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Editorial

Zika virus and congenital malformations in perspective

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Zika virus (ZIKV) has been on the agenda of virologists since many years already. Laboratories dealing with imported tropical diseases consider ZIKV infection among the differential diagnoses in cases of fever after travel to tropical Africa and Asia. The confirmation of autochthonous cases of this Old World flavivirus infection in Brazil in May 2015 was however surprising [1]. Already one month later, in June 2015, a rapid communication in *Eurosurveillance* provided a hint towards the unrecognised dimension of the outbreak. The relatively inconspicuous disease had already made it to Europe by March 2015, in the blood of an Italian traveller returning from Brazil [2]. One of the possible reasons for the 'arbovirus community' to stay somewhat inert about the outbreak initially was the introduction of chikungunya virus to Central and South America about one year earlier [3]. It seemed that ZIKV was just another example of an Aedes-transmitted and primate-associated arbovirus that had made it into the virgin soil environment of the neotropics, and moreover a harmless one from a clinical perspective.

A new dimension to the emergence of ZIKV was added in October 2015, when the Brazilian Ministry of Health (MoH) expressed concern about an increased incidence of microcephaly in newborns in the north-eastern part of the country [4]. These cases followed the assumed arrival and spread of ZIKV with a delay that made congenital infection plausible. Microcephaly had never been reported in connection with ZIKV infection before – but admittedly had not been assessed in any systematic way during previous outbreaks. In this issue of *Eurosurveillance,* Besnard et al. present a summary of 19 cases with a wide range of congenital cerebral abnormalities with and without microcephaly [5]. In all of these cases, the times of gestation most vulnerable for neurological fetopathies fell into the height of the 2013 to 2014 ZIKV outbreak in French Polynesia.

There is a whole number of viruses causing congenital neural malformations in humans. Cytomegalovirus, parvovirus B19 and varicella zoster virus are of highest concern in Europe. Rubella virus, the most relevant example historically, is today very rare as a cause of embryo- and fetopathy in Europe thanks to comprehensive vaccination programmes.

The threat of cerebral malformations in connection with ZIKV is difficult to express in numbers. The emotional component not only for expecting mothers and the possibility to miss a window of opportunity for study and intervention have already triggered ad hoc funding programmes and pragmatic approaches to extract information from available data [6]. It is not in spite of the emotional component, but because of it, that we should look at the problem from a rational perspective.

Most viral infections that cause fetopathy have a low manifestation index. Almost certainly, also the ZIKV will cause harm in only a small proportion of fetuses in the many pregnant women recently and currently exposed to the virus. Based mainly on data from the outbreak in French Polynesia, a recent study projected that women infected by ZIKV during the first trimester of pregnancy may have a risk of fetal microcephaly of 1% [6].

In the presently affected regions, where people are similarly exposed and immunologically naïve towards ZIKV infection, high rates of unnoticed infection are to be expected - including in pregnant women. The 6,158 suspected cases of microcephaly reported in Brazil by 9 March 2016, are likely to represent only a small fraction of the many pregnant women who got infected during 2015 [7].

Another issue is the reporting bias and classification of microcephaly. In autumn 2015, the Brazilian MoH strengthened and emphasised microcephaly surveillance, whereas notifications before this time occurred on a more routine basis. The media coverage of the ZIKV infection /microcephaly connection contributed an additional stimulus for reporting. A preliminary research manuscript by Rocha et al. suggests that the

Brazilian MoH reporting criterion for suspected cases of microcephaly, based on cranial circumference lower than 32 cm at birth, might be inappropriate for the most affected population in north-eastern Brazil. In a worst case scenario raised by the authors, this criterion may trigger the formal notification of up to 10% of all newborns as suspected cases of microcephaly [8]. Of the 6,158 suspected cases notified so far, 4,249 remain under investigation [7]. Of the 1,908 cases evaluated, 1,163 have not been confirmed as cases of microcephaly [7]. There seems to be an over-notification of suspected cases of microcephaly, irrespective of ZKIV diagnosis.

The literature now contains several reports on cases with microcephaly and ZIKV detection in amniotic fluid, blood, and even central nervous tissue of fetuses with signs of microcephaly. The paper by Besnard et al. includes eight cases of microcephaly [5]. Of the five cases that were tested virologically, four yielded ZIKV by RT-PCR and maternal history was positive for symptoms compatible with ZIKV infection during pregnancy. With every new case report published, we perceive the link between ZIKV infection and microcephaly to become stronger. There is probably truth to this. However, we should remember that case reports do not establish a causative link between the virus and microcephaly. As always at the beginning of epidemics, studies tend to focus on cases but not controls. What fraction of healthy pregnancies might reveal evidence for ZIKV infection if sampled at the peak of an outbreak, assuming attack rates of 10% or even higher in the adult population? At the time of writing, 583 cases of microcephaly in Brazil have been completely investigated including objective neurological criteria and virological laboratory tests. Only 67 (11.5%) were confirmed positive by laboratory tests [7].

A recent correlative analysis noted 2.8 cases of microcephaly per 10,000 births in federal states of Brazil with obvious ZIKV circulation, vs 0.6 cases per 10,000 in states without laboratory evidence for the virus [9]. These numbers suggest a 4.7-fold increase in ZIKV-affected regions overall. In the two most affected states in the north-east, the rate was increased up to ca 18 and 24-fold, respectively. This local concentration is remarkable. According to the latest epidemiological update by the Pan American Health Organization (PAHO), 80% of suspected cases and 97% of confirmed cases in Brazil are reported from the north-east region still. Will the incidence in other ZIKA-affected regions catch up?

The increase of microcephaly may represent a complex effect on the local population that could include other factors such as unrecognised or underdiagnosed pathogens. These factors may promote microcephaly alone or in concert with Zika virus infection. The Bulletin of the World Health Organization (WHO) Zika Open [10] carries a research manuscript that follows the incidence of microcephaly from 2012 to 2015 in Paraiba, the Brazilian federal state that was second most affected [11]. Using data from prospectively-designed birth cohorts, the study reveals a stark increase of microcephaly incidence already by end of 2012 and a second peak by mid-2014. Neither of the peaks can be explained by the presumed introduction of ZIKV by mid of 2014. A third peak of incidence recorded for the second half of 2015 is the strongest peak. Only this peak plausibly correlates with Zika outbreaks. We should remain open for additional explanations for the increased incidence of microcephaly observed in north-eastern Brazil.

Also, beyond microcephaly, we should not forget other neurological symptoms and malformations. Besnard et al. demonstrate that ZIKV infection was not confirmed in any of the 11 cases with non-microcephalic abnormalitities [5]. However, another recent study found a number of non-microcephalic cerebral malformations in fetuses and newborns with signs of ZIKV infection [12]. Much more worrying with regard to numbers is the perspective of sequelae, due to impairment of the central nervous system, in form of deficits that may come to show as children develop. The available reports on fetal neurotropic infection call for neuro-psychiatric follow-up of birth cohorts.

Most countries in Middle America seem to be over their recent peak of incidence of ZIKV infections by now, March 2016. We will thus be able to observe over the next coming months whether the incidence of microcephaly will increase in these areas that have been newly affected by ZIKV since the end of 2015. Animal experiments conducted to provide evidence of causation of microcephaly may unfortunately not be concluded earlier than observations in humans. It is our responsibility as public health scientists to secure epidemiological evidence by careful design of prospective, controlled observational trials. Until we have results, it should not make a difference whether exposure prophylaxis is implemented based on evidence, or out of an abundance of caution.

Conflict of interest

None declared.

Authors' contributions

Christian Drosten wrote the editorial.

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Detection of *mcr-1* colistin resistance gene in polyclonal *Escherichia coli* isolates in Barcelona, Spain, 2012 to 2015

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Colistin resistance was detected in 53 of 10,011 *Escherichia coli* (0.5%) by prospective phenotypic testing of consecutive clinical isolates in a single hospital in Barcelona, Spain (2012–15). The *mcr-1* gene was retrospectively identified by PCR and sequencing in 15 of 50 available isolates. Each isolate had a unique PFGE pattern except for two. This clonal diversity supports the hypothesis of horizontal dissemination of the *mcr-1* gene in the local study population.

Following the report on the plasmid-mediated colistin resistance gene *mcr-1* in China [1], several authors have reported the detection of this gene in *Escherichia coli* isolates of animal origin [1-5]. Currently there have been few reports of detections in humans and these involve mainly multidrug-resistant (MDR) Gram-negative bacilli [3,5-7]. To date, *mcr-1* has been detected in at least five European countries in animals and humans, and often in association with recent travel to Asia [3,5-7]. In this context, we describe *mcr-1* detection in unselected clinical isolates of *E. coli* in Barcelona in samples from 2012 to 2015.

Laboratory investigation

A total of 10,011 *E. coli* were isolated between January 2012 and December 2015 from clinical specimens in our institution, a tertiary referral teaching hospital covering an area of 407,902 inhabitants in Barcelona, Spain. Only one isolate per patient was included. Isolates from colonisation screenings were not considered. Antibiotic susceptibility testing was performed by disc diffusion according to guidelines from the Clinical and Laboratory Standards Institute (CLSI) [8]. As a first approach to screen colistin resistance, a 10 µg disc of colistin was used. Isolates displaying an inhibition zone ≤ 12 mm (n = 61) were selected for further testing of minimal inhibitory concentration (MIC) by gradient diffusion (Etest, bioMérieux, France). Both diffusion methods were performed on Mueller Hinton agar (bioMérieux, France). MIC results of colistin were interpreted following the EUCAST breakpoints for *Enterobacteriaceae* [9]. Resistance to colistin was detected in 53 *E. coli* isolates (0.5%). Of these, 40 were isolated from urine specimens, eight from blood cultures and the remaining five from other clinical specimens. The average age of the patients with infections caused by colistin-resistant *E. coli* was 70.9 years (range: 6–99 years). The male:female ratio was 1:2.

By amplification and Sanger sequencing, we searched for the presence of the *mcr-1* gene in our collection of colistin-resistant *E. coli* isolates (only 50 isolates were available). The amplification of *mcr-1* was performed as described by Liu et al. [1]. This gene was detected in 15 isolates; the amplified fragments had 100% sequence homology with the previously described *mcr-1* [1].

The patients' average age was 62 years (range: 6–97), eight of them were male and seven were female. Patients were not epidemiologically linked (Table). One patient was referred from a nursing home, and nine had had at least one hospital admission during the previous year. No travel abroad was recorded in any of the patients. The rate of positivity corresponded to 0.15% of the total of *E. coli* isolates within the period studied. Seven *mcr-1*-harbouring isolates were not MDR according to international definitions [10]. Only two were extended-spectrum beta-lactamase carriers and one had an AmpC overproduction profile (Table). Tested by Etest, the MIC to colistin ranged from 4 mg/L to 12 mg/L. The *mcr-1*-positive isolates were typed by pulsed-field gel electrophoresis (PFGE); each isolate had a unique PFGE pattern except for two.

Discussion

Colistin is one of the last resorts to treat infections caused by MDR Gram-negative bacilli. Resistance to colistin is rarely reported in *E. coli*, especially in non-MDR isolates from humans [11]. Until recently, this resistance was considered to be based solely on

Table

Characteristics of *Escherichia coli* isolates harbouring *mcr-1* and epidemiological data of the patients, Barcelona, 2012–15 (n = 15)

Date of isolation	Isolation site	Classification of infectiona	Colistin MIC (mg/L)	Antimicrobial resistance pattern
27/12/2012	Blood	Community-acquired	8	AMP-SXT
09/01/2013	Sputum	Hospital-acquired (haematology)	4	AMP-CTX-CAZ-FEP CIP-SXT (ESBL)
26/02/2013	Blood	Community-acquired	4	AMP-SXT
01/03/2013	Blood	Hospital-acquired (oncology)	12[b]	AMP-GEN-TOB
07/03/2013	Blood	Healthcare-associated	6	AMP-CTX-CAZ-FEP (ESBL)
12/03/2013	Sputum	Healthcare-associated	12	AMP-AMC-CTX-CAZ-SXT
08/06/2013	Urine	Community-acquired	4	AMP-NAL
07/07/2013	Blood	Community-acquired	4[b]	AMP-GEN-TOB
01/11/2013	Sputum	Hospital-acquired (recovery room)	4	AMP-CIP
22/05/2014	Urine	Hospital-acquired (neurosurgery)	4	AMP-NAL-SXT
22/08/2014	Urine	Healthcare-associated	6	AMP-NAL-GEN-TOB-SXT
06/10/2014	Surgical wound	Healthcare-associated	8	AMP-CIP-GEN-TOB
14/03/2015	Urine	Healthcare-associated	4	AMP-CIP-GEN-TOB-SXT
29/03/2015	Urine	Hospital-acquired (cardiology)	4	AMP-CIP-GEN-SXT
16/06/2015	Urine	Healthcare-associated[c]	4	AMP-NAL

AMP: ampicillin; AMC: amoxicillin/clavulanic acid; CAZ: ceftazidime; CIP: ciprofloxacin; CTX: cefotaxime; FEP: cefepime; GEN: gentamicin; MIC: minimum inhibitory concentration; NAL: nalidixic acid; SXT: trimethoprim-sulfamethoxazole; TOB: tobramycin; ESBL: extended spectrum beta-lactamase.

[a] Classification of the infection according to the place of acquisition. When hospital-acquired, the hospital ward where the clinical specimen was taken is shown in brackets.

[b] Isolates sharing the same PFGE pattern.

[c] This patient was referred from a nursing home.

genomic mutations in several genes involved in the synthesis of lipopolysaccharide [12]. Since Liu et al. reported plasmid-mediated colistin resistance in *E. coli* isolates [1], the whole scenario has changed and the possibility of horizontal gene transfer needs to be considered. These plasmids carry the *mcr-1* gene coding for a phosphoethanolamine transferase, an enzyme related to changes in lipid A [1]. Despite the large amount of information on *mcr-1* obtained in only a few months, the real prevalence of this gene in clinical isolates is not yet known. Most reports are retrospective, mainly refer to faecal carriers and describe scattered colistin-resistant isolates randomly collected [3,5-7,13]. We here describe *mcr-1* prevalence in colistin-resistant clinical isolates of *E. coli*. As a limitation, no other mechanisms of colistin resistance were searched for in the present study. However, the high percentage of *mcr-1* among our colistin-resistant isolates is noteworthy.

The clonal diversity shown in the present report supports the hypothesis of horizontal dissemination of *mcr-1* gene-related colistin resistance in *E. coli* isolated from our urban patient population in Barcelona. Colistin is not always tested in non-MDR *E. coli* isolates of human origin. This may explain why the previous reports describing *mcr-1* in humans mainly referred to MDR *E. coli* isolates [3,5-7]. Technical variability among methods for colistin susceptibility testing is notorious [14]. Given the discrepancies between the international committees and the lack of colistin breakpoints for *Enterobacteriaceae* in CLSI, we considered it convenient to apply a screening method. Although disc diffusion is not recommended to test colistin susceptibility, it was useful for an initial screening followed by confirmation using a MIC method.

The fact that seven of 15 *mcr-1*-harbouring strains were not MDR may not seem clinically relevant. However, horizontal spread is important epidemiologically. Screening of colistin resistance in human isolates of *Enterobacteriaceae* should be encouraged in order to know the real extent of a problem that may get worse given the constant exchange of resistance genes across microbiomes (i.e. food animals, the environment and human populations). The broad veterinary use of colistin and the increasing reports of colistin resistance in *Enterobacteriaceae* isolates from food animals are a matter of concern [15]. Spain is one of the European countries with larger use of polymyxins in veterinary medicine [16]. This fact may correlate with the high rates of colistin-resistant *Salmonella* spp. isolates in farm animals previously reported in our country [17]. The use of colistin in humans varies depending on the type of institution involved and their corresponding antimicrobial policy. In our hospital, it has increased 14-fold (0.10 to 1.47 defined daily dose /100 occupied bed-days) from 2007 to 2014.

Considering that the emerging plasmid-mediated resistance to colistin has already spread across microbiomes and considering the selective pressure that the veterinary use of this antibiotic may exert, action is urgent at a global level. Otherwise we may soon face a situation without useful antibiotics to treat infections caused by MDR Gram-negative bacteria.

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Conflict of interest

None declared.

Authors' contributions

NP, AR and BM conceived and designed the study; NP and AR performed the antimicrobial susceptibility tests; JRN and ME performed the molecular assays; MT collected the epidemiological data; NP, AR, MT, PC and BM wrote the manuscript.

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Predominance of influenza A(H1N1)pdm09 virus genetic subclade 6B.1 and influenza B/Victoria lineage viruses at the start of the 2015/16 influenza season in Europe

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Influenza A(H1N1)pdm09 viruses predominated in the European influenza 2015/16 season. Most analysed viruses clustered in a new genetic subclade 6B.1, antigenically similar to the northern hemisphere vaccine component A/California/7/2009. The predominant influenza B lineage was Victoria compared with Yamagata in the previous season. It remains to be evaluated at the end of the season if these changes affected the effectiveness of the vaccine for the 2015/16 season.

For the current northern hemisphere season, several reports have indicated intense influenza activity [1-5]. We analysed virological surveillance data from 20 European countries to study the genetic and antigenic characteristics of the circulating influenza viruses and compare them with the vaccine viruses and previously circulating strains.

Virological influenza surveillance in Europe, influenza season 2015/16

Virological influenza surveillance data in the World Health Organization (WHO) European Region are collected on a weekly basis and reported to The European Surveillance System (TESSy), a database hosted by the European Centre for Disease Prevention and Control (ECDC), as previously described [6]. From week 40/2015 to week 4/2016, 49 Member States of the Region reported influenza virus detections to TESSy, including 20 Member States (Belgium, Croatia, Czech Republic, Denmark, Finland, Germany, Greece, Ireland, Latvia, Netherlands, Norway, Portugal, Romania, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, and the United Kingdom (UK)) that also reported antigenic or genetic characterisation data.

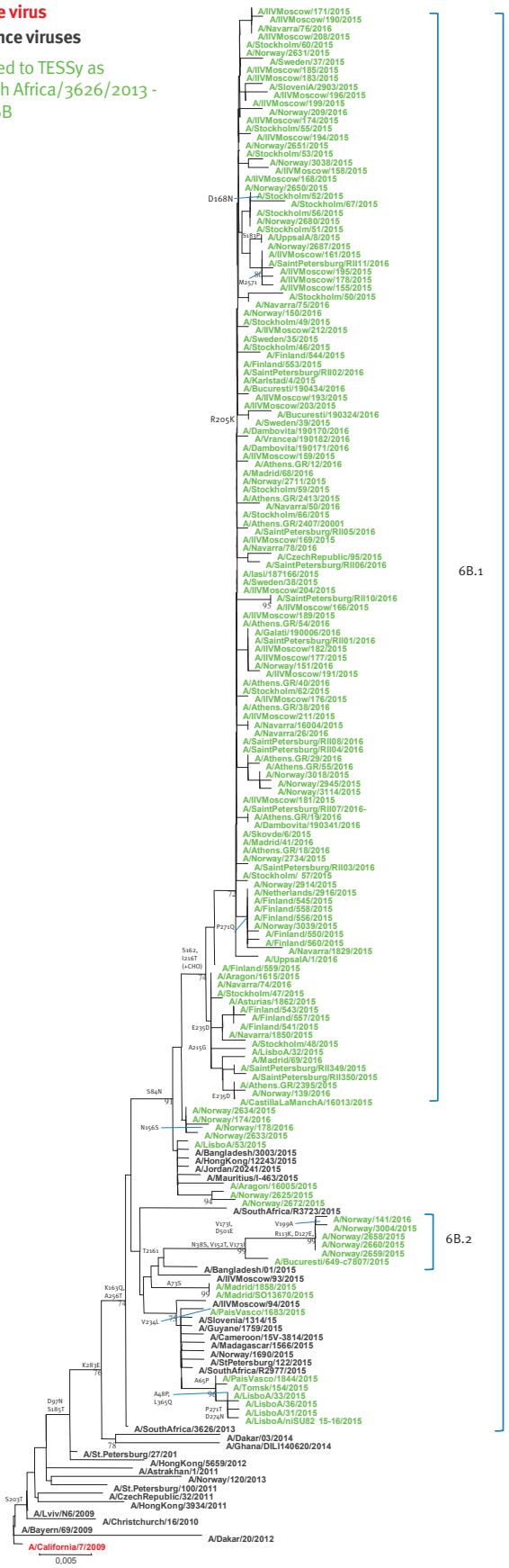
The antigenic and genetic reporting categories for TESSy are predefined by the WHO Collaborating Centre for Reference and Research on Influenza, London, for each influenza season. For antigenic characterisation, to denote a virus isolate as being like a vaccine or reference virus its haemagglutination inhibition (HI) titre with post-infection ferret antiserum raised against the reference virus should differ by no more than fourfold. For genetic characterisation, the allocation to reporting category is based on the phylogenetic and amino acid sequence analyses of haemagglutinin (HA) gene.

The summary analysis of the data are presented weekly in the Joint ECDC–WHO Regional Office for Europe weekly 'Flu News Europe' (http://flunewseurope.org/). Data on detections, antigenic and genetic characterisations were extracted on 8 February 2016 for analysis.

Between week 40/2015 and week 4/2016, influenza viruses were detected in 1,879 (19%) of 9,882 sentinel specimens tested in the 20 countries also reporting on virus characterisation. Of these 1,879 specimens, 1,512 (80%) were positive for type A influenza virus and 367 (20%) for type B. Of 1,441 subtyped influenza A viruses, 1,268 (88%) were A(H1N1)pdm09. Of 129 type B viruses with known lineage, 115 (89%) were of the B/Victoria/2/1987 lineage.

Figure 1

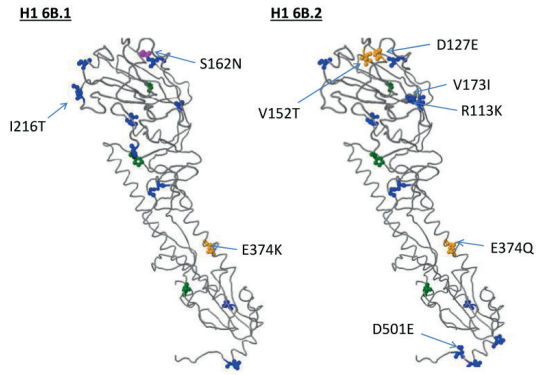
Phylogenetic analysis of A(H1N1)pdm09 haemagglutinin (HA) nt sequences reported from European countries, between week 40/2015 and 4/2016



Some sequences obtained in this study were not used to construct the phylogenetic tree because they were identical and redundant. The sequences used for the phylogenetic analysis were moreover only those of suitable length, and encode HA1 amino acids 3–327. These included sequences reported by the Czech Republic, Finland, Greece, Netherlands, Norway, Portugal, Romania, Russia, Slovenia, Spain and Sweden as well as sequences from reference A(H1N1)pdm09 viruses. The tree was constructed with the neighbour-joining method, using Kimura-2 parameter-corrected distances and bootstrapped with 1,000 replicates, Molecular Evolutionary Genetics Analysis (MEGA) software version 5.0.

