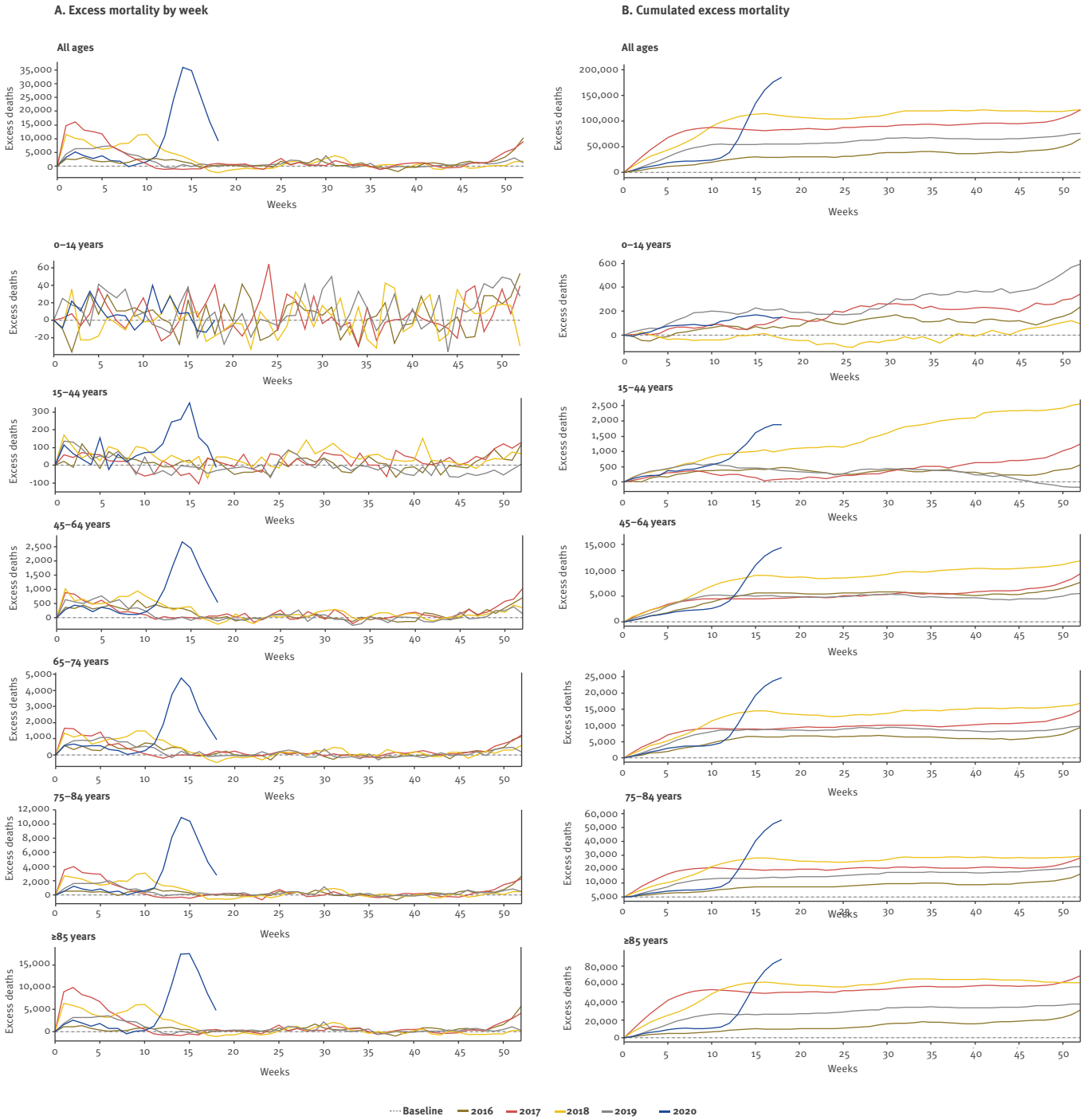


EuroMOMO: European monitoring of excess mortality for public health action.

Substantial excess mortality is defined as an excess level equivalent to four z-scores above the expected baseline.

Figure 2

EuroMOMO pooled estimates of excess[a] all-cause mortality shown combined for all ages and by age group, from week 1 to week 18 for year 2020, and week 1 to week 52 for the years 2016, 2017, 2018, 2019, respectively



EuroMOMO: European monitoring of excess mortality for public health action.