Figure 2

Protein structure model (FluSurver-JSmol) of the haemagglutinin protein monomer of A(H1N1)pdm09 subclade 6B.1, represented by A/Norway/2650/2015 (left), and subclade 6B.2, represented by A/Norway/2658/2015 (right)



Amino acid differences compared with A/California/07/2009 are indicated in colour. Well-known differences are marked in blue. Common variant marker positions are indicated in green. Amino acid involved in virulence or antigenic drift is marked in orange. Amino acid not previously associated with a specific feature is marked in grey. Amino acid that creates a new potential N-glycosylation site is marked in magenta.

Virus characterisation

Between weeks 40/2015 and 4/2016, 447 (24%) of 1,879 influenza viruses were attributed to a genetic group by 16 countries (Belgium, Czech Republic, Denmark, Finland, Germany, Greece, Ireland, Netherlands, Norway, Portugal, Romania, Russia, Slovenia, Spain, Sweden and UK), and 429 (23%) were attributed to an antigenic category by also 16 reporting countries (Croatia, Czech Republic, Denmark, Finland, Germany, Greece, Latvia, Netherlands, Portugal, Romania, Russia, Slovakia, Slovenia, Spain, Switzerland and UK) (Table 1).

The majority (68%) of all genetic characterisations were reported from Norway (n=84), Spain (n=66), Germany (n=54), Russia (n=54) and Sweden (n=46). The majority (70%) of antigenic reports were from Russia (n=124), Portugal (n=99) and Germany (n=78). For 150 viruses, reported in strain-based manner, both genetic and antigenic data were available.

All 313 A(H1N1)pdm09 viruses characterised genetically fell in clade 6, subgroup 6B, represented by A/South Africa/3626/2013. Viruses falling in this genetic subgroup, were all attributed to an antigenic category A/California/7/2009 that corresponds to the component included in the 2015/16 northern hemisphere vaccines.

Of the 77 A(H3N2) viruses attributed to a genetic group, 50 (65%) fell into genetic subgroup 3C.2a (represented by A/Hong Kong/4801/2014) that has been shown to be antigenically similar to A/Hong Kong/4801/2014 and also to the current A(H3N2) vaccine virus A/Switzerland/9715293/2013 (Table 1). Twenty-six A(H3N2) viruses fell into the vaccine virus category of 3C.3a subgroup. Viruses in subgroup 3C.3b (represented by A/Stockholm/28/2014) constituted a substantial part (98/401) of the A(H3N2) viruses in Europe in the 2014/15 season [7], but none were yet reported by week 4/2016 (Table 1). Of 20 A(H3N2) viruses attributed to an antigenic category, 14 were A/Switzerland/9715293/2013-like and thus similar to the northern hemisphere 2015/16 vaccine component and six were A/Hong Kong/4801/2014-like, similar to the southern hemisphere 2016 vaccine component and recommendation for northern hemisphere 2016/17 season.

All of the 44 B/Victoria lineage viruses characterised genetically to date fell in the clade 1A, represented by B/Brisbane/60/2008 which is included in quadrivalent vaccines for northern hemisphere 2015/16. The 13 B/Yamagata lineage viruses all genetically resembled B/Phuket/3073/2013 recommended for inclusion in trivalent vaccines for northern hemisphere 2015/16. Thirty influenza B viruses were antigenically characterised, 29 as B/Brisbane/60/2008-like and one as B/Phuket/3073/2013-like.

Analysis of A(H1N1)pdm09 HA gene sequences from 12 countries (Czech Republic, Finland, Greece, Ireland, Netherlands, Norway, Portugal, Romania, Russia, Slovenia, Spain and Sweden) reported to TESSy, with provision of accession numbers in publicly accessible databases, confirmed that all these analysed viruses possessed the signature amino acid variations that define subgroup 6B viruses: D97N, K163Q, S185T, K283E and A256T [7-9]. All 215 analysed sequences, apart from two viruses isolated in Russia, also carried P83S and I321V substitutions in HA1.

The majority of sequences (173 of 215 TESSy-reported viruses) also possessed the amino acid signature of subclade 6B.1 and formed a separate branch in the phylogenetic analysis (Figure 1, Figure 2). The 6B.1 subclade is characterised by the amino acid substitutions S84N (present in a wider subgroup), S162N and I216T [8]. Six viruses carried amino acid substitutions V152T, V173I, D501E (the latter in HA2) characterising 6B.2 subclade. In addition the five most recently sampled of these six 6B.2 viruses all possessed the R113K, D127E and E374Q substitutions (Figure 1).

The highest number of accumulated variations in the known antigenic sites were observed in the antigenic site Ca. All subgroup 6B viruses possessed the K163Q substitution, while the vast majority (173/215; 80%) also possessed the S162N substitution in HA1, resulting in a gain of a potential glycosylation site. Additional

Table 1

Viruses attributed to genetic and antigenic groups[a], weeks 40/2015–04/2016

Genetic group	Number of viruses	Antigenic group	Number of viruses
A(H1N1)pdm09 A/South Africa/3626/2013 (subgroup 6B)[b]	313	A(H1N1)pdm09 A/California/7/2009-like	379
A(H3N2) A/Hong Kong/4801/2014 (subgroup 3C.2a)[b]	50	A(H3N2) A/Hong Kong/4801/2014-like	6
A(H3N2) A/Samara/73/2013 (subgroup 3C.3)[c]	1	No separate antigenic category; expected to resemble A/Stockholm/28/2014	–
A(H3N2) A/Stockholm/28/2014 (subgroup 3C.3b)[c]	0	A(H3N2) A/ Stockholm/28/2014-like	0
A(H3N2) A/Switzerland/9715293/2013 (subgroup 3C.3a)[b]	26	A(H3N2) A/Switzerland/9715293/2013	14
B/Phuket/3073/2013 (Yamagata lineage clade 3)[b]	13	B/Phuket/3073/2013 (Yamagata lineage) -like	1
B/Brisbane/60/2008 (Victoria lineage clade 1A)[d]	44	B/Brisbane/60/2008 (Victoria lineage) -like	29

The viruses which were genetically characterised are not necessarily the same than the viruses that were antigenically characterised.

[a] Genetic and antigenic groups used for reporting into The European Surveillance System are defined by World Health Organization Collaborating Centre for Reference and Research on Influenza for each influenza season. For antigenic characterisation, to denote a virus isolate as being like a vaccine or reference virus its haemagglutination inhibition (HI) titre with post-infection ferret antiserum raised against the reference virus should differ by no more than fourfold. For genetic characterisation, the allocation to reporting category is based on the phylogenetic and amino acid sequence analyses of haemagglutinin (HA) gene.

[b] These genetic groups contain viruses with antigenic properties similar to the viruses included in the trivalent influenza vaccine for 2015/16.

[c] These genetic groups contain viruses with antigenic properties dissimilar to the viruses included in the trivalent influenza vaccine for 2015/16.

[d] Viruses in this genetic group have antigenic properties similar to those of the vaccine component (B/Brisbane/60/2008) recommended for use in quadrivalent influenza vaccines for 2015/16.

variations observed were S162K, D168N, K170E, R205K, A215G, E235D and a partial A139D. Cb antigenic site variation A73S was observed in four viruses from Spain, one of which also possessed substitution N156K in Sa antigenic site. Another Norwegian virus had a N156S substitution in Sa antigenic site. Notably, all 6B.2 viruses and also two of the 6B viruses not belonging to any of the newly identified subgroups possessed substitutions affecting the loop that consists of amino acid positions 151 to 159 located adjacent to the receptor binding site.

When comparing the A(H1N1)pdm09 strains with the corresponding strain in the current northern hemisphere influenza vaccine, A/California/7/2009, the HA1 sequences (nt 1–981, amino acids 1–327) exhibited nt similarity of 96.8 to 98.0% and deduced amino acid similarity of 95.4 to 96.3%. Viruses within subclade 6B.1 exhibited higher HA nt heterogeneity, with similarities ranging between 98.8 and 100%, while within subclade 6B.2 strains exhibited higher nt similarity, ranging between 99.3 and 100%, as the group consists of fewer sequences and most of them from one region only. The viruses analysed phylogenetically are listed in Table 2.

Discussion

Continuous surveillance of influenza viruses is essential for detecting emerging new variant strains and providing viruses for vaccine production [10]. In Europe, within the detected A subtypes, influenza A(H1N1)pdm09 predominated during 2010/11, 2012/13 and 2013/14 seasons and concerned 97% [11], 62% [12] and 53% [13] of subtyped influenza viruses respectively, with variation in country-specific proportions. The A(H1N1)pdm09 vaccine component A/California/7/2009 has not been changed since the 2009 pandemic and the circulating A(H1N1)pdm09 viruses have remained antigenically similar to the virus included in the vaccines throughout the influenza 2009/10 to 2015/16 seasons. However, since 2013, several reports have indicated the emergence of an expanding subgroup of A(H1N1)pdm09 viruses, designated 6B [1,8,9]. This subgroup appeared in 2012/13 and became predominant in 2013/14 [14].

In this study, we observe the further emergence of a subclade within the 6B subgroup, designated 6B.1 [15], which accounted for the majority of the A(H1N1)pdm09 viruses detected across the WHO European Region during the first weeks of the 2015/16 influenza season. In addition, the surveillance data show a change in the predominant B virus lineage from B/Yamagata which predominated in the preceding three seasons in Europe to B/Victoria.

Our data are preliminary for this season and are based on influenza surveillance without detailed reporting of clinical symptoms or vaccination status. Our genetic analysis was only based on the HA gene and does not extend to changes e.g. in genes encoding internal proteins of influenza viruses. The data reported to TESSy do not include antigenic titres and therefore no direct analysis of antigenic properties was possible. However, the antigenic reports rely on national influenza centres' antigenic analysis that the viruses reported as like to vaccine virus were not more than fourfold different in HI titres from the vaccine or reference viruses.

Table 2a

Details of the A(H1N1)pdm09 sequences retrieved from the Global Initiative on Sharing All Influenza Data (GISAID)'s EpiFlu Database or GenBank, for haemagglutinin-gene-based phylogenetic analysis in this study

ID	Country	Strain name	Collection date	Originating laboratory	Submitting laboratory
EPI685415	Netherlands	A/Netherlands/2916/2015	6/11/2015	National Institute for Public Health and the Environment (RIVM)	National Institute for Public Health and the Environment (RIVM)
EPI674853	Sweden	A/Stockholm/46/2015	9/10/2015	–	Swedish Institute for Infectious Disease Control
EPI674745	Sweden	A/Stockholm/47/2015	22/10/2015	–	Swedish Institute for Infectious Disease Control
EPI674753	Sweden	A/Stockholm/48/2015	24/10/2015	–	Swedish Institute for Infectious Disease Control
EPI674841	Sweden	A/Karlstad/4/2015	25/10/2015	–	Swedish Institute for Infectious Disease Control
EPI674777	Sweden	A/Stockholm/49/2015	7/11/2015	–	Swedish Institute for Infectious Disease Control
EPI686820	Sweden	A/Skovde/6/2015	18/11/2015	–	Swedish Institute for Infectious Disease Control
EPI686772	Sweden	A/Stockholm/ 57/2015	18/11/2015	–	Swedish Institute for Infectious Disease Control
EPI674785	Sweden	A/Stockholm/50/2015	11/11/2015	–	Swedish Institute for Infectious Disease Control
EPI674793	Sweden	A/Stockholm/51/2015	11/11/2015	–	Swedish Institute for Infectious Disease Control
EPI674801	Sweden	A/Stockholm/52/2015	11/11/2015	–	Swedish Institute for Infectious Disease Control
EPI674847	Sweden	A/Stockholm/53/2015	12/11/2015	–	Swedish Institute for Infectious Disease Control
EPI674809	Sweden	A/Stockholm/55/2015	10/11/2015	–	Swedish Institute for Infectious Disease Control
EPI686764	Sweden	A/Stockholm/56/2015	18/11/2015	–	Swedish Institute for Infectious Disease Control
EPI686799	Sweden	A/Stockholm/59/2015	19/11/2015	–	Swedish Institute for Infectious Disease Control
EPI686828	Sweden	A/Stockholm/60/2015	25/11/2015	–	Swedish Institute for Infectious Disease Control
EPI686844	Sweden	A/Stockholm/62/2015	26/11/2015	–	Swedish Institute for Infectious Disease Control
EPI687173	Sweden	A/Stockholm/66/2015	23/11/2015	–	Swedish Institute for Infectious Disease Control
EPI687199	Sweden	A/Stockholm/67/2015	21/11/2015	–	Swedish Institute for Infectious Disease Control
EPI674825	Sweden	A/Sweden/35/2015	12/11/2015	–	Swedish Institute for Infectious Disease Control
EPI686852	Sweden	A/Uppsala/8/2015	27/11/2015	–	Swedish Institute for Infectious Disease Control
EPI686892	Sweden	A/Sweden/37/2015	26/11/2015	–	Swedish Institute for Infectious Disease Control
EPI686900	Sweden	A/Sweden/38/2015	2/12/2015	–	Swedish Institute for Infectious Disease Control
EPI686908	Sweden	A/Sweden/39/2015	2/12/2015	–	Swedish Institute for Infectious Disease Control
EPI694343	Sweden	A/Uppsala/1/2016	11/1/2016	–	Swedish Institute for Infectious Disease Control
EPI671518	Norway	A/Norway/2625/2015	21/10/2015	Sorlandet Sykehus HF, Dept. of Medical Microbiology	Norwegian Institute of Public Health
EPI675750	Norway	A/Norway/2659/2015	3/11/2015	Drammen Hospital / Vestreviken HF, Department for Medical Microbiology section Drammen	Norwegian Institute of Public Health
EPI675751	Norway	A/Norway/2660/2015	3/11/2015	Drammen Hospital / Vestreviken HF, Department for Medical Microbiology section Drammen	Norwegian Institute of Public Health

ID: identity; SAR: Special Administrative Region; WHO: World Health Organization.

Table 2B

Details of the A(H1N1)pdm09 sequences retrieved from the Global Initiative on Sharing All Influenza Data (GISAID)'s EpiFlu Database or GenBank, for haemagglutinin-gene-based phylogenetic analysis in this study

ID	Country	Strain name	Collection date	Originating laboratory	Submitting laboratory
EPI675754	Norway	A/Norway/2672/2015	1/11/2015	Oslo University Hospital, Ulleval Hospital, Dept. of Microbiology	Norwegian Institute of Public Health
EPI675756	Norway	A/Norway/2680/2015	12/11/2015	Ostfold Hospital - Fredrikstad, Dept. of Microbiology	Norwegian Institute of Public Health
EPI675760	Norway	A/Norway/2687/2015	13/11/2015	–	Norwegian Institute of Public Health
EPI695284	Norway	A/Norway/2711/2015	18/11/2015	–	Norwegian Institute of Public Health
EPI695299	Norway	A/Norway/2914/2015	14/12/2015	Sorlandet Sykehus HF, Dept. of Medical Microbiology	Norwegian Institute of Public Health
EPI695310	Norway	A/Norway/3004/2015	15/12/2015	Innlandet Hospital Trust, Division Lillehammer, Department for Microbiology	Norwegian Institute of Public Health
EPI695311	Norway	A/Norway/3018/2015	26/12/2015	–	Norwegian Institute of Public Health
EPI695313	Norway	A/Norway/3038/2015	26/12/2015	Aalesund sjukehus	Norwegian Institute of Public Health
EPI695343	Norway	A/Norway/174/2016	7/1/2016	St. Olavs Hospital HF, Dept. of Medical Microbiology	Norwegian Institute of Public Health
EPI695344	Norway	A/Norway/178/2016	6/1/2016	Health Forde, Department of Microbiology	Norwegian Institute of Public Health
EPI695349	Norway	A/Norway/209/2016	8/1/2016	Stavanger Universitetssykehus, Avd. for Medisinsk Mikrobiologi	Norwegian Institute of Public Health
EPI677648	Finland	A/Finland/541/2015	9/11/2015	Helsinki University Central Hospital, Laboratory Services (HUSLAB)	National Institute for Health and Welfare
EPI677651	Finland	A/Finland/543/2015	19/11/2015	Helsinki University Central Hospital, Laboratory Services (HUSLAB)	National Institute for Health and Welfare
EPI678232	Finland	A/Finland/544/2015	13/11/2015	Helsinki University Central Hospital, Laboratory Services (HUSLAB)	National Institute for Health and Welfare
EPI696158	Russia	A/Tomsk/154/2015	19/11/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696470	Russia	A/IIV-Moscow/211/2015	23/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696478	Russia	A/IIV-Moscow/212/2015	23/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI690291	Spain	A/Aragon/16005/2015	21/12/2015	Servicio de Microbiología Hospital Miguel Servet	Instituto de Salud Carlos III
EPI671520	Norway	A/Norway/2631/2015	26/10/2015	Sorlandet Sykehus HF, Dept. of Medical Microbiology	Norwegian Institute of Public Health
EPI671521	Norway	A/Norway/2633/2015	27/10/2015	Haukeland University Hospital, Dept. of Microbiology	Norwegian Institute of Public Health
EPI671522	Norway	A/Norway/2634/2015	27/10/2015	Haukeland University Hospital, Dept. of Microbiology	Norwegian Institute of Public Health
EPI671525	Norway	A/Norway/2650/2015	3/11/2015	Ostfold Hospital - Fredrikstad, Dept. of Microbiology	Norwegian Institute of Public Health
EPI675748	Norway	A/Norway/2651/2015	2/11/2015	Mikrobiologisk laboratorium, Sykehuset i Vestfold	Norwegian Institute of Public Health
EPI675749	Norway	A/Norway/2658/2015	4/11/2015	Drammen Hospital / Vestreviken HF, Department for Medical Microbiology section Drammen	Norwegian Institute of Public Health
EPI695334	Norway	A/Norway/139/2016	4/1/2016	Haukeland University Hospital, Dept. of Microbiology	Norwegian Institute of Public Health
EPI695336	Norway	A/Norway/141/2016	4/1/2016	Unilabs Telelab, Laboratory for Medical Microbiology	Norwegian Institute of Public Health

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Table 2c

Details of the A(H1N1)pdm09 sequences retrieved from the Global Initiative on Sharing All Influenza Data (GISAID)'s EpiFlu Database or GenBank, for haemagglutinin-gene-based phylogenetic analysis in this study

ID	Country	Strain name	Collection date	Originating laboratory	Submitting laboratory
EPI695339	Norway	A/Norway/150/2016	12/1/2016	Stavanger Universitetssykehus, Avd. for Medisinsk Mikrobiologi	Norwegian Institute of Public Health
EPI695340	Norway	A/Norway/151/2016	12/1/2016	Stavanger Universitetssykehus, Avd. for Medisinsk Mikrobiologi	Norwegian Institute of Public Health
EPI695287	Norway	A/Norway/2734/2015	13/11/2015	Innlandet Hospital Trust, Division Lillehammer, Department for Microbiology	Norwegian Institute of Public Health
EPI695304	Norway	A/Norway/2945/2015	16/12/2015	–	Norwegian Institute of Public Health
EPI695314	Norway	A/Norway/3039/2015	28/12/2015	Aalesund sjukehus	Norwegian Institute of Public Health
EPI695326	Norway	A/Norway/3114/2015	28/12/2015	Drammen Hospital / Vestreviken HF, Department for Medical Microbiology section Drammen	Norwegian Institute of Public Health
EPI678234	Finland	A/Finland/545/2015	19/11/2015	Helsinki University Central Hospital, Laboratory Services (HUSLAB)	National Institute for Health and Welfare
EPI678238	Finland	A/Finland/550/2015	4/12/2015	National Institute for Health and Welfare	National Institute for Health and Welfare
EPI678240	Finland	A/Finland/553/2015	6/12/2015	NordLab Oulu	National Institute for Health and Welfare
EPI693689	Finland	A/Finland/556/2015	16/12/2015	National Institute for Health and Welfare	National Institute for Health and Welfare
EPI687734	Finland	A/Finland/557/2015	15/12/2015	National Institute for Health and Welfare	National Institute for Health and Welfare
EPI693690	Finland	A/Finland/558/2015	19/12/2015	National Institute for Health and Welfare	National Institute for Health and Welfare
EPI693691	Finland	A/Finland/559/2015	14/12/2015	National Institute for Health and Welfare	National Institute for Health and Welfare
EPI693692	Finland	A/Finland/560/2015	18/12/2015	National Institute for Health and Welfare	National Institute for Health and Welfare
EPI674284	Portugal	A/Lisboa/31/2015	19/11/2015	Instituto Nacional de Saude	INSA National Institute of Health Portugal
EPI674285	Portugal	A/Lisboa/32/2015	18/11/2015	Instituto Nacional de Saude	INSA National Institute of Health Portugal
EPI678690	Portugal	A/Lisboa/33/2015	25/11/2015	Instituto Nacional de Saude	INSA National Institute of Health Portugal
EPI678691	Portugal	A/Lisboa/36/2015	2/12/2015	Instituto Nacional de Saude	INSA National Institute of Health Portugal
EPI692997	Portugal	A/Lisboa/53/2015	22/12/2015	Instituto Nacional de Saude	INSA National Institute of Health Portugal
EPI678693	Portugal	A/Lisboa/niSU82_15–16/2015	2/12/2015	Instituto Nacional de Saude	INSA National Institute of Health Portugal
EPI699780	Greece	A/Athens.GR/18/2016	4/1/2016	Hellenic Pasteur Institute	Hellenic Pasteur Institute
EPI699778	Greece	A/Athens.GR/19/2016	4/1/2016	Hellenic Pasteur Institute	Hellenic Pasteur Institute
EPI699774	Greece	A/Athens.GR/29/2016	7/1/2016	Hellenic Pasteur Institute	Hellenic Pasteur Institute
EPI699772	Greece	A/Athens.GR/38/2016	7/1/2016	Hellenic Pasteur Institute	Hellenic Pasteur Institute
EPI699770	Greece	A/Athens.GR/40/2016	7/1/2016	Hellenic Pasteur Institute	Hellenic Pasteur Institute
EPI699766	Greece	A/Athens.GR/54/2016	8/1/2016	Hellenic Pasteur Institute	Hellenic Pasteur Institute
EPI699764	Greece	A/Athens.GR/55/2016	8/1/2016	Hellenic Pasteur Institute	Hellenic Pasteur Institute
EPI670326	Romania	A/Iasi/187166/2015	13/10/2015	Cantacuzino Institute	Cantacuzino Institute
EPI690111	Romania	A/Bucuresti/649-c7807/2015	22/12/2015	Cantacuzino Institute	Cantacuzino Institute
EPI699023	Romania	A/Bucuresti/190460/2016	19/1/2016	Cantacuzino Institute	Cantacuzino Institute
EPI696174	Russia	A/IIV-Moscow/158/2015	12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation

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Table 2d

Details of the A(H1N1)pdm09 sequences retrieved from the Global Initiative on Sharing All Influenza Data (GISAID)'s EpiFlu Database or GenBank, for haemagglutinin-gene-based phylogenetic analysis in this study

ID	Country	Strain name	Collection date	Originating laboratory	Submitting laboratory
EPI696182	Russia	A/IIV-Moscow/159/2015	12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696198	Russia	A/IIV-Moscow/161/2015	14/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696246	Russia	A/IIV-Moscow/169/2015	17/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696270	Russia	A/IIV-Moscow/174/2015	16/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696278	Russia	A/IIV-Moscow/176/2015	15/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696286	Russia	A/IIV-Moscow/177/2015	16/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696326	Russia	A/IIV-Moscow/183/2015	21/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696382	Russia	A/IIV-Moscow/191/2015	20/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696414	Russia	A/IIV-Moscow/195/2015	22/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI687093	Russia	A/Saint-Petersburg/RII349/2015	25/11/2015		WHO National Influenza Centre Russian Federation
EPI696574	Russia	A/Saint-Petersburg/RII350/2015	30/11/2015	WHO National Influenza Centre Russian Federation	WHO National Influenza Centre Russian Federation
EPI696486	Russia	A/Saint-Petersburg/RII01/2016	19/12/2015	WHO National Influenza Centre Russian Federation	WHO National Influenza Centre Russian Federation
EPI696494	Russia	A/Saint-Petersburg/RII02/2016	21/12/2015	WHO National Influenza Centre Russian Federation	WHO National Influenza Centre Russian Federation
EPI696502	Russia	A/Saint-Petersburg/RII03/2016	21/12/2015	WHO National Influenza Centre Russian Federation	WHO National Influenza Centre Russian Federation
EPI696510	Russia	A/Saint-Petersburg/RII04/2016	21/12/2015	WHO National Influenza Centre Russian Federation	WHO National Influenza Centre Russian Federation
EPI696166	Russia	A/IIV-Moscow/155/2015	7/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696518	Russia	A/Saint-Petersburg/RII05/2016	14/12/2015	WHO National Influenza Centre Russian Federation	WHO National Influenza Centre Russian Federation
EPI696526	Russia	A/Saint-Petersburg/RII06/2016	14/12/2015	WHO National Influenza Centre Russian Federation	WHO National Influenza Centre Russian Federation
EPI696534	Russia	A/Saint-Petersburg/RII07/2016	21/12/2015	WHO National Influenza Centre Russian Federation	WHO National Influenza Centre Russian Federation
EPI696542	Russia	A/Saint-Petersburg/RII08/2016	22/12/2015	WHO National Influenza Centre Russian Federation	WHO National Influenza Centre Russian Federation
EPI696558	Russia	A/Saint-Petersburg/RII10/2016	23/12/2015	WHO National Influenza Centre Russian Federation	WHO National Influenza Centre Russian Federation
EPI696566	Russia	A/Saint-Petersburg/RII11/2016	24/12/2015	WHO National Influenza Centre Russian Federation	WHO National Influenza Centre Russian Federation
EPI696222	Russia	A/IIV-Moscow/166/2015	16/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696238	Russia	A/IIV-Moscow/168/2015	15/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation

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Table 2e

Details of the A(H1N1)pdm09 sequences retrieved from the Global Initiative on Sharing All Influenza Data (GISAID)'s EpiFlu Database or GenBank, for haemagglutinin-gene-based phylogenetic analysis in this study

ID	Country	Strain name	Collection date	Originating laboratory	Submitting laboratory
EPI696254	Russia	A/IIV-Moscow/171/2015	17/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696294	Russia	A/IIV-Moscow/178/2015	17/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696318	Russia	A/IIV-Moscow/182/2015	18/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696334	Russia	A/IIV-Moscow/185/2015	18/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696366	Russia	A/IIV-Moscow/189/2015	19/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696374	Russia	A/IIV-Moscow/190/2015	20/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696398	Russia	A/IIV-Moscow/193/2015	21/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696406	Russia	A/IIV-Moscow/194/2015	20/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696422	Russia	A/IIV-Moscow/196/2015	22/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696430	Russia	A/IIV-Moscow/199/2015	22/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696446	Russia	A/IIV-Moscow/203/2015	22/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696454	Russia	A/IIV-Moscow/204/2015	22/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI696462	Russia	A/IIV-Moscow/208/2015	23/12/2015	D.I. Ivanovsky Research Institute of virology MoPH of RF,Moscow	WHO National Influenza Centre Russian Federation
EPI686526	Spain	A/Madrid/1858/2015	22/12/2015	Servicio de Microbiología Hospital Ramón y Cajal	Instituto de Salud Carlos III
EPI690296	Spain	A/Madrid/1859/2015	23/12/2015	Servicio de Microbiología Hospital Ramón y Cajal	Instituto de Salud Carlos III
EPI672780	Spain	A/Madrid/SO13656/2015	21/10/2015	Instituto de Salud Carlos III	Instituto de Salud Carlos III
EPI674599	Spain	A/Madrid/SO13670/2015	20/10/2015	Instituto de Salud Carlos III	Instituto de Salud Carlos III
EPI680490	Spain	A/Madrid/SO13763/2015	8/12/2015	Instituto de Salud Carlos III	Instituto de Salud Carlos III
EPI699957	Spain	A/Madrid/41/2016	13/1/2016	Instituto de Salud Carlos III	Instituto de Salud Carlos III
EPI699959	Spain	A/Madrid/68/2016	12/1/2016	Servicio de Microbiología Hospital Ramón y Cajal	Instituto de Salud Carlos III
EPI699960	Spain	A/Madrid/69/2016	12/1/2016	Servicio de Microbiología Hospital Ramón y Cajal	Instituto de Salud Carlos III
EPI690298	Spain	A/Navarra/16004/2015	27/12/2015	Servicio de Microbiología Complejo Hospitalario de Navarra	Instituto de Salud Carlos III
EPI686527	Spain	A/Navarra/1829/2015	15/12/2015	Servicio de Microbiología Complejo Hospitalario de Navarra	Instituto de Salud Carlos III
EPI686528	Spain	A/Navarra/1850/2015	17/12/2015	Servicio de Microbiología Complejo Hospitalario de Navarra	Instituto de Salud Carlos III

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Table 2f

Details of the A(H1N1)pdm09 sequences retrieved from the Global Initiative on Sharing All Influenza Data (GISAID)'s EpiFlu Database or GenBank, for haemagglutinin-gene-based phylogenetic analysis in this study

ID	Country	Strain name	Collection date	Originating laboratory	Submitting laboratory
EPI690302	Spain	A/Navarra/26/2016	3/1/2016	Servicio de Microbiología Complejo Hospitalario de Navarra	Instituto de Salud Carlos III
EPI699967	Spain	A/Navarra/50/2016	11/1/2016	Servicio de Microbiología Complejo Hospitalario de Navarra	Instituto de Salud Carlos III
EPI699973	Spain	A/Navarra/74/2016	14/1/2016	Servicio de Microbiología Complejo Hospitalario de Navarra	Instituto de Salud Carlos III
EPI699974	Spain	A/Navarra/75/2016	12/1/2016	Servicio de Microbiología Complejo Hospitalario de Navarra	Instituto de Salud Carlos III
EPI699975	Spain	A/Navarra/76/2016	14/1/2016	Servicio de Microbiología Complejo Hospitalario de Navarra	Instituto de Salud Carlos III
EPI699977	Spain	A/Navarra/78/2016	14/1/2016	Servicio de Microbiología Complejo Hospitalario de Navarra	Instituto de Salud Carlos III
EPI672781	Spain	A/PaisVasco/1683/2015	21/10/2015	Servicio de Microbiología Hospital Donostia	Instituto de Salud Carlos III
EPI686529	Spain	A/PaisVasco/1844/2015	15/12/2015	Servicio de Microbiología Hospital Donostia	Instituto de Salud Carlos III
EPI687827	Slovenia	A/Slovenia/2903/2015	26/10/2015	Laboratory for Virology, National Institute of Public Health	Crick Worldwide Influenza Centre
KU558983	Czech Republic	A/Czech Republic/95/2015	1/12/2015	National Institute of Public Health	National Institute of Public Health
EPI699832	Greece	A/Athens.GR/2395/2015	23/12/2015	Hellenic Pasteur Institute	Hellenic Pasteur Institute
EPI699830	Greece	A/Athens.GR/2407/2015	28/12/2015	Hellenic Pasteur Institute	Hellenic Pasteur Institute
EPI699827	Greece	A/Athens.GR/2413/2015	29/12/2015	Hellenic Pasteur Institute	Hellenic Pasteur Institute
EPI699824	Greece	A/Athens.GR/12/2016	5/1/2016	Hellenic Pasteur Institute	Hellenic Pasteur Institute
EPI698911	Romania	A/Dambovita/190170/2016	18/1/2016	Cantacuzino Institute	Cantacuzino Institute
EPI698910	Romania	A/Galati/190006/2016	8/1/2016	Cantacuzino Institute	Cantacuzino Institute
EPI699021	Romania	A/Vrancea/190182/2016	18/1/2016	Cantacuzino Institute	Cantacuzino Institute
EPI699023	Romania	A/Bucuresti/190324/2016	19/1/2016	Cantacuzino Institute	Cantacuzino Institute
EPI699059	Romania	A/Bucuresti/190434/2016	23/1/2016	Cantacuzino Institute	Cantacuzino Institute
EPI698912	Romania	A/Dambovita/190171/2016	18/1/2016	Cantacuzino Institute	Cantacuzino Institute
EPI699024	Romania	A/Dambovita/190341/2016	21/1/2016	Cantacuzino Institute	Cantacuzino Institute
EPI699000	Romania	A/Vrancea/190181/2016	11/1/2016	Cantacuzino Institute	Cantacuzino Institute
EPI672779	Spain	A/Aragon/1615/2015	29/9/2015	Servicio de Microbiología Hospital Miguel Servet	Instituto de Salud Carlos III
EPI690293	Spain	A/Asturias/1862/2015	17/12/2015	Servicio de Microbiología Hospital Central Universitario de Asturias	Instituto de Salud Carlos III
EPI699955	Spain	A/Baleares/16036/2015	30/12/2015	Servicio de Microbiología Hospital Universitario Son Espases	Instituto de Salud Carlos III
EPI699956	Spain	A/Baleares/35/2016	5/1/2016	Servicio de Microbiología Hospital Universitario Son Espases	Instituto de Salud Carlos III
EPI690295	Spain	A/CastillaLaMancha/16013/2015	30/12/2015	Instituto de Salud Carlos III	Instituto de Salud Carlos III
EPI624748	Russia	A/St-Petersburg/122/2015	26/2/2015	WHO National Influenza Centre Russian Federation	Crick Worldwide Influenza Centre
EPI624673	Cameroon	A/Cameroon/15V-3814/2015	7/5/2015	Centre Pasteur du Cameroun	Crick Worldwide Influenza Centre
EPI624730	Norway	A/Norway/1690/2015	17/3/2015	WHO National Influenza Centre	Crick Worldwide Influenza Centre

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Table 2G

Details of the A(H1N1)pdm09 sequences retrieved from the Global Initiative on Sharing All Influenza Data (GISAID)'s EpiFlu Database or GenBank, for haemagglutinin-gene-based phylogenetic analysis in this study

ID	Country	Strain name	Collection date	Originating laboratory	Submitting laboratory
EPI630638	Mauritius	A/Mauritius/I-463/2015	18/5/2015	Central Health Laboratory	Crick Worldwide Influenza Centre
EPI621835	Madagascar	A/Madagascar/1566/2015	15/4/2015	Institut Pasteur de Madagascar	Crick Worldwide Influenza Centre
EPI630634	Hong Kong SAR	A/Hong Kong/12243/2015	14/6/2015	Government Virus Unit	Crick Worldwide Influenza Centre
EPI630684	South Africa	A/South Africa/R3723/2015	29/6/2015	Sandringham, National Institute for Communicable D	Crick Worldwide Influenza Centre
EPI630676	South Africa	A/South Africa/R2977/2015	5/6/2015	Sandringham, National Institute for Communicable D	Crick Worldwide Influenza Centre
EPI630652	Slovenia	A/Slovenia/1314/15	5/3/2015	Laboratory for Virology, National Institute of Public Health	Crick Worldwide Influenza Centre
EPI624706	Russia	A/IIV-Moscow/94/2015	12/3/2015	Ivanovsky Research Institute of Virology RAMS	Crick Worldwide Influenza Centre
EPI624704	Russia	A/IIV-Moscow/93/2015	10/3/2015	Ivanovsky Research Institute of Virology RAMS	Crick Worldwide Influenza Centre
EPI589565	Jordan	A/Jordan/20241/2015	22/3/2015	Laboratory Directorate	Crick Worldwide Influenza Centre
EPI253705	Germany	A/Bayern/69/2009	1/1/2009	Robert-Koch-Institute	Robert-Koch-Institute
EPI278607	New Zealand	A/Christchurch/16/2010	12/7/2010	Canterbury Health Services	WHO Collaborating Centre for Reference and Research on Influenza
EPI319590	Russia	A/Astrakhan/1/2011	28/2/2011	WHO National Influenza Centre Russian Federation	National Institute for Medical Research
EPI319527	Russia	A/St. Petersburg/27/2011	14/2/2011	WHO National Influenza Centre Russian Federation	National Institute for Medical Research
EPI416411	Norway	A/Norway/120/2013	2/1/2013	WHO National Influenza Centre	National Institute for Medical Research
EPI574439	Ghana	A/Ghana/DILI-14–0620/2014	7/7/2014	University of Ghana	National Institute for Medical Research
EPI390473	Hong Kong SAR	A/Hong Kong/5659/2012	21/5/2012	Government Virus Unit	National Institute for Medical Research
EPI326206	Hong Kong SAR	A/Hong Kong/3934/2011	29/3/2011	Government Virus Unit	National Institute for Medical Research
EPI466626	South Africa	A/South Africa/3626/2013	6/6/2013	Sandringham, National Institute for Communicable D	National Institute for Medical Research
EPI539472	Senegal	A/Dakar/04/2014	3/2/2014	Institut Pasteur de Dakar	National Institute for Medical Research
EPI417122	Senegal	A/Dakar/20/2012	9/12/2012	Institut Pasteur de Dakar	National Institute for Medical Research
EPI319447	Czech Republic	A/Czech Republic/32/2011	18/1/2011	National Institute of Public Health	National Institute for Medical Research
EPI215957	Ukraine	A/Lviv/N6/2009	27/10/2009	Ministry of Health of Ukraine	National Institute for Medical Research
EPI320141	Russia	A/St. Petersburg/100/2011	14/3/2011	Russian Academy of Medical Sciences	Centers for Disease Control and Prevention
EPI626148	Bangladesh	A/Bangladesh/3003/2015	4/5/2015	Institute of Epidemiology Disease Control and Research (IEDCR) and Bangladesh National Influenza Centre (NIC)	Centers for Disease Control and Prevention
EPI626140	Bangladesh	A/Bangladesh/01/2015	10/5/2015	Institute of Epidemiology Disease Control and Research (IEDCR) and Bangladesh National Influenza Centre (NIC)	Centers for Disease Control and Prevention
EPI176620	United States	A/California/07/2009	9/4/2009	Naval Health Research Center	Centers for Disease Control and Prevention
EPI624468	French Guiana	A/Guyane/1759/2015	9/4/2015	Institut Pasteur	Institut Pasteur

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The data supporting the predominance of the 6B.1 subclade stem from the subset of 12 European countries that reported virus characterisation data referring to sequences available in publically accessible databases. These countries are well spread across Europe which corroborates the conclusion of widespread 6B.1 subclade circulation. Data from the WHO Collaborating Centres indicate that the new subgroup remains antigenically similar to the vaccine component A/California/7/2009 [1], but some recent A(H1N1)pdm09 viruses within the 6B.1 and 6B.2 subclades reacted poorly with sera from individuals vaccinated with A/California/7/2009-like-strain-containing vaccine [15].

The emergence of a new A(H1N1)pdm09 subclade may eventually affect the susceptibility of the population to the currently circulating A(H1N1)pdm09 viruses, e.g. by viruses drifting closer to become immune escape variants. It is not clear whether the emergence and predominance of subclade 6B.1 has been driven by immune selection or what its impact on vaccine effectiveness may be and this needs assessment e.g. by generating lineage-specific estimation of vaccine effectiveness. Early vaccine effectiveness estimates for A(H1N1)pdm09 this season compared with the previous ones are not significantly different [16] from previous seasons. As to the severity observed this season [1-4], similar observations have been made also in earlier seasons e.g. in 2010/11 in the United Kingdom, which experienced notably severe A(H1N1)pdm09 impact in the first post-pandemic season.

Notably, recent studies have demonstrated that antigenic change in A(H1N1)pdm09 viruses is mainly caused by single amino acid substitutions affecting the loop located adjacent to the receptor binding site [17]; eight of the 215 analysed 2015/16 viruses possessed such substitutions, all six of the viruses in subclade 6B.2 and two in 6B subgroup, that do not belong to any of the newly emerged subclades.

Further enhancement of the antigenicity and virulence of influenza virus has been attributed to shielding of the major antigenic epitopes by alteration of N-linked glycosylation sites [18]. D127E substitution seen in 6B.2 has been associated with antigenic change of other influenza viruses through modelling [17]. The change at position 173 (V173I) also in the 6B.2 subclade of viruses is located in antigenic site Ca1 (position 169–173), and therefore a change here could contribute to antigen drift. It has been proposed that the evolution of A(H1N1)pdm09 will involve the acquisition of additional glycosylations, as for former seasonal A(H1N1) HA [19]. Noteworthy, 80% of the analysed HA sequences have gained a potential glycosylation site S162N. No D222G/E/N substitutions were detected, nor N129D which was recently identified in India in two severe or fatal cases [9]. If the emerging groups continue to diversify from the vaccine component, their antigenic properties may change and the vaccine effectiveness might be reduced. WHO recommended not to change the vaccine component of A(H1N1)pdm09 for the northern hemisphere 2016/17 season [20].

Early vaccine effectiveness estimates for 2015/16 are not yet available for A(H3N2) and B viruses which have been detected in lower numbers in most countries. The B/Victoria virus component is available only in the quadrivalent vaccines in the northern hemisphere for this season. As the majority of the countries use trivalent vaccines, the lineage switch from B/Yamagata to B/Victoria may contribute to lower vaccine effectiveness against influenza B. For A(H3N2), the current component of influenza vaccines is expected to have improved vaccine effectiveness compared with the two previous seasons [21,22]. In the southern hemisphere, seasonal influenza vaccine has been demonstrated to have an overall effectiveness against A(H3N2) of 36% (95% confidence interval (CI): 11–54)) for general practice encounters and 50% (95%-CI: 20–68) for hospitalisations in 2015 [23]. Despite the changes in the genetic makeup of influenza A(H1N1)pdm09 viruses and the predominance of B/Victoria lineage over B/Yamagata lineage, seasonal influenza vaccine remains the single most effective measure to prevent severe outcomes of influenza.

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Conflict of interest

None declared.

Authors' contributions

Eeva Broberg: Data extraction, data maintenance, first draft of the manuscript, study design, revisions of the article. Angeliki Melidou: data processing, phylogenetic analysis and text. Katarina Prosenc: data processing, analysis, text. Karoline Bragstad: data processing, amino acid analysis, text. Olav Hungnes: data processing, analysis, text. ECDC and WHO Regional Office for Europe staff: influenza surveillance data maintenance, management and analysis. Country experts: surveillance systems, data collection, data analysis at national level and reporting to TESSy.

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Research article

Congenital cerebral malformations and dysfunction in fetuses and newborns following the 2013 to 2014 Zika virus epidemic in French Polynesia

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We detected an unusual increase in congenital cerebral malformations and dysfunction in fetuses and newborns in French Polynesia, following an epidemic of Zika virus (ZIKV), from October 2013 to March 2014. A retrospective review identified 19 cases, including eight with major brain lesions and severe microcephaly, six with severe cerebral lesions without microcephaly and five with brainstem dysfunction without visible malformations. Imaging revealed profound neurological lesions (septal and callosal disruption, ventriculomegaly, abnormal neuronal migration, cerebellar hypoplasia, occipital pseudocysts, brain calcifications). Amniotic fluid was drawn from seven cases at gestation weeks 20 to 29. ZIKV RNA was detected by RT-PCR and infectious ZIKV isolates were obtained in four of five microcephalic, but not in two non-microcephalic cases with severe brain lesions. Medical termination of pregnancy was performed in eleven cases; two cases with brainstem dysfunction died in the first months of life; six cases are alive, with severe neurological impairment. The results show that four of seven tested fetuses with major neurological injuries were infected with ZIKV in utero. For other non-microcephalic, congenital abnormalities we were not able to prove or exclude ZIKV infection retrospectively. The unusual occurrence of brain malformations or dysfunction without microcephaly following a ZIKV outbreak needs further studies.