[a] Excess is defined as >2 z scores.

compare the results to the same period of the previous 4 years (2016, 2017, 2018, and 2019).

Relevance of excess mortality monitoring during the coronavirus disease pandemic in Europe

Following a coronavirus disease (COVID-19) outbreak in China in late December 2019, the causative virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), spread rapidly to become a major global public health emergency [1,2]. On 11 March 2020, COVID-19 was declared a pandemic [3], which is currently still ongoing. In Europe, the first COVID-19 cases were reported in January 2020 in France [4]. During the following weeks, occurrences of cases and fatalities with rapidly increasing numbers were observed across many European countries [5,6]. By the end of June 2020 [7], about 1.6 million confirmed COVID-19 cases and 177,000 deaths had been officially reported from European Union (EU)/European Economic Area (EEA) countries and the United Kingdom (UK).

The official national statistics on COVID-19 cases and deaths among European countries are heterogeneous, partly due to the differences in applied testing strategies and access to testing, and use of different reporting modalities. In this situation, numbers of excess all-cause deaths can provide a more complete and timely proxy measure of the mortality burden of COVID-19 in the population, in particular when there are no other factors known to cause excess mortality, such as seasonal influenza [8].

Since 2009, following the influenza A(H1N1)pdm09 pandemic, the EuroMOMO network (www.euromomo.eu) has monitored the weekly all-cause excess mortality in a large number of countries across Europe. EuroMOMO uses a statistical algorithm, which allows to compare and pool national mortality estimates [9]. The EuroMOMO mortality outputs form part of the routine monitoring of seasonal influenza severity in Europe, producing weekly and end-of-season reports to inform national and international public health agencies, and to evaluate mortality signals within and between countries in a systematic and timely manner [10-12]. Such outputs are particularly useful in the context of an emerging pandemic caused by a new infectious agent, where the true mortality burden is difficult to ascertain and compare between countries.

Estimating the number of all-cause deaths in EuroMOMO countries

Countries participating in the EuroMOMO network collect weekly data from civil registers or other official reporting sources on the number of deaths of all causes. The all-cause excess mortality, defined as the observed minus the expected numbers of deaths, is estimated using the EuroMOMO statistical algorithm, previously described in detail [9]. The EuroMOMO hub compiles these weekly data from individual countries and conducts a pooled analysis using an age-stratified method [13].

Currently, the following 24 European countries or federal states participate with their weekly data submission: Austria, Belgium, Denmark, England (UK), Estonia, Finland, France, Germany (Berlin and Hesse), Greece, Hungary, Ireland, Italy (19 cities), Luxembourg, Malta, the Netherlands, Northern Ireland (UK), Norway, Portugal, Scotland (UK), Spain, Sweden, Switzerland and Wales (UK). Ireland has encountered additional delays in death registrations during the pandemic period, hence the included numbers for this country are not yet complete.

We present preliminary pooled European-wide mortality estimates from the EuroMOMO network for 2020. The pooled estimates cover the period until the end of week 18 (3 May)/2020, based on data received by the end of week 23 (7 June) of this year. Estimates are shown for all ages combined, and by the age groups 0–14, 15–44, 45–64, 65–74, 75–84, and ≥ 85 years. In addition to weekly all-cause mortality estimates, we also calculate the weekly and cumulative excess all-cause mortality for 2020 up to week 18, and compare the results with the same period in each of the previous 4 years (2016, 2017, 2018, and 2019) using our standard approach.

Due to delay in death registration, the data for the most recent weeks beyond week 18 2020 are not included in the present report, but are available from the EuroMOMO website, where estimates corrected for delay in registration using a country-specific adjustment function are shown.

Ethical statement

Ethical approval was not needed for the study, which is based on surveillance data only.

Pooled estimates of all-cause excess mortality

All-cause mortality started to exceed normal expected levels in Italy around week 10 (1–8 March)/2020. In the following weeks, excess mortality was also detected in several other EuroMOMO countries, including the following: Belgium, England (UK), France, the Netherlands, Northern Ireland (UK), Portugal, Scotland (UK), Spain, Sweden, Switzerland and Wales (UK). While, during the same period of the COVID-19 pandemic, several other countries experienced no or only very limited excess mortality including: Austria, Denmark, Estonia, Finland, Germany (Berlin and Hesse federal states), Greece, Hungary, Luxembourg, Malta and Norway.

The pooled mortality estimates for the 24 participating European countries or federal states showed an increasing trend during the first weeks of March 2020, and an excess mortality level higher than four z-scores above the baseline (defined as 'substantial excess') in week 11 (9–15 March)/2020 (Figure 1). The mortality was

highest among individuals aged 65 years and older, but some countries also observed marked excess deaths among those aged 45–64 years, and some countries (in particular England and Spain) even noted excess mortality in the age group 15–44 years, also reflected in the overall pooled estimates. No excess mortality was observed in children aged 0–14 years.

Mortality increased steeply in the next 3 weeks and peaked in all countries during week 14 (30 March–5 April)/2020, when a total of 88,581 deaths (all ages) was reached, translating into a z-score of 58. By week 15 (6–12 April)/2020 the mortality started a rapid decline, affecting all age groups except the 0–14 years where no excess mortality had been observed; however, by week 18/2020 a substantial mortality for all ages combined, of around 60,000 deaths, was still seen, corresponding to a z-score of 16 above the baseline.

Figure 2 shows the weekly and cumulative pooled excess all-cause mortality estimates observed during the COVID-19 pandemic in comparison to the previous 4 years, from week 1 to week 18. At the peak level of mortality, in week 14, an excess of 35,802 deaths across all ages was estimated, of which 32,815 (92%) were persons aged ≥ 65 years. In comparison, the highest excess mortality in any week during the previous 4 years reached 16,165 deaths (all ages) in week 2 in 2017, i.e. during the severe 2016/17 influenza season [11] (Figure 2A).

The cumulative excess mortality from week 1 to week 18/2020 reached a total of 185,287 deaths (all ages), including 24,438 (13%) in persons aged 65–74 years, 55,226 (30%) in persons aged 75–84 years, and 88,598 (48%) in persons aged ≥ 85 years. The cumulative deaths in the younger age groups reached 14,339 (8%) in 45–64 year-old persons and 1,843 (1%) in 15–44 year-old persons. This period of the year includes a part of the usual influenza season. In comparison, the cumulative excess deaths (all ages) by week 18 reached 55,441 in 2019, 110,483 deaths in 2018, 83,009 deaths in 2017 and 29,849 deaths in 2016 (Figure 2B).

Discussion

Soon after its detection in China in late 2019, COVID-19 was found to lead to a considerable morbidity and mortality burden. A systematic review and meta-analysis resulted in an overall estimated proportion of severe cases of 25.6% and a case fatality rate (CFR) of 3.6%, with more severe clinical symptoms and higher CRF among older patients and patients with underlying medical conditions [14]. As increasing age and comorbidity appear to pose a risk for fatal outcome, it can be argued that COVID-19 mainly leads to death in patients with an expected short life span so that the overall excess mortality at the population level may be relatively limited. However, our analysis suggests that transmission of COVID-19 indeed has had a marked impact on all-cause mortality in the European population, despite the extensive societal preventive measures taken and the increase of treatment capacity in affected countries. We observed steep peaks in excess mortality in the age groups 65–74 years, 75–84 years and ≥ 85 years, respectively, considerably exceeding the excess mortality levels observed during any of the past influenza seasons monitored by EuroMOMO. Excess mortality was also observed in persons aged 45–64 years and 15–44 years, although to a much smaller extent than was seen among elderly people. No excess mortality was observed in children under 15 years old.

When facing a new viral pandemic such as COVID-19, with many unknowns regarding biology and transmission potential, estimating the impact on public health in terms of disease severity and mortality is critical. With limited testing capacity, changing testing strategies and different surveillance and reporting systems, the officially reported mortality statistics based on individual COVID-19 death reports will inevitably be heterogeneous and incomplete. In this situation, estimating excess all-cause mortality using a standard approach across countries provides a powerful tool to rapidly obtain unbiased estimates of the COVID-19 mortality burden, and how it affects different age groups and different countries and areas. The mortality impact of the COVID-19 epidemic was clearly demonstrated by reports of the excess all-cause mortality estimates by the Ministry of Health in Italy in March 2020 [15,16], and by weekly all-cause mortality reports published early in the epidemic by the national health authorities of several other European countries.