Introduction

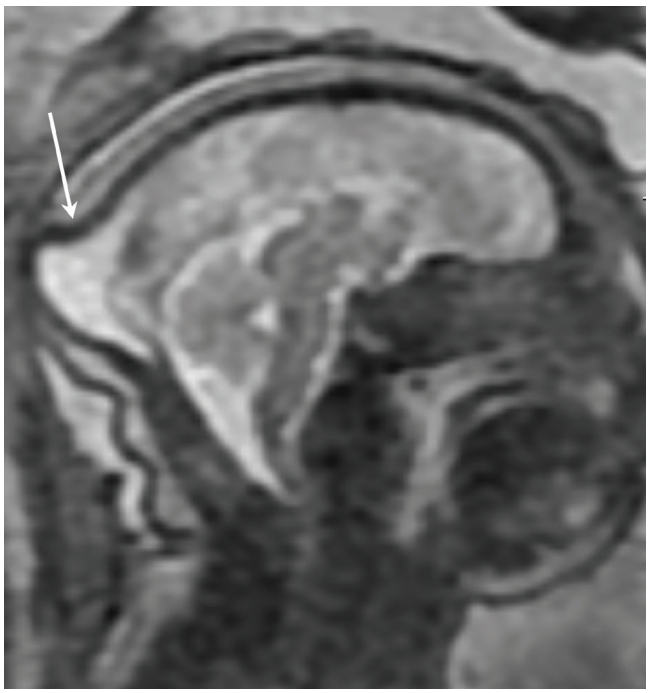
A Zika virus (ZIKV) outbreak in French Polynesia from October 2013 to March 2014 resulted in 8,750 suspected cases reported through the general practitioners-based (25 to 45 sentinel practitioners) sentinel surveillance system for infectious diseases. The system exists since 2009 and syndromic cases definitions are basis for weekly reporting. An estimated 32,000 suspected cases sought medical care and more than half of the population might have been infected [1,2]. ZIKV is an emerging arbovirus that before 2013 was considered to cause only mild disease, characterised by fever, rash, joint pain, and conjunctivitis.

From 2014 to 2015, following the ZIKV epidemic, we observed an unusual increase in annual congenital cerebral malformations (two-fold), brainstem dysfunction (31-fold), and severe microcephaly (14-fold) among fetuses and newborns (data not shown). Following the announcement by the Brazilian Government in November 2015 of a dramatic increase in the incidence of microcephaly possibly associated with an ongoing ZIKV outbreak [3,4], we notified the World Health Organization (WHO) of this unusual cluster of congenital neurological abnormalities in our islands [5].

French Polynesia consists of five archipelagos in the South Pacific Ocean, with 118 islands, of which 76 are inhabited. The total population was 271,800 in 2014

Figure 1

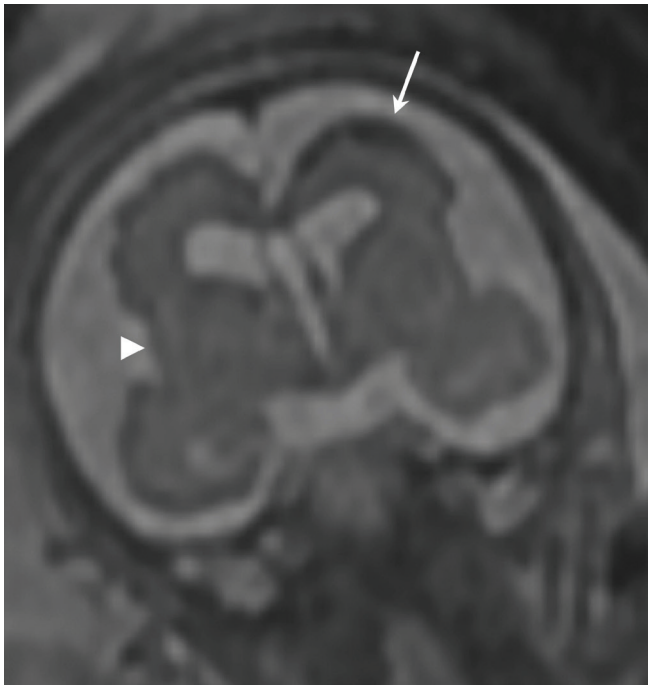
Prenatal MRI T2-weighted performed at 30 weeks plus 5 days of gestation on fetus with congenital cerebral malformations following the 2013–2014 Zika virus outbreak, French Polynesia, 2014 to 2015



This midsagittal slice shows very small volume of supratentorial structures and the pericerebral space is enlarged. We see a notch over the fetal skull (arrow). The corpus callosum and the cavum septi pellucidi are absent.

Figure 2

Prenatal MRI T2-weighted performed at 28 weeks plus 4 days of gestation on fetus with congenital cerebral malformations following the 2013–2014 Zika virus outbreak French Polynesia, 2014 to 2015



This coronal slice shows very irregular cortex (arrow), which is suggestive of diffuse polymicrogyria with bilateral opercular dysplasia (arrowhead) and enlarged pericerebral space. The corpus callosum and the cavum septi pellucidi are seen.

and 70% reside on Tahiti. Approximately 4,200 births are recorded per year (2014: 4,161 births), mainly in Tahiti, with 60% at the Centre Hospitalier de Polynésie Française (CHPF), 30% in two private clinics in Tahiti, and 10% in two distant district hospitals.

We report here a retrospective case series of 19 fetal and newborn cases with congenital cerebral malformations and dysfunction and detail the neurological lesions identified and the corresponding virological results.

Methods

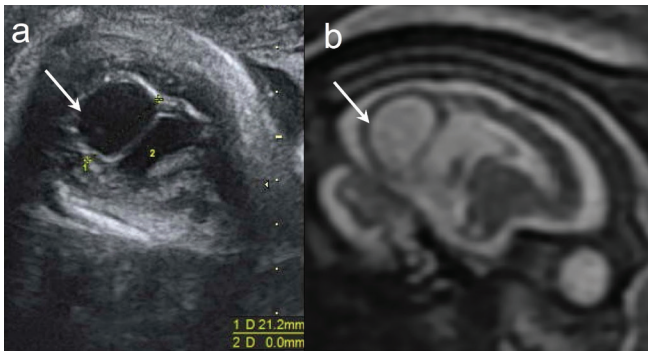
We conducted a retrospective review of congenital cerebral malformations and dysfunction, detected in a prenatal and neonatal population in French Polynesia from March 2014 to May 2015. We included pregnancies beginning between June 2013 and August 2014, the period which corresponded to the largest estimated circulation period of ZIKV. There is no territorial register of congenital malformations, thus the prenatal cases were collected through the non-computerised charts of the prenatal diagnostic unit of the CHPF and the neonatal cases were recorded through the hospital Programme de Médicalisation des Systèmes d'Information (PMSI).

Prenatal monitoring

According to the recommendations of the Haute Autorité de Santé, France [6], three prenatal ultrasounds (US) scans, trisomy screening and serological assays for, hepatitis B, HIV, rubella, toxoplasmosis and syphilis are performed for all pregnant mothers. When fetal anomalies are detected by routine antenatal US scan, the pregnant woman is referred to the CHPF prenatal diagnostic unit, where a second US evaluation is conducted by an expert obstetrician and a nurse experienced in prenatal diagnosis. Once cerebral congenital malformations, for example, are confirmed, they perform an amniocentesis and prescribe magnetic resonance imaging (MRI) of the fetal brain. Then, as all cases of severe congenital abnormalities, they are reviewed by the Prenatal Multidisciplinary Diagnostic Centre of East Paris, France. According to the French law, the committee determines if termination of pregnancy (TOP) can be performed, until full gestational term, upon parental request. An average of 20 annual TOPs are performed (range 12–26) for various severe fetal malformations that constitute a substantial risk of serious motor or cognitive disabilities. After the TOP procedure, pictures of the fetus are taken and skeletal X-rays are performed. Weight, height and head circumference are measured. No autopsy is done as there is no fetopathologist in French Polynesia. Except for

Figure 3

Prenatal ultrasound (a) performed at 27 weeks of gestation and prenatal MRI T2-weighted (b) performed at 30 weeks plus 1 day of gestation on fetus with congenital cerebral malformations following the 2013–2014 Zika virus outbreak, French Polynesia, 2014 to 2015



These para-sagittal slices show a large occipital subependymal pseudocyst (arrow) facing the enlarged occipital horn and pericerebral space is enlarged.

formalin-fixed, paraffin-embedded placenta samples, no fetal tissue is stored.

Postnatal diagnosis

There is only one neonatal intensive care unit (ICU) in French Polynesia. It is situated at CHPF and takes care of all severe neurological malformations and dysfunction. We collected all cases by reviewing the coding of hospitalised patients [7]. When congenital cerebral malformations are detected *in utero*, systematic control after birth of brain imaging (US, MRI or CT-scan) are performed to confirm and assess the brain lesions.

Case definition

We included cases with congenital microcephaly, defined by a head circumference below the third percentile for gestational age and sex, according to the Association des Utilisateurs de Dossiers Informatisés en Pédiatrie, Obstétrique et Gynécologie (AUDIPOG) charts [8], cases with brain lesions without microcephaly and cases with congenital brainstem dysfunction characterised by a deficiency of coordination between sucking, swallowing and breathing, with no visible cerebral malformation and a normal birth head circumference. We assigned them to three groups:

Group 1: fetuses (Group 1a) and newborns (Group 1b) with severe cerebral lesions and microcephaly;

Group 2: fetuses with severe cerebral lesions without microcephaly;

Group 3: newborns with congenital brainstem dysfunction.

Cases with proven aetiology and usual and isolated neurological anomalies without brain damage were excluded.

We retrospectively reviewed the medical records of all mothers and fetuses or newborns with their clinical, serological and radiological (US scans and MRI) data. The imaging examinations were reviewed by radiologists of CHPF and of Hospital Armand-Trousseau, Paris.

We contacted mothers of cases identified and asked about symptoms of ZIKV infection during their pregnancy. Informed written consent was obtained from mothers for all investigations regarding available samples, and publication. Our study was approved by the Ethics Committee of French Polynesia.

Laboratory investigations

Amniotic fluids were analysed for karyotype (comparative genomic hybridisation (CGH) array was not routinely performed) and PCR for cytomegalovirus (CMV). Between June 2013 and August 2014, no virological testing for ZIKV was performed in amniotic fluids and pregnant women.

Retrospectively, we collected seven of thirteen available amniotic fluids, drawn from 11 mothers with fetuses with congenital brain malformations (5/5 in Group 1a; 2/6 in Group 2) and 2 mothers with fetuses with polyhydramnios (0/2 in Group 3), reflecting future brainstem dysfunction, and tested them by RT-PCR for dengue virus (DENV), enteroviruses (EV), herpes simplex virus (HSV), lymphocytic choriomeningitis virus (LCMV), rubella, and varicella zoster virus (VZV). ZIKV was tested with RT-PCR (RealStar ZIKV RT-PCR 1.0, Altona Diagnostics, Germany) and cultured on Vero cells and C6/36 cell lines. ZIKV serology was investigated with ELISA to detect flavivirus antibodies and seroneutralisation assays to further characterise the probable flavivirus.

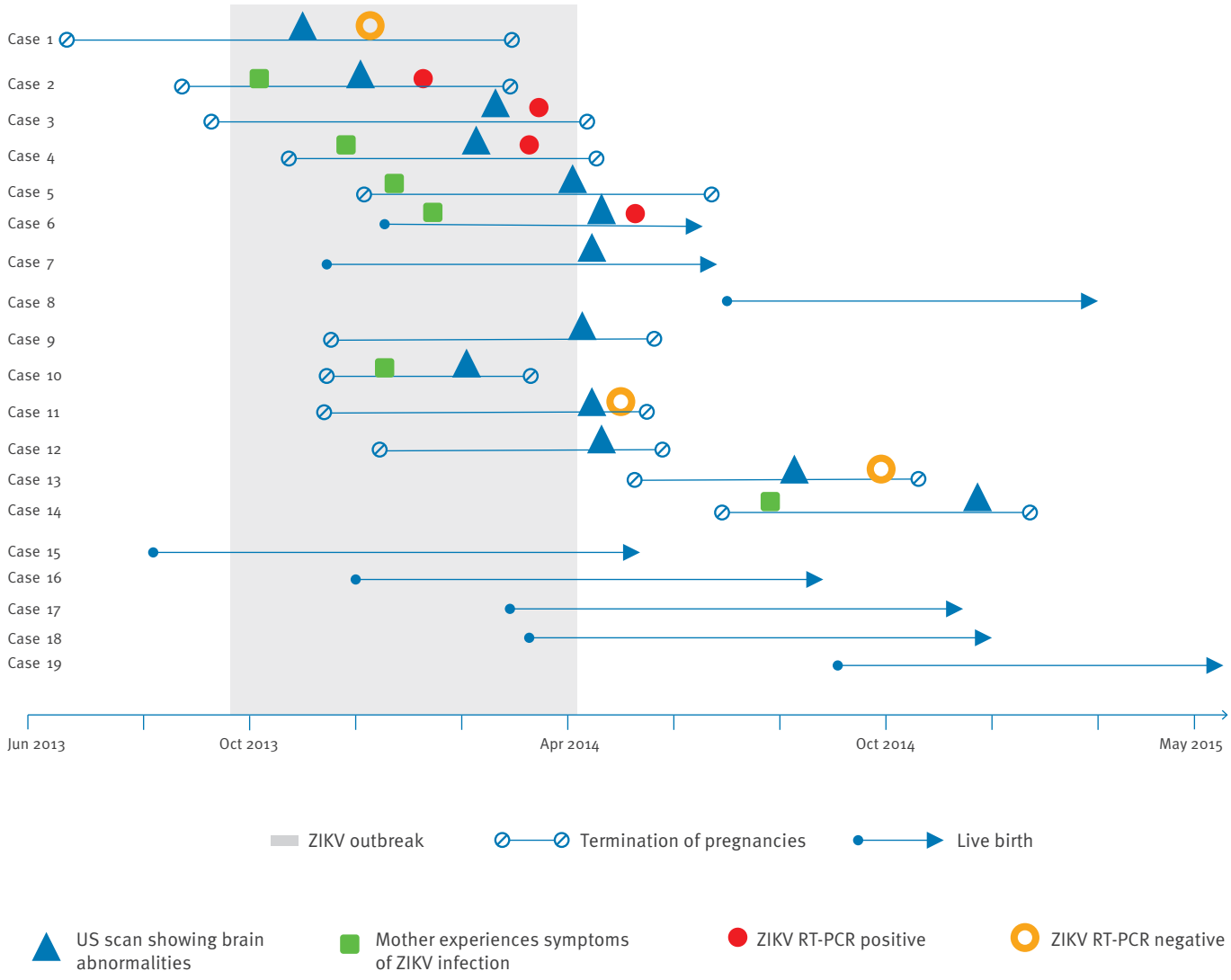
Results

There were 4,787 births during the study period March 2014 to May 2015, and we observed 33 cases with congenital brain malformations or dysfunction. We excluded four cases with proven aetiology (one toxoplasmosis, two chromosomal abnormalities, one myopathy) and ten with usual and isolated neurological anomalies without brain damage (three intraventricular haemorrhages, three corpus callosum agenesis, one neural tube defect and three polymalformative syndromes during the first trimester of pregnancy). The remaining unusual 19 congenital cerebral malformations and dysfunction were included in our study: eight in Group 1 i.e. five fetuses in Group 1a and three newborns in Group 1b; six fetuses in Group 2 and five newborns in Group 3 (Table 1).

The mean age of the mothers in Group 1 was 29.7 years (range 22.8–38.9), 26.9 years in Group 2 (range 15.7–38.0), and 30.8 years in Group 3 (range 20.2–39.0). There was no relevant medical or family genetic history in any of the mothers. Alcohol or maternal cocaine use during pregnancy was denied in our series.

Figure 4

Chronological sequence of Zika virus infection and pregnancy in 19 cases, French Polynesia, 2014 to 2015



US: ultrasound; ZIKV: Zika virus.

Symbols for ZIKV PCR indicate the time when amniotic fluid samples were collected; testing was undertaken retrospectively in December 2015.

Information about ZIKV infection symptoms in mothers was obtained retrospectively in December 2015.

There was no seroconversion for CMV, hepatitis B, HIV, rubella, syphilis and toxoplasmosis in any of the mothers; for 7 of 13 samples of amniotic fluid, PCR for EV, rubella, LCMV, HSV, VZV and DENV was negative.

Imaging findings for all cases are summarised in Table 2. Skeletal X-rays were done for all fetuses and revealed an intracranial calcification in one case (Case 5), which was also observed on prenatal US scan.

Below we describe in detail the findings in the different Groups.

Group 1a

The five fetuses in this group exhibited severe signs of brain injury associated with microcephaly, visualised within the second trimester of pregnancy (after 20 weeks of gestation (WG)) by US scan and confirmed by MRI in four cases. In all cases, in addition to microcephaly, imaging findings (fetal US or MRI) showed multiple severe cerebral injuries including profound destruction of median structures and evidence of interruption of brain development (Figures 1, 2, 3) including absence (or rupture) of the corpus callosum (n = 3) and of cavum septi pellucidi (n = 3), ventriculomegaly superior or equal to 12 mm (norm: ‹10mm, mild ventriculomegaly 10–12mm) (n = 3), occipital subependymal pseudocysts (n = 2), opercular dysplasia (agyria,

Table 1

Main characteristics of fetuses and newborns with congenital cerebral malformations and dysfunction following the 2013–2014 Zika virus outbreak, French Polynesia, 2014 to 2015 (n=19)

	Date of conception	Gestational term at birth or TOP[a], WG + D	Head circumference at birth, percentile	RT-PCR ZIKV in amniotic fluid	Karyotype	Birth weight, percentile
Group 1a: Fetal brain abnormalities and microcephaly						
Case 1	17 Jun 2013	39+4a	<3°	Neg	46 XY	10°
Case 2	15 Aug 2013	30+1a	<3°	Pos	46 XX	50°
Case 3	29 Sep 2013	31+4a	<3°	Pos	46 XY	75°
Case 4	22 Oct 2013	26+1a	NA	Pos	46 XY	75°
Case 5	20 Dec 2013	21+4a	NA	Pos	46 XY	5°
Group 1b: Newborns with cerebral lesions and severe microcephaly						
Case 6	7 Oct 2013	41	<3°	NA	NA	4°
Case 7	10 Nov 2013	36+4	<3°	NA	NA	11°
Case 8	22 Jul 2014	36+2	<3°	NA	46 XY	32°
Group 2: Fetal brain abnormalities without microcephaly						
Case 9	25 Oct 2013	24a	NA	NA	46 XY	10°
Case 10	5 Nov 2013	26+6a	NA	NA	46 XX	25°
Case 11	24 Nov 2013	29+3a	5°	NA	46 XY	63°
Case 12	18 Dec 2013	21+5a	5°	Neg	46 XX	5°
Case 13	17 May 2014	23+5a	90°	Neg	46XY	80°
Case 14	22 Aug 2014	26 a	25°	NA	46 XX	60°
Group 3: Newborns with congenital brainstem dysfunction						
Case 15	5 Sep 2013	39+4	45°	NA	46 XX	25°
Case 16	20 Dec 2013	39+3	45°	NA	46 XX	4°
Case 17	18 Mar 2014	35+4	75°	NA	46 XX	67°
Case 18	11 Mar 2014	40+1	7°	NA	46 XY	15°
Case 19	7 Sep 2014	39	70°	NA	46 XY	51°

D: day; NA: not available; Neg: negative; Pos: positive; TOP: termination of pregnancy; WG: weeks of gestation.
[a] Term (WG+D) at TOP.

polymicrogyria) (n=4), vermian dysgenesis (n=3), enlarged pericerebral space (n=4) and parenchymal calcifications (n=5). In each of the five cases at least five of these lesions were present and all pregnancies were terminated. Extracranial findings were micropenis (Case 1) and hypotrophy below the third percentile with intestinal hyperechogenicity (Case 5). At delivery, head circumferences were below the third percentile, but not measured on two fetuses. Placental microcalcifications were observed in three cases either on US scan and histology (Case 3) or histologically only (Cases 4 and 5).

Retrospectively, ZIKV RNA was detected by RT-PCR and infectious ZIKV was isolated in four of five amniotic fluid samples (Cases 2, 3, 4, 5). When interviewed, the mothers of Cases 2, 4 and 5 reported clinical infection in the first trimester of pregnancy, the mother of Case 3 could not be reached. The chronological sequence of maternal symptoms, fetal malformations discovery and PCR results are summarised in Figure 4.

Group 1b

Microcephaly was diagnosed in utero for two of the three cases (Case 7 and 8) but mothers did not wish TOP. Microcephaly was diagnosed for one case (Case 6) on US scan at 34 WG and the mother did not wish further investigation. MRI was performed in utero for one case (Case 7), and one month after birth in another (Case 8); in both cases MRI confirmed severe microcephaly and cerebral lesions with mainly, occipital pseudocysts and abnormal gyration (Table 2). All three cases were born with a head circumference of 27 cm, which was substantially below the third percentile. At birth, one case was hypotrophic with birth weight at fourth percentile (Case 6) and one had micropenis and severe vision deficiency due to bilateral ocular atrophy and hearing loss suspected on potential recalls (Case 8). No cutaneous lesion or malformation were observed. All three cases demonstrated severe neurological outcome manifest by delayed motor and cognitive development, failure to thrive due to swallowing difficulties and epilepsy.

Laboratory tests were normal for Cases 7 and 8, for Case 6, none were available. No amniotic fluids nor placental samples were taken from any of the three cases in this group. One mother (Case 6) reported clinical signs of ZIKV infection in early pregnancy, but no

Table 2

Imaging findings in fetuses and newborns with congenital cerebral malformations and dysfunction following the 2013–2014 Zika virus outbreak, French Polynesia, 2014–2015 (n=19)

Prenatal findings										
	Term at MRI, WG + D	Term at US scan, WG + D	Gyration	VM (mm)	OPC	ACC	ASP	Vermis (percentile)	EPS	Calcifications
Group 1a: Fetal brain abnormalities and microcephaly										
Case 1	35+3	34	PMG	13	0	0	0	‹3°	+	+
Case 2	28+4	29+3	PMG/OD	12	+	0	0	N	+	+
Case 3	30+1	29+2	PMG/OD	15	+	+	+	‹3°	+	+
Case 4	25	22	PMG/OD	10	0	+	+	N	+	+
Case 5	0	21	NA	10	0	+	+	0	0	+
Group 1b: Newborns with cerebral lesions and severe microcephaly										
Case 6	0	34+2	NA	10	NA	NA	NA	N	NA	NA
Case 7	32+4	30+5	PMG/OD	14	+	+	+	N	+	+
Group 2: Fetal brain abnormalities without microcephaly										
Case 9	24	21+4	OD	23	0	0	Rupture	N	0	0
Case 10	0	24	NA	16	0	+	+	0	0	0
Case 11	28+2	28	OD	22	0	0	Rupture	N	0	0
Case 12	0	19+6	NA	12	0	0	0	‹3°	0	0
Case 13	0	22+5	NA	15	0	+	+	N	0	0
Case 14	0	24+5	NA	17	0	+	+	N	0	0
Post-natal findings										
	Age at MRI (month)	Age at CT scan (month)	Gyration	VM (mm)	OPC	ACC	ASP	Vermis (percentile)	ECM	Calcifications
Group 1b: Newborns with cerebral lesions and severe microcephaly										
Case 8	1	0	PMG/OD	N	+	0	0	N	0	0
Group 4: Newborns with congenital brainstem dysfunction										
Case 15	1	19	N	N	0	0	0	N	0	+
Case 16	1	NA	N	N	0	0	0	N	+	0
Case 17	1	14	N	N	0	0	0	N	+	+
Case 18	0.3	NA	N	N	0	0	0	N	+	0
Case19	1	8	N	N	0	0	0	N	0	0

ACC: absence of corpus callosum; ASP: absence of cavum septi pellucidi; D: day of gestation; ECM: enlarged cisterna magna; EPS: enlarged pericerebral space; MRI: magnetic resonance imaging; N: normal; NA: not available; OD: opercular dysplasia; OPC: occipital pseudo-cyst; PMG: polymicrogyria; 0: none; +: present; VM: ventriculomegaly; WG: weeks of gestation.

serological test was performed. The two other mothers (Cases 7 and 8) were asymptomatic during pregnancy.

Group 2

For the six fetuses in this group, the head circumferences at birth were above the third percentile (range 5–90) or looked normal on pictures. However, all had at least two severe brain damages on imaging (Table 2): ventriculomegaly ≥12 mm (n=6), absence of the corpus callosum and cavum septi pellucidi (n=3), opercular dysplasia (n=2), and vermian agenesis (n=2). These severe lesions led to TOP for all of them. Four of the six fetuses, additionally had extra-cerebral lesions: facial dysmorphia, laparoschisis and fetal akinesia with multiple pterygyum (Case 9); hydrops fetalis, fetal akinesia, rachischisis (Case 10); fetal akinesia (Case 11), and rachischisis (Case 12).

Three cases showed placental calcifications (Case 12, 13 and 14).

Viral tests were done on two amniotic fluids available (cases 12 and 13) and PCR was negative for ZIKV. Two mothers (Cases 11 and 14) reported symptoms of ZIKV infection in the first trimester (Figure 4).

Group 3

The five newborns in this group had clinical evidence of brainstem dysfunction, manifest by absence of sucking and swallowing, but without microcephaly or severe cerebral radiological anomalies. Prenatal US scan revealed in three cases polyhydramnios as consequence of early swallowing deficiency (Cases 17, 18, 19). Head circumference was normal at birth for all cases. They were admitted to the neonatal ICU, due to severe feeding disorders, need for frequent aspiration and cardiac dysautonomia. Two cases (Cases 15 and 17) had an associated Pierre Robin Sequence (PRS) with retrognathia, glossoptosis and posterior U-shaped

cleft palate, and a Moebius syndrome i.e. palsy of sixth and seventh cranial nerves. One of them (Case 17) required a tracheostomy and the other also had club-feet and epilepsy (Case 15). One case had renal hypoplasia (Case 16). Cardiac dysautonomia manifested by attacks of bradycardy or tachycardy, was constantly present in all five cases.

All five were extensively investigated in Hôpital Necker, Paris, or Starship Hospital, Auckland, where most aetiologies for the brainstem dysfunction i.e. infectious, syndromic, genetic, metabolic were ruled out. Two cases (Cases 16 and 18) died in their first year of life at three (Case 16) and nine months (Case 18), respectively, likely due to parasympathetic cardiac dysregulation and respiratory distress. CGH array was normal for the three cases still alive. The two cases with PRS and Moebius syndrome (Cases 15 and 17) had periventricular and bulbar microcalcifications on CT-scans. Three cases had benign isolated enlarged cisterna magna on MRI. All five cases required gastrostomy tube feeding and frequent pharyngeal aspirations. Two (Cases 18 and 19) needed transient non-invasive positive pressure ventilation. The three living cases (Cases 15, 17, 19) are beginning to swallow and those with PRS have delayed neurological development.

No amniotic fluid was available for any of the five cases. Pregnancies were uneventful, besides urinary tract infection for one mother (Case 16). Mothers reported no ZIKV infection symptoms during pregnancy. In-house ELISA flavivirus serology revealed IgG in four of the five mothers. Two of them had neutralising antibodies for dengue serotypes 1–4, but no ZIKV neutralising antibodies. In the two others, no neutralising antibodies were detected for ZIKV, DEN, West Nile and Japanese encephalitis virus.

Discussion

Congenital cerebral malformations are rather rare in French Polynesia and are not collected in a register of congenital malformations. After 2011, when the prenatal diagnosis unit records started, about 15 congenital cerebral malformations were observed in 2012 and 2013, respectively and according to PMSI data, between 2001 and 2013, only two cases of brainstem dysfunction were noted in 2009, leading to an average annual incidence rate of 0.34 cases per 10,000 births. Seven cases of congenital microcephaly were reported in the same period, equivalent to an average annual incidence rate of 1.2 cases per 10,000 live births. The present cluster of severe cerebral malformations with a 14-fold increase in congenital microcephaly and 31-fold in brainstem dysfunction was spatially and temporally associated with a large outbreak of ZIKV in French Polynesia.

In Brazil, the detection of ZIKV genome in amniotic fluid [9-11] and in fetal brains of children with microcephaly [10,12,13], concurrent with widespread local ZIKV transmission [3,4,9] strengthened the hypothesis of teratogenicity of ZIKV. In our series, four fetuses who were infected *in utero* with ZIKV had typical symptoms of viral fetopathy, with microcephaly, severe brain lesions, intrauterine growth retardation and placental calcifications. Infectious virus was isolated from the amniotic fluid of four microcephaly cases, and clinical symptoms of ZIKV infection in the first trimester of pregnancy were reported by three of their mothers. However, ZIKV was not detected in the available amniotic fluids of three other cases tested (one microcephalic, two non-microcephalic), and their mothers were asymptomatic for ZIKV infection, as 80% of the population infected with ZIKV [2]. This finding raises the question about the possible correlation between maternal symptoms, probably associated with high viraemia, and the risk of transplacental transmission.

In the three microcephalic newborns in Group 1b, no further malformations were associated, besides micropenis. Another case of micropenis was found in Group 1a; a possible explanation for this finding could be central hormonal deficiency. Although there was no laboratory evidence for ZIKV infection in all three cases in Group 1b and no ZIKV infection symptoms reported in two of three mothers, the severity of the microcephaly and of the radiologic brain lesions similar to those of group 1a, supports the hypotheses of probable ZIKV infection in the context of the ZIKV outbreak.

Of six cases with cerebral congenital malformations without microcephaly in Group 2, two mothers reported ZIKV infection symptoms in pregnancy. Unlike the other mothers of the same group, none of these mothers had fetuses with extracerebral malformation, besides fetal akinesia which could have been due to severe brain damages or peripheral neuropathy. One case with hydrops and brain lesions had not been explored for ZIKV infection. In Brazil, a fetus with hydrops, hydranencephaly and cranial calcifications was associated with a positive RT-PCR of ZIKV in brain and amniotic fluid [13].

The wide spectrum of clinical and neuroradiological findings in our microcephalic and non-microcephalic foetuses and newborns series suspected to be associated with ZIKV infection, may reflect the timing and severity of fetal viral infection. Early viral infection within the first trimester of pregnancy may interfere with neurocorticogenesis by impairing neuronal proliferation and migration between 12 and 24 WG, which leads to severe microcephaly [14] and neuronal cell migration disorders, whereas second semester infection may cause brainstem dysfunction without obvious lesions and intellectual disabilities. Microcephaly has been diagnosed as early as 21 WG in one fetus. By 25 to 30 WG, cortical gyrification can be analysed by MRI and in microcephalic fetuses, migration disorders were constantly present. The first signs of cerebral damage can be identified by the second trimester of pregnancy by US scan [15] when it reveals ventriculomegaly, destruction of the corpus callosum and/or of

the cavum septi pellucidi and cerebral cysts. Occipital subependymal pseudocysts, which seemed to be another frequent hallmark visible on US scans in four of seven of our explored microcephalic cases, may suggest the consequences of the destruction of the germinative zone by the ZIKV, similar to what was observed in CMV infection [16].

Congenital brainstem dysfunction includes several symptoms such as sucking deficiency, aspiration, upper airways obstruction, and a possible PRS with a posterior and vertical position of the tongue [17]. It may be isolated or syndromic (CHARGE syndrome, etc.) [18]. Besides genetic causes, it could be due to an injury of the brainstem during embryonic or fetal life, such as an ischaemic stroke, misoprostol teratogenicity, maternal cocaine use or later viral infection [19] of the brainstem. The latter can be suspected for two of our cases with microcalcifications in the pons region. There was no evidence of ZIKV congenital infection, nor other proven aetiology, in any of our patients with congenital brainstem dysfunction (Group 3), and their mothers did not report any viral symptoms consistent with ZIKV infection during pregnancy.

The main limitations of our study are those of a retrospective review with bias of collecting data and loss of biological fluids which are stored for one year only. We have chosen to include only the severe brain malformations or dysfunction in our study. A surveillance artefact could result from missing data due to lack of an official register of congenital malformations, or due to non-exhaustive PMSI data and the risk that less severe cases who are not hospitalised were not notified which would have led to less cases having been noted. As concerns severe cases, the nature of the symptoms seems to make it unlikely that we have missed such cases while as pointed out above, milder cases could have been missed. Other postnatal lesions such as hearing, visual, cognitive impairments and epilepsy that could manifest later in life might be reported in further prospective studies in populations exposed to ZIKV.

Conclusion

ZIKV was for many years thought to be a benign febrile illness. In line with findings in Brazil, we retrospectively identified an unusual and heterogeneous cluster of congenital brain malformations and brainstem dysfunction in fetuses and newborns over a limited period following a ZIKV epidemic in French Polynesia. Except for four cases of fetal microcephaly, presenting with infectious virus in amniotic fluid and further confirming the Brazilian experience [9-13], we found no evidence of ZIKV vertical transmission for additional 15 cases; however, we were only able to perform ZIKV RT-PCR for three of them and we have no more opportunity to prove or to exclude the ZIKV infection in the 12 remaining cases. The spacio-temporal association might explain brain malformations in context of a viral outbreak. A case–control study is planned to look for other risk factors for these unexplained fetal and newborn abnormalities.

Given the potentially substantial public health implications of our findings, we strongly encourage researchers and clinicians to ensure that non-microcephalic congenital abnormalities, extra-cerebral malformations and brainstem dysfunction are included in their viral investigations for the potential teratogenicity of ZIKV.

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Conflict of interest

None declared.

Authors' contributions

MB and LM collected the data.

DEG, PGA and CG reviewed the imaging examinations.

SL, FR, ILG performed the viral investigations.

FBB reviewed the placenta samples.

VA managed the patients and helped with diagnostic and therapeutic care.

MLM and JMJ counselled for the prenatal management of fetuses.

HPM supervised the investigation, retrospective work and performed the epidemiological study.

MB, DEG, VA, FR and HPM wrote the paper.

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Research article

Effectiveness of seasonal influenza vaccine in preventing laboratory-confirmed influenza in primary care in the United Kingdom: 2015/16 mid-season results

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In 2015/16, the influenza season in the United Kingdom was dominated by influenza A(H1N1)pdm09 circulation. Virus characterisation indicated the emergence of genetic clusters, with the majority antigenically similar to the current influenza A(H1N1)pdm09 vaccine strain. Mid-season vaccine effectiveness (VE) estimates show an adjusted VE of 41.5% (95% confidence interval (CI): 3.0–64.7) against influenza-confirmed primary care consultations and of 49.1% (95% CI: 9.3–71.5) against influenza A(H1N1)pdm09. These estimates show levels of protection similar to the 2010/11 season, when this strain was first used in the seasonal vaccine.

Introduction

The United Kingdom (UK) has had for many years an influenza vaccination programme using inactivated influenza vaccine targeted at individuals at higher risk of severe disease such as the elderly and under 65-year-olds in a clinical risk group. The 2015/16 influenza season is the third where an intranasally administered live attenuated influenza vaccine was provided to children [1]. This winter has been characterised by circulation of mainly influenza A(H1N1)pdm09, with evidence of hospitalisations and admissions to the intensive care unit (ICU) particularly in younger adults 15 to 64 years of age [2]. Influenza A(H1N1)pdm09 previously circulated in the UK in 2013/14, 2012/13 and particularly in 2010/11, the first post-pandemic season where particular impact was seen in younger adults. The 2015/16 season has also seen a large number of school and hospital outbreaks with evidence of excess all-cause mortality in 15 to 64 year-olds using the EuroMoMo standard algorithm [2].

The UK has long-standing systems to measure influenza vaccine effectiveness (VE) in the middle and at the end of the season [3,4]. The aims of the present study were to provide early season estimates of influenza VE to inform influenza prevention and control measures both for the remainder of this season and for the World Health Organization (WHO) northern hemisphere meeting that was held in February 2016 to decide influenza vaccine composition for the forthcoming 2016/17 season.

Methods

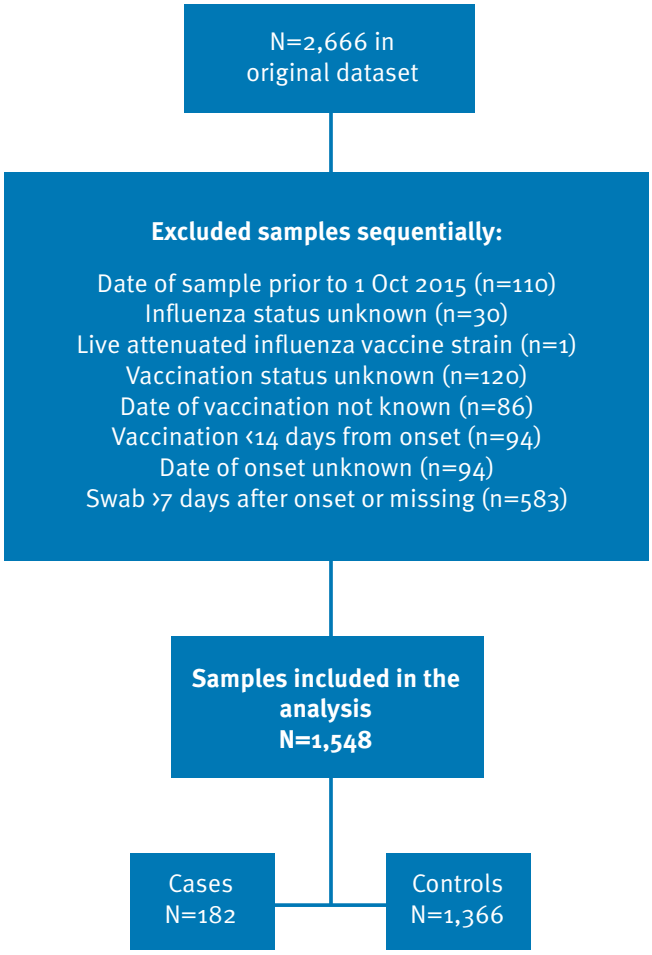
Study population and period

Five primary care influenza sentinel swabbing surveillance schemes from England (two schemes), Scotland, Wales and Northern Ireland provided data. Information on the Royal College of General Practitioners (RCGP) Research and Surveillance Centre (RSC), Public Health England (PHE) Specialist Microbiology Network (SMN), Public Health Wales, Public Health Agency (PHA) of Northern Ireland and Health Protection Scotland (HPS) schemes have been provided in earlier publications [4].

The time of investigation ran from 1 October 2015 to 22 January 2016. Patients were swabbed during their consultation, with verbal consent. Cases were defined as patients presenting to a general practitioner (GP) in a participating practice with an acute influenza-like

Figure 1

Specimen inclusion and exclusion criteria, interim 2015/16 influenza vaccine effectiveness evaluation, United Kingdom, 1 October 2015–22 January 2016 (n = 2,666)



illness (ILI) who tested positive for influenza A or B viruses by real-time PCR. Controls were individuals presenting with ILI in the same period who tested negative for influenza. ILI was defined as an individual presenting in primary care with an acute respiratory illness with physician-diagnosed fever or complaint of feverishness.

A standardised form was completed by the GP during the consultation. Demographic, epidemiological and clinical information was collected from participants, including date of birth, sex, defined underlying clinical risk group, date of specimen collection, date of onset of respiratory illness, and influenza vaccination status for the 2015/16 season with vaccination dates and route of administration (injection/intranasal). It was also recorded (in England, Scotland and Northern Ireland) whether the patient was resident in an area where a primary school-age programme was in operation.

Laboratory methods

Sentinel samples from the GP surveillance networks were sent to the national laboratories as previously described [4]. Laboratory confirmation was undertaken at all sites using comparable real-time PCR methods capable of detecting circulating influenza A and influenza B viruses and other respiratory viruses [5,6]. In addition, hospital diagnostic laboratories submitted samples in which influenza virus had been detected to the reference laboratories from a selection of cases (including severe cases and vaccinated cases) for further strain characterisation. Influenza viruses from all sources (both sentinel and non-sentinel) were isolated from PCR-positive samples in Madin-Darby canine kidney epithelial (MDCK) cells or MDCK cells containing the cDNA of human 2,6-sialtransferase (SIAT1) cells as previously described [7,8].

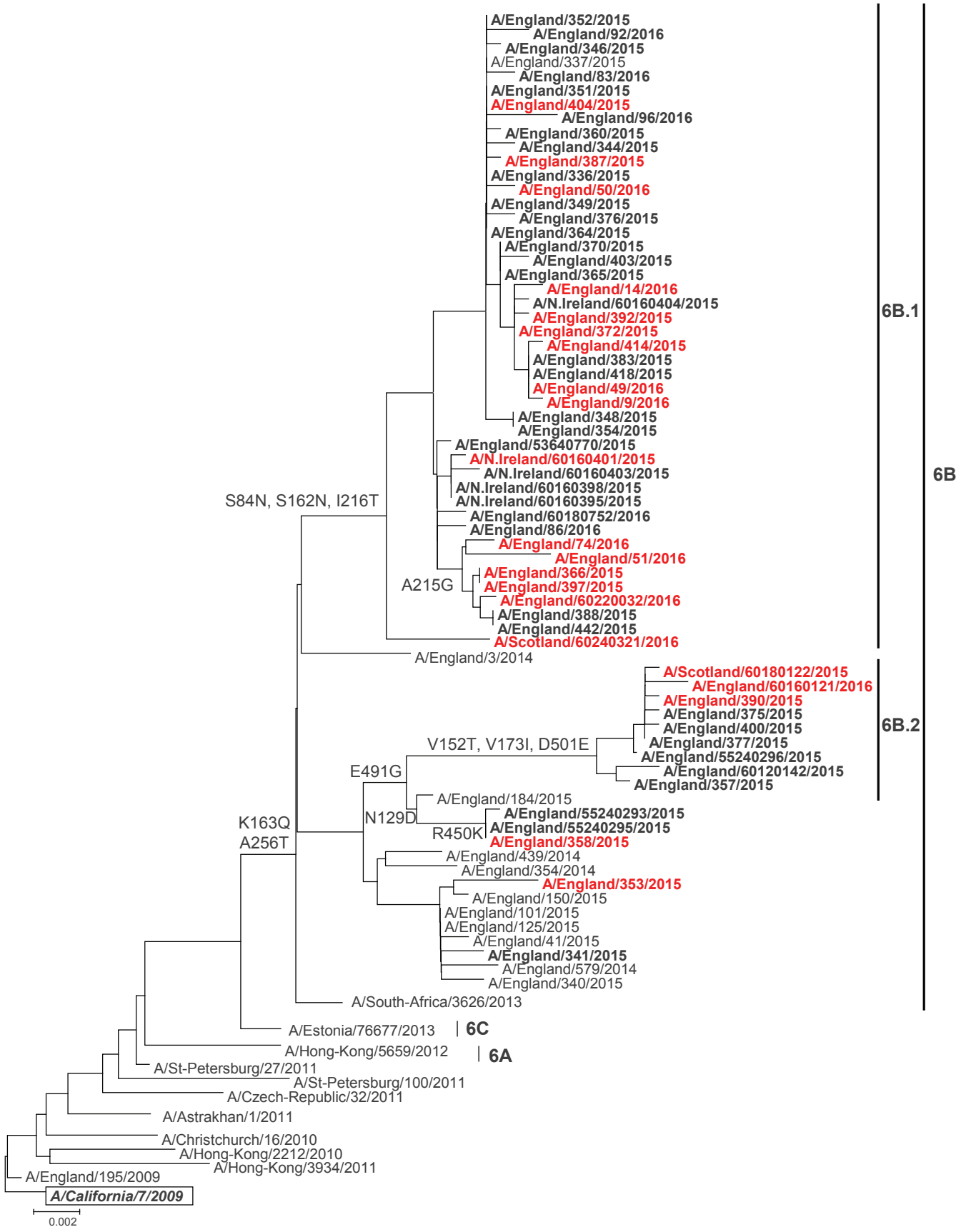
Influenza A(H1N1)pdm09 virus isolates with a haemagglutination titre ≥ 40 were characterised antigenically using post-infection ferret antisera in haemagglutination inhibition (HI) assays, with turkey red blood cells [9]. Reference virus strains used for HI assays included A/California/7/2009 (vaccine strain) grown in embryonated chicken eggs, and other A(H1N1)pdm09 England strains were grown in embryonated chicken eggs or tissue culture cells. The fold difference between the homologous HI titre for egg-grown A/California/7/2009 and the HI titre for each clinical isolate was calculated to determine antigenic similarity of clinical isolates to the vaccine strain.

Nucleotide sequencing of the haemagglutinin (HA) gene of a subset of influenza A(H1N1)pdm09 viruses selected to be representative of the range of the patients' age, date of sample collection, geographical location and antigenic characterisation of the virus isolate, if performed, was undertaken (primer sequences available on request), and phylogenetic trees were constructed with a neighbour-joining algorithm available in the Mega 6 software (http://www.megasoftware.net) [10]. HA sequences from reference strains used in the phylogenetic analysis were obtained from the EpiFlu database of the Global Initiative on Sharing Avian Influenza Data (GISAID) (Table 1).