All-cause excess mortality is estimated in the current study. Considering the limited occurrence of seasonal influenza during the peak time of the COVID-19 mortality in the participating countries, and the absence of other major public health events, the estimated excess mortality can primarily be attributed to COVID-19. Some of these deaths may be directly related to COVID-19; others indirectly due to delays in accessing healthcare for other illnesses, and others due to other factors. The COVID-19 pandemic in Europe is not over yet, and in the coming weeks and months, as the national mortality data become more complete, more definitive estimates of the mortality burden of COVID-19 in Europe will be available and comparisons to previous influenza epidemics/pandemics and other public health events can be made. Similarly, observed discrepancies between all-cause mortality estimates and officially notified mortality statistics can be evaluated, to guide future COVID-19 case reporting and surveillance efforts.

In the current COVID-19 pandemic situation, the EuroMOMO system has proven to be a valuable tool for timely detection and reporting of excess all-cause mortality across many parts of Europe in a coordinated and consistent manner. National and international organisations, the general public, media and others have largely drawn on EuroMOMO as a source of timely and

easily accessible information about the evolving pandemic. The EuroMOMO network welcomes any country within Europe to become part of the network and thereby contribute to an even wider geographical coverage of the ongoing monitoring of the COVID-19 pandemic, from which new waves of transmission could occur. Importantly, the EuroMOMO statistical algorithm applied at the national level data provides countries with a simple and easy-to-use national mortality monitoring system. These mortality data are crucial for early warning and impact assessment, informing policy decisions and public health action.

Note

The members of the ECDC Public Health Emergency Team for COVID-19 are affiliated with the European Centre for Disease Prevention and Control, Solna, Sweden.

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Conflict of interest

None declared.

Authors' contributions

LSV drafted the first version of the manuscript. LSV and JN performed the analyses and provided the graphs and figures. LSV, JN, LR, DS, NB, TB, GD, TV, OL, TM, AF, CCS, MH, HU, TL, KG, AP, LD, JO, FD, FN, PH, TV, KE, LA, RAW, RT, SPS, APR, AL, CDS, AF, IG, CJ, DP, MS, NA, MO, DFPM, SK, SJO, RP, PP, NBu, CA, TGV and KM provided data and/or contributed in the writing of the manuscript and approved the final version. The authors alone are responsible for the views presented in this manuscript and they do not necessarily reflect the views, decisions or policies of the institutions with which the authors are affiliated.

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Department of Infectious Diseases Control, Flanders
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Medical and Public Health Services, Ministry of Health, Nicosia
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Czech Republic

Zprávy CEM (The Bulletin of Centre for Epidemiology and Microbiology)
Státní zdravotní ústav (National Institute of Public Health), Prague
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Department of Infectious Disease Epidemiology and Prevention, Statens Serum Institut, Copenhagen.
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Monthly to quarterly, online. In Icelandic and English.
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Ireland

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Health Protection Surveillance Centre, Dublin
Monthly, online. In English.
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Notiziario dell'Istituto Superiore di Sanita
Istituto Superiore di Sanita, Reparto di Malattie Infettive, Rome
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http://www.sva.lv/epidemiologija/bileteni

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Center for Communicable Disease Prevention and Control, Vilnius
Online. In Lithuanian.
http://www.ulac.lt/index.php?pl=26

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http://www.cnscbt.ro/

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Institute of Public Health, Center for Infectious Diseases, Ljubljana
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Sweden

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Weekly, online. In Swedish.
https://www.folkhalsomyndigheten.se/nyheter-och-press/

European Union

Europa is the official portal of the European Union. It provides up-to-date coverage of main events and information on activities and institutions of the European Union.
http://europa.eu

European Commission - Public Health

The website of the European Commission Directorate General for Health and Consumer Protection (DG SANCO).
http://ec.europa.eu/health/

Health-EU Portal

The Health-EU Portal (the official public health portal of the European Union) includes a wide range of information and data on health-related issues and activities at both European and international levels.
http://ec.europa.eu/health-eu/

European Centre for Disease Prevention and Control

European Centre for Disease Prevention and Control (ECDC)
The European Centre for Disease Prevention and Control (ECDC) was established in 2005. It is an EU agency that aims to strengthen Europe's defences against infectious diseases. It is located in Stockholm, Sweden.
http://www.ecdc.europa.eu

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