The HA sequences from England obtained in this study, which were also used in the phylogenetic analysis, were deposited in GISAID under the following accession numbers: EPI679151, EPI679186, EPI679213, EPI679221, EPI679245, EPI679266, EPI679300, EPI679313, EPI711775, EPI711780, EPI711788, EPI711796, EPI711804, EPI711812, EPI711820, EPI711828, EPI711834, EPI711842, EPI711850, EPI711858, EPI711866, EPI711873, EPI711881, EPI711888, EPI711893, EPI711901, EPI711909, EPI711917, EPI711925, EPI711930, EPI711938, EPI711943, EPI711951, EPI711959, EPI711967, EPI711975, EPI711983, EPI711991, EPI711996, EPI712002, EPI712007, EPI712012, EPI712020, EPI712028, EPI712036, EPI712044, EPI712052, EPI712060, EPI712068, EPI712076, EPI712084, EPI712092, EPI712100, EPI712108, EPI712116, EPI712121, EPI712129, EPI712137, EPI712142, EPI712150, EPI712166, EPI712167, EPI712168, EPI712169, EPI712170, EPI712171, EPI712172, EPI712311.

Figure 2

Phylogenetic analysis of full length haemagglutinin gene comparing reference sequences from the GISAID EpiFlu database and influenza A(H1N1)pdm09 sequences from patients, United Kingdom, 2015/16 influenza season



The tree was built using a neighbour-joining algorithm, with vaccine strain A/California/07/2009 selected as the root. Signature amino acid substitutions characterising genetic groups are annotated at the root of each cluster. 2015/16 UK samples are highlighted in bold. Sentinel samples are highlighted in red.

Table 1

Influenza A(H1N1)pdm09 haemagglutinin sequences obtained from GISAID used in the phylogenetic analysis

Virus isolate	Segment ID/ Accession number	Country	Collection date (year-month-day)	Originating laboratory	Submitting laboratory
A/Astrakhan/1/2011	EPI319590	Russian Federation	2011-Feb-28	WHO National Influenza Centre, Saint Petersburg, Russian Federation	National Institute for Medical Research, London, UK
A/St. Petersburg/27/2011	EPI319527	Russian Federation	2011-Feb-14	WHO National Influenza Centre, Saint Petersburg, Russian Federation	National Institute for Medical Research, London, UK
A/England/3/2014	EPI503206	United Kingdom	2014-Jan-08	Microbiology Services Colindale, Public Health England, London, UK	National Institute for Medical Research, London, UK
A/Estonia/76677/2013	EPI466545	Estonia	2013-Mar-13	Health Protection Inspectorate, Tallin, Estonia	National Institute for Medical Research, London, UK
A/Hong Kong/5659/2012	EPI390473	Hong Kong (SAR)	2012-May–21	Government Virus Unit, Hong Kong (SAR)	National Institute for Medical Research, London, UK
A/Hong Kong/3934/2011	EPI326206	Hong Kong (SAR)	2011-Mar-29	Government Virus Unit, Hong Kong (SAR)	National Institute for Medical Research, London, UK
A/Hong Kong/2212/2010	EPI279895	Hong Kong (SAR)	2010-Jul-16	Government Virus Unit, Hong Kong (SAR)	National Institute for Medical Research, London, UK
A/Czech Republic/32/2011	EPI319447	Czech Republic	2011-Jan-18	National Institute of Public Health, Prague, Czech Republic	National Institute for Medical Research, London, UK
A/England/195/2009	EPI178507	United Kingdom	2009-Apr-28	Microbiology Services Colindale, Public Health England, London, UK	National Institute for Medical Research, London, UK
A/St. Petersburg/100/2011	EPI316435	Russian Federation	2011-Mar-14	Russian Academy of Medical Sciences, Saint Petersburg, Russian Federation	Centers for Disease Control and Prevention, Atlanta, US
A/South Africa/3626/2013	EPI577031	South Africa	2013-Jun-06	National Institute for Medical Research, London, UK	Centers for Disease Control and Prevention, Atlanta, US
A/Christchurch/16/2010	EPI280344	New Zealand	2010-Jul-12	WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia	Centers for Disease Control and Prevention, Atlanta, US
A/California/07/2009	EPI177294	United States	2009-Apr-09	Naval Health Research Center, San Diego, US	Centers for Disease Control and Prevention, Atlanta, US

GISAID: Global Initiative on Sharing All Influenza Data; SAR: Special Administrative Regions of the People's Republic of China; UK: United Kingdom; US: United States; WHO: World Health Organization.

Statistical methods

Patients were defined as vaccinated if the date of vaccination with the 2015/16 seasonal vaccine was at least 14 days before onset of illness. Those vaccinated less than 14 days before onset of illness and those with unknown date of vaccination were excluded. Those with unknown date of onset or onset date more than seven days before the swab was taken were also excluded.

VE was estimated by the test-negative case control design. In that design, VE is calculated using odds ratios (OR) as 1–(OR) obtained using multivariable logistic regression models with influenza A PCR results (influenza B numbers were too small to examine) and seasonal vaccination status as the linear predictor. VE was also calculated separately for influenza A(H1N1) pdm09. In the analyses evaluating VE for a specific type or strain, those positive for other virus types were excluded from the analysis. For this mid-season analysis, we fixed the variables for adjusted estimates based on past seasons as age (coded into standard age groups, <5, 5–17, 18–44, 45–64 and ≥65 years),

sex, surveillance scheme (RCGP, SMN, HPS, Wales, Northern Ireland), residence in an area where a primary school-age programme operated and date of sample collection (month). All statistical analyses were carried out in Stata version 13 (StataCorp, College Station, Texas).

Results

The reasons for study inclusion and exclusion are outlined in Figure 1.

Of the 2,666 swabbed individuals, 1,548 individuals were included in the study. Their details were stratified according to the swab result. There were a total of 1,366 controls, 20 influenza B detections, 152 influenza A(H1N1)pdm09 detections, 3 influenza A(H3N2) detections and nine influenza A(unknown) detections. Influenza A(H1N1)pdm09 positivity rates were highest by age in younger than five years (16.8%) and in 18 to 44 year-olds (10.9%), by vaccine status in those who were unvaccinated (11.1%) compared with vaccinated (5.6%), and in non-pilot (14.1%) compared with pilot areas (6.3%). Overall positivity rates differed significantly by age group (highest in <5 year-olds), sex (higher in males), risk group (higher in those without a risk factor), month (highest in January), scheme, vaccination status (highest in unvaccinated) and area of primary school-age programme (highest in non-pilot areas) (Table 2).Numbers and row percentages (to indicate positivity rates) are shown.

Influenza A(H1N1)pdm09 strain characterisation from sentinel and non-sentinel samples

Since the start of the 2015/16 winter influenza season in week 40 2015, the PHE Respiratory Virus Unit has characterised a total of 274 influenza A(H1N1)pdm09 viruses from all sources; 103 genetically (of which nine (9%) from sentinel sources), 210 antigenically (of which 46 (22%) sentinel sources) and 39 both antigenically and genetically (of which three (8%) from sentinel sources).

The A(H1N1)pdm09 viruses genetically characterised to date all belonged in the genetic subgroup 6B (Figure 2), which had been the predominant genetic subgroup in the 2014/15 season. Some heterogeneity has been seen in HA of the current season's A(H1N1) pdm09 viruses, with some genetic subgroups becoming evident: the HA genes of more than 85% of A(H1N1) pdm09 viruses fell into genetic cluster 6B.1, characterised by the amino acid changes S84N, S162N (with gain of a potential glycosylation site) and I216T, with a subset in this cluster having the substitution A215G. Less than 10% of viruses fell into a second emerging cluster (6B.2), and had the amino acid substitutions V152T, V173I, E491G and D501E in the HA gene, or a third minor cluster with substitutions N129D, R450K and E491G. A few viruses from this season did not show any of these changes or have substitution S84N alone, and clustered with A(H1N1)pdm09 viruses from season 2014/15 (6B subgroup).

Of 210 viruses analysed by HI assay using ferret post-infection sera, more than 90% were antigenically similar to the A/California/7/2009 northern hemisphere 2015/16 A(H1N1)pdm09 vaccine strain. In the period 1 October to 30 November 2015, 6% (2/32) of isolates had an eightfold or greater reduction in reactivity to antiserum raised to egg-grown A/California/7/2009 virus, compared with 11% (19/178) that had an eightfold or greater reduction in the period 1 December 2015 to 22 January 2016.

Model fitting for vaccine effectiveness estimation

The variables included in the multivariable model (age group, sex, month of sample collection, surveillance scheme and primary school-age programme area) were all significantly associated with swab positivity and were confounders for the vaccine effects (changed estimates by more than 5%) with the exception of primary school-age programme area. Information on risk group was missing for 53 samples (3.4%) and as in previous seasons' analyses [4] was not included in the final model.

Vaccine effectiveness estimates against influenza A(H1N1)pdm09, influenza A (all types) and all influenza are shown in Table 3. It was not possible to estimate effectiveness against influenza A(H3N2) or influenza B due to inadequate sample number. The adjusted VE of influenza vaccine against any influenza was 41.5% (95% confidence interval (CI): 3.0–64.7) and was very similar for A(H1N1)pdm09 at 49.1% (95% CI: 9.3–71.5).

Discussion

In a season dominated by circulation of influenza A(H1N1)pdm09, we found an overall VE of 41.5% in preventing laboratory-confirmed influenza infection resulting in a primary care consultation; it was 49.1% specifically against A(H1N1)pdm09, reflecting the fact that A(H1N1)pdm09 was the dominant circulating strain at this stage of the season. We also found some early evidence of circulation of A(H1N1)pdm09 genetic variants, but with no evidence of loss of effectiveness of the 2015/16 vaccine.

The UK, together with other European Union Member States, the United States, Canada and Australia has well established systems to generate interim estimates of seasonal influenza VE. These early results are used to optimise in-season control and prevention measures, to inform other countries before their influenza season and to contribute to the WHO deliberations on the influenza vaccine composition for the northern hemisphere. The UK, as other countries in Europe, has experienced a season dominated by circulation of influenza A(H1N1)pdm09 with reports of increases in hospitalisations and ICU admissions mainly in younger adults [11]. Although concerns have been expressed

Table 2

Details for influenza A and B cases (n = 182) and controls (n = 1,366), United Kingdom, 1 October 2015–22 January 2016

	Control (n = 1,366)	Influenza B[a] (n = 20)	A(H1N1)[a] (n = 152)	A(H3N2) (n = 3)	A (unknown) (n = 9)	p value[b]
Age						
<5	163 (83.2%)	2 (1.0%)	33 (16.8%)	0 (0.0%)	0 (0.0%)	
5–17	193 (91.9%)	1 (0.5%)	16 (7.6%)	0 (0.0%)	0 (0.0%)	
18–44	502 (86.6%)	12 (2.1%)	63 (10.9%)	0 (0.0%)	3 (0.5%)	0.001
45–64	315 (88.0%)	4 (1.1%)	32 (8.9%)	2 (0.6%)	5 (1.4%)	
≥ 65	192 (95.0%)	1 (0.5%)	7 (3.5%)	1 (0.5%)	1 (0.5%)	
Missing	1 (50.0%)	0 (0.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	
Sex						
Female	840 (90.3%)	8 (0.9%)	73 (7.8%)	2 (0.2%)	7 (0.8%)	0.002
Male	522 (85.2%)	12 (2.0%)	78 (12.7%)	1 (0.2%)	2 (0.3%)	
Missing	4 (80.0%)	0 (0.0%)	1 (20.0%)	0 (0.0%)	0 (0.0%)	
Surveillance scheme						
Northern Ireland	33 (63.5%)	4 (7.7%)	9 (17.3%)	0 (0.0%)	6 (11.5%)	
RCGP	540 (87.8%)	4 (0.7%)	69 (11.2%)	2 (0.3%)	0 (0.0%)	<0.001
SMN	58 (75.3%)	1 (1.3%)	18 (23.4%)	0 (0.0%)	0 (0.0%)	
Scotland	701 (92.8%)	10 (1.3%)	42 (5.6%)	1 (0.1%)	3 (0.4%)	
Wales	34 (69.4%)	1 (2.0%)	14 (28.6%)	0 (0.0%)	0 (0.0%)	
Risk group						
No	908 (86.5%)	17 (1.6%)	119 (11.3%)	2 (0.2%)	6 (0.6%)	<0.001
Yes	414 (93.0%)	3 (0.7%)	25 (5.6%)	1 (0.2%)	2 (0.4%)	
Missing	44 (83.0%)	0 (0.0%)	8 (15.1%)	0 (0.0%)	1 (1.9%)	
Onset to swab						
0–1 days	145 (86.3%)	0 (0.0%)	21 (12.5%)	0 (0.0%)	2 (1.2%)	0.400
2–4 days	713 (87.7%)	12 (1.5%)	84 (10.3%)	3 (0.4%)	3 (0.4%)	
5–7 days	508 (89.6%)	8 (1.4%)	47 (8.3%)	0 (0.0%)	4 (0.7%)	
Vaccination status						
Unvaccinated	1,055 (87.0%)	16 (1.3%)	135 (11.1%)	2 (0.2%)	7 (0.6%)	0.013
Vaccinated (14–91 days ago)	280 (92.7%)	3 (1.0%)	17 (5.6%)	1 (0.3%)	1 (0.3%)	
Vaccinated(>91 days ago)	31 (93.9%)	1 (3.0%)	0 (0.0%)	0 (0.0%)	1 (3.0%)	
Primary school-age programme area						
No	594 (84.7%)	6 (0.9%)	99 (14.1%)	2 (0.3%)	0 (0.0%)	<0.001
Yes	768 (91.1%)	14 (1.7%)	53 (6.3%)	1 (0.1%)	9 (1.1%)	
Missing	4 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Month of event						
October	300 (98.7%)	1 (0.3%)	1 (0.3%)	1 (0.3%)	1 (0.3%)	
November	380 (96.4%)	5 (1.3%)	7 (1.8%)	2 (0.5%)	0 (0.0%)	<0.001
December	446 (85.9%)	5 (1.0%)	67 (12.9%)	0 (0.0%)	1 (0.2%)	
January	240 (72.5%)	9 (2.7%)	77 (23.3%)	0 (0.0%)	7 (2.1%)	

RCGP: Royal College of General Practitioners' Research and Surveillance Centre scheme; SMN: Public Health England Specialist Microbiology Network.

[a] Two people tested positive for both influenza B and A(H1N1)pdm09.

[b] Positive vs negative for influenza.

Numbers and row percentages (to indicate positivity rates) are shown.

about a possible increase in virulence, the epidemiological observations are consistent with earlier seasons in the UK dominated by circulation of A(H1N1)pdm09, in particular in 2010/11, the first post-pandemic season, but also to a lesser extent in 2012/13 and 2013/14.

Although evidence of heterogeneity has been seen in the HA gene of A(H1N1)pdm09 viruses genetically characterised from all sources to date this season, more than 90% of the 210 viruses analysed by HI assays were antigenically similar to the A/California/7/2009 northern hemisphere 2015/16 (H1N1)pdm09 vaccine strain, suggesting little change in the antigenic properties

Table 3

Samples positive (cases) and negative (controls) for influenza, by vaccination status and vaccine effectiveness estimate, United Kingdom, 1 October 2015–22 Jan 2016 (n = 1,548)

	Cases (vac/unvacc)	Controls (vac/unvacc)	Crude VE (95% CI)	Adjusted VE [a] (95% CI)
Influenza A and B	24/158	311/1,055	48.5% (19.4–67.1)	41.5% (3.0–64.7)
Influenza A(H1N1)pdm09	17/135	311/1,055	57.3% (28.1–74.6)	49.1% (9.3–71.5)
Influenza A	20/144	311/1,055	52.9% (23.5–71.0)	47.3% (9.0–69.5)

CI: confidence interval; VE: vaccine effectiveness.

[a] Adjusted for age group, sex, month, surveillance scheme and primary school-age programme area.

of circulating strains. Similar observations have been reported from other European countries [11]. The full picture of virological genetic variation requires further detailed analysis, which is not possible at this stage of the winter season.

In support of the antigenic characterisation findings, we demonstrate that the influenza vaccine has been effective in preventing laboratory-confirmed primary care consultations this season. The adjusted VE against all influenza for all age groups was very similar to that against influenza A(H1N1)pdm09 reflecting the fact that A(H1N1)pdm09 has been the dominant circulating virus strain this season. Indeed, the result is not significantly different to that observed for the UK mid-season estimate in 2010/11, when A(H1N1)pdm09 was the dominant circulating strain with an estimate against A(H1N1)pdm09 of 51% (95% CI 29 to 66%) [12], and in 2012/13 with an end of season estimate of 73% (95% CI: 37 to 89) [4]. The results were also not significantly different from the VE against influenza A(H1N1)pdm09 of 44.2% (95% CI: −3.1 to 69.8%) recently reported for the current season by the European I-MOVE network [13] and the recent estimate from Canada of 64% (95% CI: 44–77%) [14]. The lack of apparent antigenic and epidemiological vaccine mismatch at this stage is encouraging.

Nonetheless, it is important to highlight lack of precision in our estimate: the lower 95% CI was 3% and the upper CI was 65%, indicating a large range of uncertainty, although we can say with confidence that the influenza vaccine has been effective so far this season. Furthermore, this mid-season analysis was done at a time when activity was still increasing and does not preclude the possibility to that there may be changes in the dominant circulating strain, with potential implications for the vaccine effectiveness. These limitations will be addressed in the end-of-season analysis which will also include stratification by age group and type of vaccine, in particular for children.

Finally, the results outlined in this paper have contributed to the recent global assessment for the coming season's influenza vaccine composition: the WHO recommended that the vaccine for the 2016/17 northern hemisphere winter should continue to include the A/California/7/2009 vaccine strain [15].

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Conflict of interest

None declared.

Authors' contributions

RGP led the drafting; FW and NA led on the statistical analysis; JE, MG and CT led on the virological analysis; all co-authors contributed epidemiological and/or virological data, contributed to the design and interpretation of the results, reviewed the early draft and approved the final version.

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A unique measles B3 cluster in the United Kingdom and the Netherlands linked to air travel and transit at a large international airport, February to April 2014

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This report describes a joint measles outbreak investigation between public health officials in the United Kingdom (UK) and the Netherlands following detection of a measles cluster with a unique measles virus strain. From 1 February to 30 April 2014, 33 measles cases with a unique measles virus strain of genotype B3 were detected in the UK and the Netherlands, of which nine secondary cases were epidemiologically linked to an infectious measles case travelling from the Philippines. Through a combination of epidemiological investigation and sequence analysis, we found that measles transmission occurred in flight, airport and household settings. The secondary measles cases included airport workers, passengers in transit at the same airport or travelling on the same flight as the infectious case and also household contacts. This investigation highlighted the particular importance of measles genotyping in identifying transmission networks and the need to improve vaccination, public health follow-up and management of travellers and airport staff exposed to measles.

Introduction

Measles is a highly contagious, acute viral illness with potential for severe complications, including pneumonia, encephalitis and death. The infectious period for measles is from four days before until four days after rash onset [1]. International travel by susceptible persons to measles-endemic areas can lead to imported sporadic cases in countries that have achieved or are close to achieving measles elimination [2,3]. Many of these sporadic cases result from measles transmission in flight or at airports [3-7]. Risk of further transmission following an importation then depends on the level of immunity in the exposed population and the responsiveness of public health agencies once a case is identified.

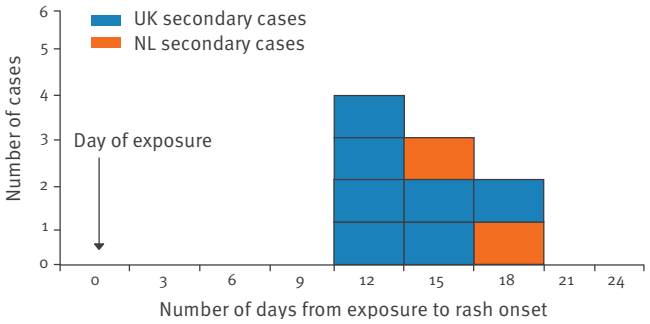
In 2014, 48 laboratory-confirmed measles importations were reported in the United Kingdom (UK) compared with 50 in 2013 and 27 in 2012. In the Netherlands in 2014, nine laboratory-confirmed measles importations were reported, compared with 31 in 2013 and eight in 2012. Vaccination coverage for two doses of the measles-mumps-rubella (MMR) vaccine is high in both the UK (88.3%) [8] and the Netherlands (92.4%) [9]. Their respective national immunisation programmes offers two doses of the MMR vaccine at 14 months and nine years in the Netherlands [10] and between 12 and 13 months and then between three years four months and five years in England [11]. Two doses of the MMR vaccine are 99% effective at preventing measles [1].

Heathrow Airport in London in the UK and Amsterdam Airport Schiphol in the Netherlands are the first and fourth busiest airports in Europe, handling ca 72.3 million and 52.6 million passengers respectively in 2013 [12,13]. As a consequence, these large airports present opportunities for measles importation and transmission, posing additional challenges for measles elimination in Europe [14].

In the event of an infectious measles case travelling on a flight, European risk assessment guidelines for infectious diseases transmitted on aircraft (RAGIDA), published by the European Centre for Disease Prevention and Control (ECDC), recommend that contact tracing should prioritise children aged below 2 years

Figure 1

Epidemic curve of secondary measles cases epidemiologically linked with the index case, week 5 to week 8 2014, United Kingdom and the Netherlands (n = 9)



NL: the Netherlands; UK: United Kingdom.

and passengers seated in the same row as the index case. Contact tracing should then proceed row by row in either direction away from the index case, in an effort to identify vulnerable susceptible contacts on the entire flight. Timeliness is crucial in these situations, as post-exposure prophylaxis (PEP) with either vaccine or immunoglobulin needs to be administered within a few days of exposure [15]. In the UK and the Netherlands, the policy for PEP is similar to the ECDC RAGIDA guidelines [10,11].

A cluster of measles cases with onset dates from 1 February to 30 April 2014 occurred in the UK and the Netherlands and was linked to an index case that travelled from the Philippines to London, via Amsterdam Airport Schiphol, in January 2014. At this time, a large measles outbreak was ongoing in the Philippines. Although the measles cluster was only recognised when genotype information on the third UK case became available, we present the key epidemiological features and lessons learnt from the investigation and management of this cluster.

Methods

Case definition

Measles is a notifiable disease in both the UK and the Netherlands. In the UK, a suspected case of measles includes any person in whom a clinician suspects measles infection, or any person with a clinically compatible rash and fever illness [16]. In the Netherlands, the European Union (EU) measles case definition is used [17]. In all cases, samples are requested from suspect cases (oral fluid samples for detection of measles IgM and/or viral RNA in the UK; oral fluids or nasopharyngeal aspirate specimens for IgM serology and/or detection of viral RNA in the Netherlands) to confirm the diagnosis. In this investigation, the outbreak case definition included laboratory-confirmed cases of measles notified between 1 February and 1 June 2014 with the measles virus MVs/Tonbridge.GBR/5.14 [B3] (i.e. an identical 450 nucleotide (nt) sequence) or laboratory-confirmed measles cases without genotyping but with a clear epidemiological link to cases with the measles virus MVs/Tonbridge.GBR/5.14 [B3].

Laboratory investigation

Serum or oral fluid samples were tested by commercial serological assay for measles IgM and IgG using standard methods. PCR testing and genotyping of oral fluid and or nasopharyngeal aspirates were conducted by the Virus Reference Department (VRD) at Public Health England (PHE) and by the Centre for Infectious Disease Research, Diagnostics and Screening at the Dutch National Institute for Public Health and the Environment (RIVM). Genotyping and strain identification is attempted on all confirmed measles cases. Measles genotyping involves amplification of the 450 nt fragment of the measles nucleocapsid (N) gene. Classification of genotypes is based on nucleic acid sequencing of the PCR products [18]. Measles sequences were entered and compared against other measles sequences in the World Health Organization (WHO) measles nucleotide surveillance database (MeaNS, http://www.who-measles.org), hosted by the VRD at PHE [19].

Public health investigation and response

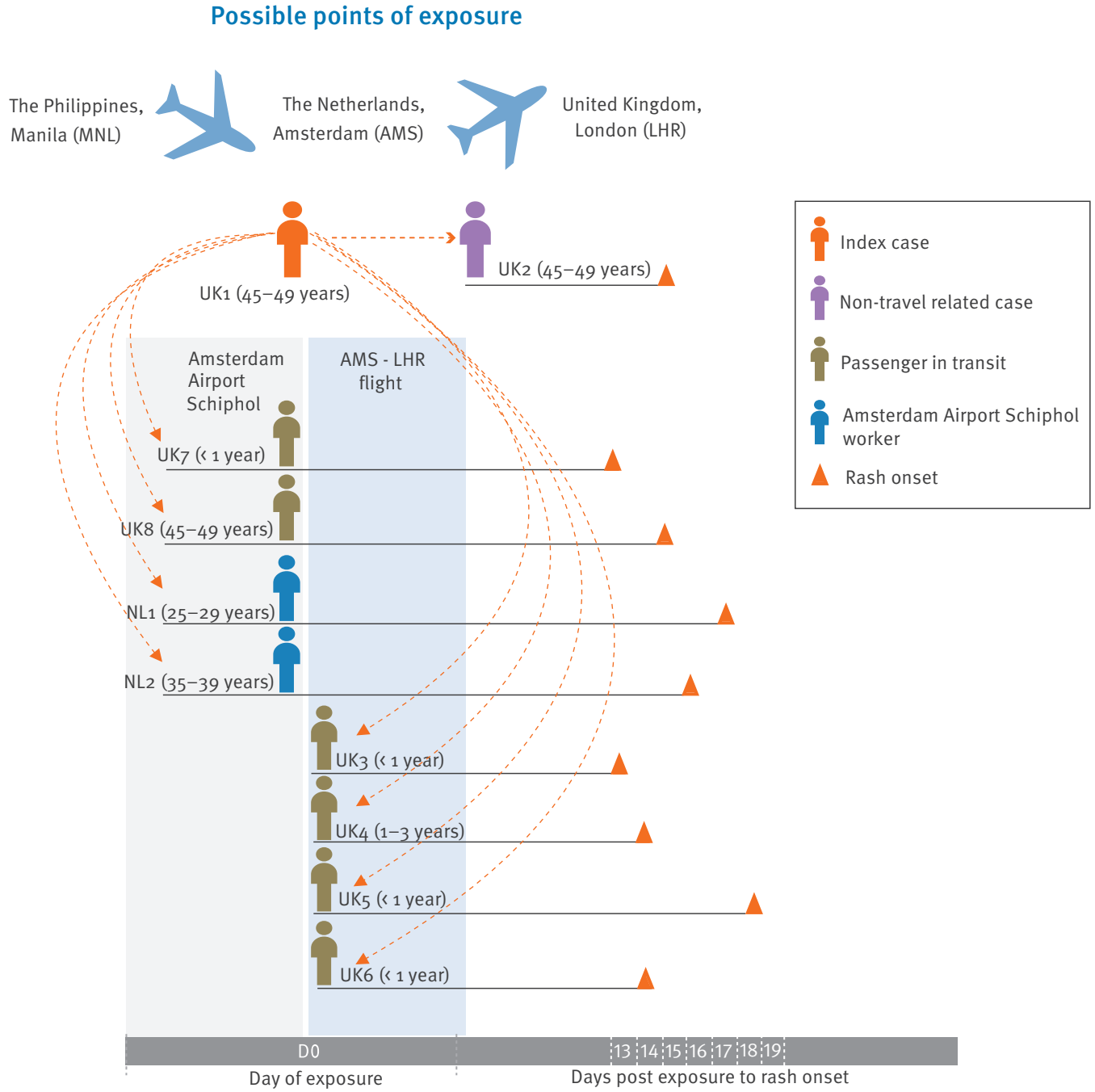
PHE is responsible for the investigation and public health management of measles cases and contacts in England. PHE interviewed the index case immediately after notification and PEP was offered to susceptible household contacts of the case. As the index case was infectious during air travel, PHE contacted the airline for passenger contact tracing. The airline initially declined to provide the passenger and crew member list to PHE but agreed to send a letter to passengers and crew members on the flight between Amsterdam Airport Schiphol and Heathrow Airport. The letter notified them about the potential measles exposure and recommended that they contact PHE or their local health service if they were pregnant, had a weakened immune system, travelled with an infant less than 6 months old, or had developed symptoms compatible with measles. PHE also notified the Dutch International Health Regulation Focal Point (IHRFP) about possible exposures during the transit of the index case at Amsterdam Airport Schiphol.

The RIVM laboratory shared genotype and epidemiological information with the WHO MeaNS network about two geographically separate clusters of the outbreak strain that were identified in March 2014. RIVM alerted Municipal Health Services in regions of the Netherlands with measles cases to conduct enhanced surveillance. Subsequent cases were captured, confirmed and sequenced.

Following the identification of multiple unlinked cases with the measles virus MVs/Tonbridge.GBR/5.14 in the UK and the Netherlands, epidemiological links between UK and Dutch cases were further investigated. All possible related cases in both the UK and the Netherlands

Figure 2

Possible point of exposure and chain of transmission among measles cases in the United Kingdom and the Netherlands, week 5 to week 8 2014



AMS: Amsterdam Airport Schiphol; LHR: Heathrow Airport; MNL: Manila Ninoy Aquino International Airport; NL: the Netherlands; UK: United Kingdom.

with the measles virus MVs/Tonbridge.GBR/5.14 were interviewed to look for common links.

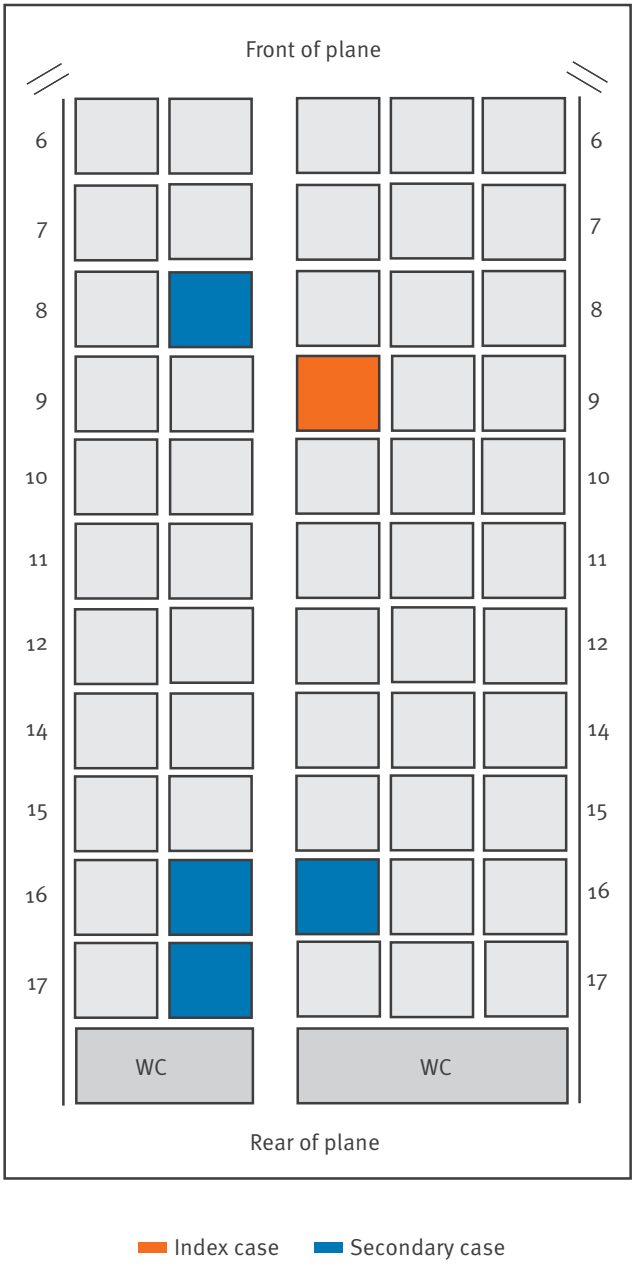
Results

Index case

The index case in this cluster (UK1) was an unvaccinated adult aged 45–49 years, born in the Philippines, but currently resident in the UK. Between December 2013 and January 2014, UK1 spent two consecutive months visiting family and friends in Manila, the Philippines. While in Manila, UK1 became unwell and developed a rash in week 5 2014, the day before returning to the UK. UK1 sought medical attention in Manila, but measles was not suspected. UK1 departed Manila for Heathrow Airport the following day, transiting via Taiwan Taoyuan International Airport and Amsterdam Airport Schiphol, before arriving in the UK on the second day after rash

Figure 3

Aircraft seating plan showing the index case and secondary cases on the flight from Amsterdam Airport Schiphol to Heathrow Airport (D0), week 5 2014



onset. The index case spent approximately five hours at Amsterdam Airport Schiphol during transit. Due to deterioration of symptoms, UK1 attended a local hospital the day after arrival in the UK (day 3 after rash onset), where measles was clinically suspected.

Laboratory results

The diagnosis of UK1 was confirmed by serology (IgM) and detection of the virus by PCR two days after hospital attendance in week 6 2015. Sequencing results showed that UK1 had been infected with a unique measles virus strain of genotype B3, MVs/Tonbridge.GBR/5.14, (GenBank KJ650198). The sequence differed by two nucleotides from the Harare B3 sequence (Mvi/Harare.ZWE/38.09), which by February 2014 had been associated with measles outbreaks in many countries, including the Philippines [20]. On 6 February 2014, the sequence was submitted to the WHO MeaNS database. At that time, there were no identical sequences available in either GenBank or MeaNS. With the identification of other cases with the same 450 nt sequence, this sequence was assigned as a new measles strain type, MVs/Tonbridge.GBR/5.14, (referred to in this report as the outbreak strain).

Overview of contacts

Following identification of UK1, a further 17 cases in the UK that met the outbreak case definition were identified between week 7 and week 12 2014. Of these 17 UK cases, 16 had the outbreak strain, and one case was not sequenced but had clear epidemiological links to the index case. Seven cases with the outbreak strain in the UK could be epidemiologically linked to UK1 (Figure 1).

Six of these UK cases (UK3–UK8) were found to have transited through or travelled from Amsterdam Airport Schiphol on the same date or flight as UK1, while one case (UK2) was a household contact of the index case (Figure 2). The remaining 10 UK cases with the outbreak strain identified through surveillance activities were investigated, but could not be epidemiologically or directly linked to UK1.

In the Netherlands, 15 cases met the outbreak case definition and were identified between week 7 and week 14 2014. Two secondary cases (NL1 and NL2) could be epidemiologically linked to UK1 (Figure 1) and worked at Amsterdam Airport Schiphol (Figure 2). Four measles cases with the outbreak strain lived in the same community as NL2, near Amsterdam Airport Schiphol. The remaining nine cases with the outbreak strain occurred in The Hague, and no formal epidemiological link with UK1 was established. An overview of cases linked to UK1, of whom six were male and three were female, is shown in Table 1.

Secondary and tertiary measles cases with the outbreak strain in the United Kingdom

UK2 was a household contact of UK1 during their infectious period in the UK. UK2 was an unvaccinated adult aged 45–49 years, with no history of recent travel outside of the UK. UK2 received a post-exposure MMR vaccination within 24 hours following contact with UK1, but developed measles rash 15 days post exposure.

UK3 was an unvaccinated infant aged < 1 year old returning from a country in southern Africa to the UK via Amsterdam Airport Schiphol. UK3 was on the same flight as UK1 from Amsterdam to London and was seated on a parent's lap. UK3 developed measles rash 13 days post exposure.

UK4 was an unvaccinated child aged 1–3 years returning from a country in southern Africa to the UK via

Table

Summary of measles cases in the United Kingdom and the Netherlands linked to air travel and transit at Amsterdam Airport Schiphol, week 5 to week 8 2014 (n=10)

Case number	Age (given as range)	Number of days post-exposure to rash onset	Measles vaccination status	Transmission setting
UK1 (index case)	45–49 years	Do	0 doses	Unknown
UK2	45–49 years	15	1 post-exposure MMR	Household contact
UK3	<1 year	13	0 doses	Airport/same flight
UK4 [a]	1–3 years	14	0 doses	Airport/same flight
UK5	<1 year	19	0 doses	Airport/same flight
UK6	<1 year	14	0 doses	Airport/same flight
UK7	<1 year	13	0 doses	Airport
UK8	45–49 years	15	0 doses	Airport
NL1	25–29 years	18	1 monovalent measles vaccine and 2 MMR doses	Airport
NL2	35–39 years	16	1 MMR dose	Airport

MMR: measles-mumps-rubella vaccine.

[a] Not genotyped, rash onset date consistent with acquisition of infection on the flight or at Amsterdam Airport Schiphol.

Amsterdam Airport Schiphol. While not genotyped, UK4 had rash onset 14 days post exposure, which is consistent with acquisition of infection on the flight or at Amsterdam Airport Schiphol, rather than in the household setting.

UK5 was an unvaccinated infant < 1 year old and a sibling of UK4. UK5 was also on the same flight as UK1 from Amsterdam to London, and was seated in a bassinet. UK5 developed measles rash 19 days post exposure.

UK6 was an unvaccinated infant aged < 1 year old returning from a country in southern Africa. UK6 was on the same flight as UK1 from Amsterdam to London, and seated on a parent's lap. UK6 developed measles rash 14 days post exposure.

Of the four UK secondary cases (UK3, UK4, UK5, UK6) who were on the same flight as UK1, only one case was seated within one row of UK1, while the remaining three cases were seated near the rear of the plane, six to seven rows from UK1 (Figure 3).

The remaining two secondary cases, an unvaccinated infant aged < 1 year old (UK7) and an unvaccinated adult aged 45–49 years (UK8) were not on the same flight as UK1. However, both cases were in transit at Amsterdam Airport Schiphol on the same day as UK1. UK7 was en route to the Caribbean and had flown with the same airline as UK1, and used the same gate area. UK7 developed a measles rash 13 days post exposure. UK8 was flying from Amsterdam Airport Schiphol to Gatwick Airport in the UK but had flown with a different airline from a different gate. UK8 developed measles rash 15 days post exposure.

The ten UK cases, who were infected with the outbreak strain but were not epidemiologically linked to the index case, were in the age range of < 1 year and 49 years. Nine cases were not known to have travelled abroad, with five cases clustered in Newcastle and two cases clustered in a different area of the country. One measles case had been on a cruise ship in Italy in late February 2014, which reported a measles outbreak with the same outbreak strain [21].

Secondary and tertiary measles cases with the outbreak strain in the Netherlands

In March 2014, RIVM identified measles cases with the same outbreak strain among two Amsterdam Airport Schiphol workers (Table 1). The outbreak strain was first identified in the Netherlands in an Amsterdam Airport Schiphol worker (NL1) aged 25–29 years. NL1 had received a single dose of monovalent measles vaccine and two doses of MMR vaccine in the past. NL1 had measles rash onset 18 days post exposure. NL1 worked at the airport on the morning that UK1 was in transit.

The second outbreak strain case was an Amsterdam Airport Schiphol worker aged 35–39 years (NL2) who had received one dose of MMR vaccine in the past. NL2 had measles rash onset 16 days post exposure and was hospitalised for four days due to general malaise and rash. NL2 was the only case in this cluster to require hospitalisation.

Between week 11 and week 14 2014, an additional four measles cases with the outbreak strain were detected in the community where NL2 lived, which was close to the airport. All four cases were unvaccinated and in the age range of 15 to 44 years.

The remaining nine Dutch cases with the outbreak strain but not epidemiologically linked to the index case were aged between < 1 year and 44 years. These cases were involved in two separate cluster events in The Hague. Five cases with the outbreak strain were involved in a kindergarten measles cluster. We were unable obtain information relating to recent travel. However, four of the five cases reported that the source of infection was at the kindergarten. Four cases were involved in a hospital measles cluster. Two of these four cases had a history of recent travel, but travelled after onset of measles symptoms.

Discussion

This report describes measles transmission in multiple settings in the UK and the Netherlands linked to an infectious traveller returning from the Philippines. Secondary transmission occurred firstly in passengers in transit and workers at Amsterdam Airport Schiphol; secondly, to passengers before or during a flight from the Netherlands to the UK and thirdly, in the household setting of the index case in the UK. One secondary case required hospitalisation due to general malaise and rash. Tertiary transmission occurred in the Netherlands in a community close to the airport. Additional cases with the outbreak strain were also identified in both the UK and the Netherlands, but could not be epidemiologically linked. We did not follow up other passengers potentially exposed on the Manila to Taipei and Taipei to Amsterdam flights, as there was no efficient method known to contact potentially exposed passengers. Therefore, there may have been additional or unreported cases linked to this cluster in the UK and the Netherlands.

The index case in this cluster developed measles in the Philippines, having spent two months there before departure to the UK, and where a large measles outbreak was ongoing [20,22]. Historically, the measles genotypes in clade B have been associated with sub–Saharan Africa [19,23], but genotype B3 is now widely distributed throughout the world, and associated with many outbreaks [24-26]. However, the outbreak strain we describe in this cluster had not been previously reported. The ability to identify the outbreak strain in the UK and subsequently in the Netherlands confirms the utility of having timely submission of sequences to the WHO MeaNS database. Exchange of information regarding epidemiological risk and exposures between the public health authorities in the UK and the Netherlands made it possible to link the cases in the Netherlands to the index case.

In a separate event in late February 2014, a measles outbreak on a cruise ship in Italy reported cases with the same strain [21]. At the time of reporting of this cruise ship outbreak, the only reference for this unique B3 sequence type was that of UK1. No epidemiological link was identified between UK1 and the cruise ship outbreak [27]. A UK case with the outbreak strain who had been on the cruise ship was later identified in March 2014. Three measles cases on the cruise ship outbreak were from the Philippines [21], which suggests the circulation of the novel B3 strain in the Philippines in early 2014.

Four UK cases in this cluster who were at the same airport or on the same flight as the index case were aged under 12 months and were not yet eligible for routine MMR vaccination in England [11]. Young unvaccinated children are at increased risk of measles and its complications [1]. Given the risk of measles while travelling to countries with endemic or epidemic transmission, current national recommendations in both the UK and the Netherlands advise that children aged over 6 months of age should be considered for an early dose of MMR vaccine before travel [10,11]. The data from this report suggests that infants travelling through major international hubs are at risk of measles infection and an early MMR vaccination should be considered by their parents or guardians.

Four adult cases (UK1, UK2, UK8, and NL2) were unvaccinated or had received only a single MMR vaccine. This represents a susceptible group of adults who were either not exposed to measles during childhood or were not fully vaccinated in routine MMR vaccination programmes. Measles is still endemic in many countries and measles outbreaks are occurring on a global scale [28]. It is therefore important that travellers obtain appropriate advice when travelling, to ensure that they are fully protected against measles and other vaccine-preventable diseases.

In this cluster, two measles cases were airport workers at Amsterdam Airport Schiphol. In a separate measles cluster in the Netherlands in January and February 2014, two other airport workers at Amsterdam Airport Schiphol, who were both unvaccinated and required hopsitalisation for measles complications, were found to have a genotype B3 strain identical to the MVi/Harare.ZWE/38.09 strain. Additional cases with the MVi/Harare.ZWE/38.09 strain were detected in their communities, indicating possible tertiary transmission. In 2013 and 2014, this strain was detected in cases imported from the Philippines into Canada, Japan and the United States, and in 10 cases in the UK [24-26]. Staff at large international airports risk possible exposure to infectious diseases from travellers. Therefore, in order to prevent the spread of measles, occupational health departments at airports could consider checking the vaccination status of airport workers to ensure they are fully vaccinated.

The recommendation by RAGIDA to prioritise contact tracing among those under 2 years of age is well-justified [15]. In this report, four of the secondary cases who contracted measles met these criteria for prioritisation. However, the RAGIDA recommendation to prioritise contact tracing of the remaining passengers by proximity to the index case may not be sufficiently sensitive for a highly communicable infection such as

measles. Firstly, measles exposure may have occurred in flight, while waiting at the gate or during the boarding process. Secondly, three out of four cases in the cluster were seated six to seven rows from the index case. Furthermore, when PHE contacted the airline to obtain passenger information, the airline was initially unwilling to release passenger details, despite assurances and regulations [29]. This hampered the targeted contact tracing as part of the public health investigation and response. Sending an email or text message to all potential exposed crew and passengers, rather than row by row as recommended in the RAGIDA guidelines, may be more feasible. This method, adopted by PHE when they are able to obtain the information in a timely manner from an airline, serves not only to rapidly identify vulnerable groups but also to remind those who are unvaccinated to receive their MMR vaccine. Early identification of potentially exposed cases could have limited further community spread. There is therefore a need for an efficient method to obtain passenger information and contact details that can be globally adopted by airline companies and enforced by their regulatory authorities. Additionally, by rapidly contacting potentially exposed cases, public health authorities could provide appropriate health messages or interventions to prevent tertiary spread in the wider community.

Conclusion

This report highlights the importance of sequence databases in epidemiological investigations and shows how a global effort to update circulating measles strains could assist in identifying the geographical origin of importations. This is particularly important as many countries progress towards measles elimination and therefore need to demonstrate absence of sustained indigenous transmission. Identification of clusters, combined with rapid public health responses, can limit further spread. However, public health response is reliant on rapidly identifying exposed cases. Therefore, improvements in vaccination of airport workers, in vaccine recommendations to those travelling and airline contact tracing should be made, to ensure exposed contacts are rapidly identified.

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Conflict of interest

None.

Authors' contributions

KEB, RB and SH coordinated the investigation. KP interviewed the index case in the UK. SM collected and interpreted case data in the UK. LN and RdS collected and interpreted case data in the Netherlands. KEB and RB coordinated genotyping and interpretation of the measles results in the UK and the Netherlands, respectively. Each author contributed to the content of the report and participated in drafting and revising the manuscript.

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Perspective

A system for automated outbreak detection of communicable diseases in Germany

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We describe the design and implementation of a novel automated outbreak detection system in Germany that monitors the routinely collected surveillance data for communicable diseases. Detecting unusually high case counts as early as possible is crucial as an accumulation may indicate an ongoing outbreak. The detection in our system is based on state-of-the-art statistical procedures conducting the necessary data mining task. In addition, we have developed effective methods to improve the presentation of the results of such algorithms to epidemiologists and other system users. The objective was to effectively integrate automatic outbreak detection into the epidemiological workflow of a public health institution. Since 2013, the system has been in routine use at the German Robert Koch Institute.

Introduction

In recent years, more and more data have been collected for the routine surveillance of infectious diseases. For instance in Germany, the Robert Koch Institute (RKI) implemented a national electronic surveillance system (SurvNet@RKI) in 2001 in response to the newly enacted Protection against Infection Act that requires regular collection of data on a number of notifiable diseases [1]. Cases are first reported by laboratories or physicians to local health authorities that may perform further investigations, and then transmitted to the RKI via the federal state health authorities. Collected information about cases includes sex, age, and subtype of the pathogen.

In addition to increasing data collection, a multitude of different outbreak detection algorithms for routinely collected public health data have been published [2]. Nonetheless, the added value of applying statistical methods for aberration detection at public health institutions is still subject to discussion because of several challenges, one of which is automating the data analysis and identifying signals without producing a plethora of signals. For instance, on October 2015, the SurvNet@RKI database contained ca 6.0 million case notifications in 88 different reporting categories such as *Salmonella* or norovirus, while outbreaks often become apparent when inspecting certain subsets of the data, e.g. within a specific geographical area or even a specific age group [3]. The problem is therefore to promptly identify these relevant subsets in the haystack of data. One statistical approach to this problem is to regularly analyse the data as multiple univariate time series in order to detect unexpected aberrations in specific subsets.

Nowadays, a semi-automatic monitoring system is in operation in many public health institutions (for examples in Europe see [4]). But because of too many signals or a misalignment between users' needs and signal presentation, the system output often has little impact on the practical work of these institutions. First attempts to focus more on the user perspective of monitoring systems are presented in Cakici et al. [4] and Kling et al. [5]. Our goal was to develop and establish an automatic information system that supports epidemiologists at the RKI in the timely detection of potential outbreaks of communicable diseases.

In this article, we present the implementation of a novel automated monitoring system at the RKI in Germany. The new system is now in routine use at the RKI for many reporting categories. Here, we describe the architecture of the system and our design decisions as well as first results and planned improvements. In sharing our experiences we aim to provide valuable information to others working on similar surveillance systems.

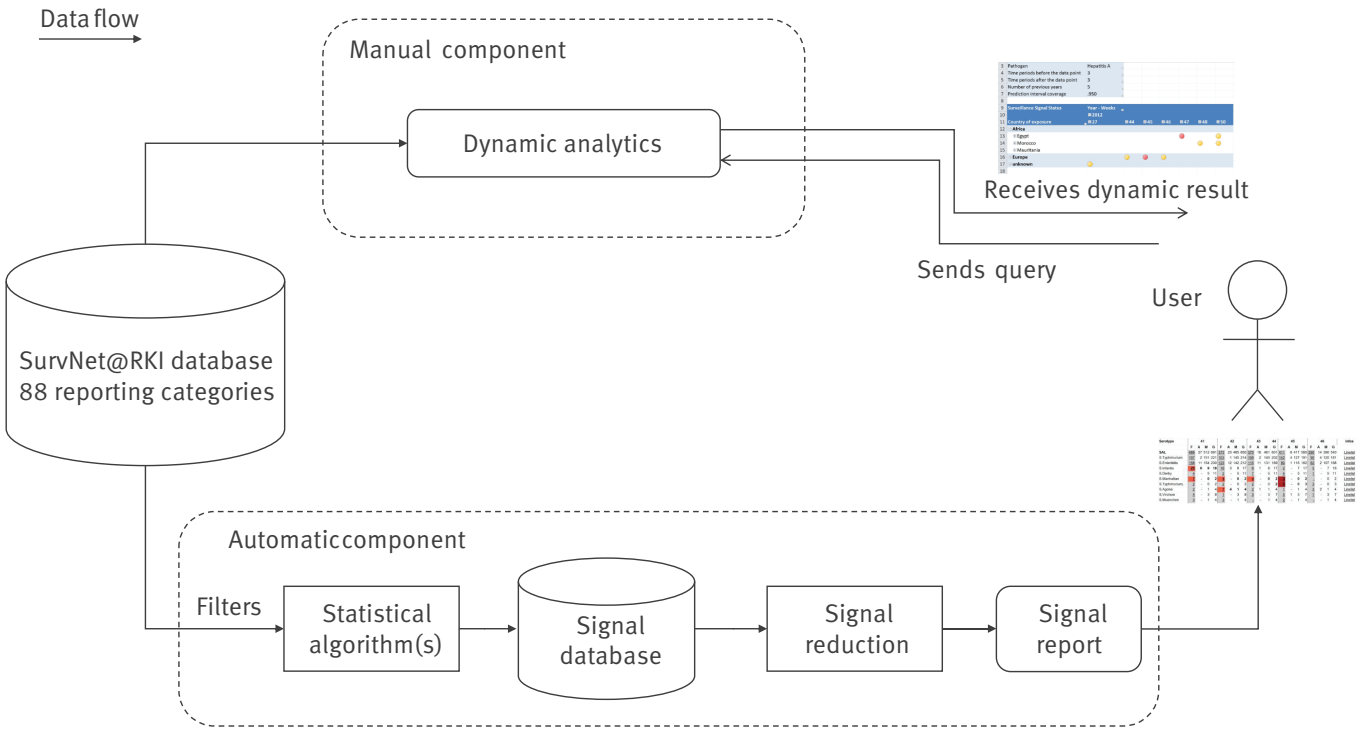
System design

Defining features of the system

We wanted to obtain results of a consistent quality as well as a standard procedure for the routine surveillance

Figure 1

Structural overview of the automatic surveillance system



The user can receive output from the automatic component of the system which consists of reports generated with predefined settings. Excerpts of such output are shown in Table 1 and Table 2. The user can also actively make ad-hoc queries to the manual component of the system the output of which is illustrated in Table 3.

workflow in our organisation. This objective lead to specific requirements for the system that were largely in line with the checklist for computer-supported outbreak detection systems formulated by Hulth et al. [6]; that article contains recommendations such as user friendliness and tight integration with the database. The development of the system and the refinements of the requirements were conducted iteratively. Based on the rapid prototyping philosophy, we initially focused on building a first prototype for one reporting category, namely *Salmonella* with its many serotypes.

Once the prototypes of the components had produced first results, we started discussing with two users, the epidemiologists in charge of *Salmonella*, the output of the system for *Salmonella*. The experiences from the first prototype lead to the design of a weekly automated report sent by email to the two epidemiologists. Once the system produced satisfactory results for this reporting category, we progressively scaled up the system to 48 reporting categories which account for roughly 80% of all received cases. Our goal has always been to create a general system for a variety of diseases instead of highly disease-specific solutions. In addition to the one-on-one discussions with the system users, we received more feedback and feature requests as the system grew.

System design

The system consists of two components: an automatic component routinely monitoring the data and a manual component which enriches data queries with ad hoc aberration detection (Figure 1). The first component automatically produces surveillance reports according to pre-defined settings. The second component allows the user to make customised queries for any time series they wish.

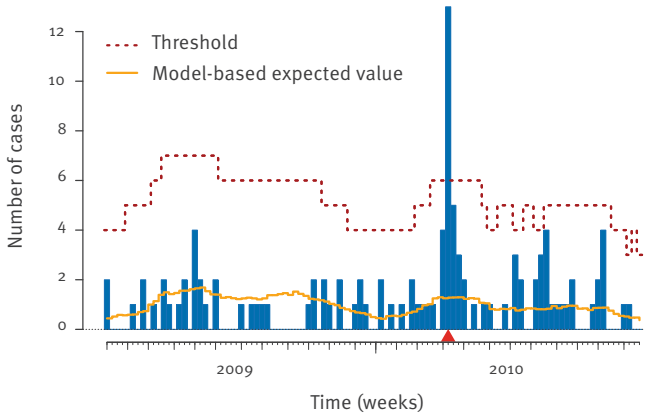
Automated analytical process

As shown in Figure 1, the automatic component consists of three subsystems: an analytical process, a signal database and a signal interface. The analytical process analyses the data with aberration detection algorithms and, in case of an unusually high number of cases, produces a signal which are stored in the signal database and communicated to the user through the signal interface.

The analytical process monitors the SurvNet@RKI case counts of the current and the six previous weeks on a daily basis for all reporting categories selected for aberration detection. Since outbreaks can occur in specific subsets of the population, e.g. at a specific location and in a specific age group, we monitor in parallel numerous time series corresponding to the respective subsets of the population in order to detect

Figure 2

Illustration of the time series detection algorithm applied to weekly cases of Salmonella Montevideo infection in Germany, 2009–2010



The algorithm is described in [7] and its open-source implementation in [8]. Blue bars indicate the observed number of cases. The red arrow indicates a signal.

signals that would be invisible when analysing the whole population. In particular, we stratify the time series by pathogen subtype (e.g. *Salmonella* serotype such as *S.* Infantis) or symptom (e.g. pneumonia), location (federal state, county), age group, sex, place of exposure. This stratification yields a set of univariate time series for each reporting category aggregated per week or month. The number of diagnostic tests performed is not a variable collected in the German mandatory reporting system. Therefore, the analysis of the numbers is sensitive to variations due to, for example, changes in laboratory procedures or in healthcare-seeking behaviour, e.g. during an outbreak with much media attention.

The system applies the implementation of the algorithm of Noufaily et al. [7] as described in Salmon et al. [8] to each time series in order to get a threshold for each observed count. The last four years of historic data are used as reference values for the algorithm. The algorithm uses an overdispersed Poisson generalised linear model with log link. The linear predictor accounts for seasonality through a 10-level factor variable, includes a time trend and uses a re-weighing scheme for taking past outliers into account. The estimates from the regression model are used to compute a threshold specific for each monitored week, defined as a quantile from the predictive distribution of the current count. A signal is generated for time t_0 if the observed number of cases exceeds the threshold. As an example, Figure 2 illustrates the detection algorithm applied to a single time series of *S.* Montevideo in Germany in 2009 and 2010 [9]. To address reporting delay, we monitor the current week and the six weeks before, i.e. it is possible to obtain a signal for one of the six previous weeks given the current data. This could mean getting a signal in week 5 of 2015 for the number of *Salmonella* infections reported during week 3 of 2015.

The automated analytical process was initially implemented solely in the statistical programming language *R* [10], using the *surveillance* package [8,11] for the detection part and other *R* packages [12] for the data pre- and post-processing steps, as well as for support for behaviour-driven software development. As in other systems [4,13], the automated component was built in a modular way so that the detection component can incorporate different detection algorithms. *R* was chosen over other programming languages as it allowed us to directly use a variety of statistical detection algorithms and visualisation procedures out of the box and because of its ability to rapidly prototype statistical procedures. During subsequent developments we ported large parts of the data management components to Microsoft C#/.NET to harmonise the system with existing information technology infrastructure at the RKI.

Signal database

The signal database stores signals generated by the analytical process. A signal corresponds to statistical evidence that the case count in a given subset of the data is higher than we would expect it to be based on historic data. A signal combines information about that data segment in which case counts were detected by a statistical algorithm, i.e. a filter on the data with a set of attributes (e.g. 'Hepatitis A; week 25 of 2013') and about the algorithm itself, its configuration and its output (e.g. the detection threshold).

This definition can be used directly to store the signals in the signal database and enables subsequent processing of the signals. This has direct advantages over analysis and communication as a combined step: the signals can have an age, they can be more or less important, they can be similar to each other and they can disappear over time when new data are received. In addition, signals can be communicated differently based on aspects such as user preferences.

Signal interface and communication

Signals are communicated to the user through predefined report templates for each reporting category. The reports display relevant signals found for a given category within a given time period. In addition to these main reports, several other reports display new signals found recently (for instance a signal at week 46 for a number of cases reported during week 45, which did not give a signal at week 45 or because of transmission delays), line lists and a spatial visualisation of the cases. The main reports are archived as Microsoft Excel files once a day and are sent by email to epidemiologists in charge of specific reporting categories once a week. Such a push/pull principle of communication was inspired by other monitoring systems such as the one described by Reis et al. [13].

Table 1

Automated outbreak detection, excerpt of the *Salmonella* report for weeks 41–46 of 2013: time series analysis at the national level

Serotype	Week 41				Week 42				Week 43				Week 44				Week 45				Week 46			
	y_t	o_t	μ_t	U_t	y_t	o_t	μ_t	U_t	y_t	o_t	μ_t	U_t	y_t	o_t	μ_t	U_t	y_t	o_t	μ_t	U_t	y_t	o_t	μ_t	U_t
Salmonella, all serotypes	466	27	512	691	373	23	485	650	370	16	461	620	356	15	439	601	411	8	417	580	290	14	390	540
S. Typhimurium	107	2	151	221	103	1	145	214	108	2	140	208	106	5	134	202	142	4	127	191	90	4	120	181
S. Enteritidis	158	11	154	230	123	12	142	212	115	11	131	194	84	4	124	189	80	1	116	182	62	2	107	168
S. Infantis	25	6	9	18	16	3	8	17	8	1	8	18	10	-	8	17	2	-	7	17	5	-	7	16
S. Derby	4	NA	5	11	2	NA	5	11	7	NA	5	11	3	NA	5	11	4	NA	5	11	1	-	5	11
S. Manhattan	7	NA	0	2	4	NA	0	2	4	NA	0	2	3	NA	0	2	3	NA	0	2	NA	NA	0	2
S. Typhimurium, monophasic	2	NA	0	2	2	NA	0	2	2	NA	0	2	6	NA	0	2	5	NA	0	3	3	NA	0	3
S. Agona	2	NA	1	4	7	4	1	4	2	1	1	4	3	2	1	4	1	NA	1	4	3	2	1	4
S. Virchow	4	NA	3	8	1	NA	3	8	3	NA	3	7	1	NA	3	7	5	1	3	7	1	NA	3	7
S. Muenchen	3	NA	1	4	3	NA	1	4	NA	NA	1	4	3	NA	1	4	2	NA	1	4	NA	NA	1	4

NA: not applicable.

Columns shaded in grey: observed count of the week. Bold: signals. Yellow: signals detected seven or more days before the current week (here, week 46 of 2013); red: newer signals.

Table 2

Automated outbreak detection, excerpt of the *Salmonella* report for weeks 41–46 of 2013: cluster analysis

			Week 41				Week 42				Week 43				Week 44				Week 45				Week 46			
Serovar	Region	Data filter	y_t	o_t	μ_t	U_t	y_t	o_t	μ_t	U_t	y_t	o_t	μ_t	U_t	y_t	o_t	μ_t	U_t	y_t	o_t	μ_t	U_t	y_t	o_t	μ_t	U_t
S. Agona	Germany	Male	1	NA	1	3	5	3	1	3	1	1	1	3	2	2	1	3	NA	NA	1	3	2	2	1	3
	Baden-Württemberg	LK Germersheim, LK Karlsruhe, LK Rastatt	NA	NA	NA	NA	6	4	0	2	1	1	0	2	2	2	0	2	NA	NA	0	1	2	2	0	1
S. Manhattan	Germany	Age 50–59 years	3	NA	0	1	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Male	3	NA	0	2	4	NA	0	2	1	NA	0	2	2	NA	0	2	2	NA	0	2	NA	NA	0	2
		Female	4	NA	0	2	NA	NA	0	2	3	NA	0	2	1	NA	0	2	1	NA	0	2	NA	NA	0	2
	Schleswig-Holstein	NA	3	NA	0	1	NA	NA	NA	NA	1	NA	0	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S. Schwarzengrund	Germany	Male	NA	NA	NA	NA	2	1	0	1	NA	NA	NA	NA	NA	NA	NA	NA	2	NA	NA	NA	NA	NA	NA	NA
S. Typhimurium, monophasic	Germany	Male	2	NA	0	2	1	NA	0	3	NA	NA	NA	NA	4	NA	0	3	3	NA	0	4	1	NA	0	3

LK: Landkreis; NA: not applicable.

Columns shaded in grey: observed count of the week. Bold: signals. Yellow: signals detected seven or more days before the current week (here, week 46 of 2013); red: newer signals.

The signal interface uses Microsoft SQL Server Reporting Services [14], mainly because it is already used at the RKI. It allows quick development of the reports that can be accessed from the Intranet through a web browser and supports the exportation of the reports as Microsoft Excel files. Furthermore, in order to support the decision on whether a signal is relevant, the user can click on any case count in the report to see the associated list of cases from the SurvNet@RKI database (line list).

Signal abstraction

During reporting, a problem arises due to the monitoring of the many time series aggregated in different ways for a reporting category: given a set of closely related signals, what signals should be shown to the user? Closely related signals could be signals for *Salmonella* in week 22 in Bavaria, for *Salmonella* in week 22 in Munich or for *Salmonella* in week 22 in Munich for male cases. Therefore, we developed a method to reduce the number of signals for reporting categories with a high number of signals, such as *Salmonella*.

Table 3

Automated outbreak detection, dynamic data query with aberration detection of hepatitis A cases associated with the country of exposure in 2012

Country of exposure	Week 27	Week 44	Week 45	Week 46	Week 47	Week 48	Week 50
Africa							
Egypt					Signal		Signal
Morocco						Signal	Signal
Mauritania							
Europe		Signal	Signal	Signal			
unknown	Signal						

The procedure makes use of the fact that each signal is associated with a filter for a set of attributes, e.g. geographical location, temporal location, sex and age group. Given a set of signals available for reporting, we first determine similar signals by partitioning the original set of signals into a set of signal groups. All signals within a specific group have equal values for a number of filter attributes. For example, we could group all signals by week so that each signal group consists of signals with the same reporting week; e.g. 2013 week 42. In the system at the RKI, we group all attributes except sex, age group and reporting location of the signal. Thus the signals within a group will not necessarily have the same values for sex, age group and location.

In a second step, we filter out some signals in each of these groups, while other similar signals are not filtered, to avoid presenting information which is not considered relevant for users. This is done by so-called filter relations which allow us to rank and compare signals according to a predefined metric. We use three different relations: 'more specific than', 'more general than' and 'more specific on the location and more general on age and sex'. The user can select between having no reduction, one of the three relations or a combination of the first two relations. In our example, the most general signal would be the signal for *Salmonella* in week 22 in Bavaria, whereas the most specific signal would be the signal for *Salmonella* in week 22 in Munich for male cases. It is therefore possible to focus the analysis of the signals on specific aspects, e.g. locating the centre of a possible outbreak by displaying only the most specific signals in terms of their filter attributes.

Manual analytical component

In addition to the automatic tool for outbreak detection, we also prepared a detection tool that can be applied to almost any subset of the data defined by the user, allowing users to screen very specific time series on demand, which was a wish expressed during meetings conducted with future users before the design of the system. This component monitors specific subsets of the data, for example case counts of hepatitis A in Berlin within the last six weeks, by comparing the current counts with past data, using a method similar to the algorithm of Stroup et al. [15].

System use

Report interface

As at October 2015, 62 users at the RKI and federal state health authorities received weekly reports from the automated component and interacted with the reports. Table 1 and Table 2 correspond to an excerpt of the Excel-based report for cases of *Salmonella* infection reported in weeks 41 to 46 in 2013. The report contains two data tables with a similar structure. For each week t, we report the number of cases y_t, the estimated expected case count μ_t, the threshold U_t and the number of cases o_t that were manually marked as being part of an outbreak in the SurvNet@RKI database. Cases are sometimes identified as a cluster by local health authorities, e.g. a cluster of cases of norovirus infection after a shared meal. Coloured cells in the Tables indicate signals for the respective week. Signals that were detected seven or more days before the current week are marked yellow, newer signals are marked red. Table 1 corresponds to the reported number of cases per serotype for the six weeks before the current week, in this example with a signal for *S.* Infantis in week 41. Table 2 displays the results of a stratified analysis as described in the previous section. In this example, we see a cluster of female cases of *S.* Manhattan infection in week 41. Some of these signals prompt further checks by epidemiologists, helped by a direct link between the signal and the corresponding cases (line list). The number of signals in a report is an interplay between the number of time series formed by the considered subgroups of sex, age and geographical location, the algorithm settings for the disease, and whether signal reduction is performed. From January to October 2015, the median number of signals over all filters in the weekly *Salmonella* report was 62.

Table 3 shows an excerpt of an output of the manual component for a query of hepatitis A cases for the year 2012 by country of exposure, which is not a time series routinely analysed by the automatic component but could be of interest in particular situations. The table displays weeks of 2012 and countries where the number of cases exceeds the upper limit of the prediction interval.

Experiences from operation

Since 2013, the monitoring system has been widely adopted at the RKI. Although it has not been formally evaluated yet, we can observe a positive user acceptance, for example supported by an increasing number of users and feedback in discussions. Furthermore, the system has contributed to several outbreak investigations. For example, it detected a large local outbreak of cryptosporidiosis in August 2013 [16]. Apart from outbreak detection, the tool has provided awareness to epidemiologists, especially those monitoring trends in frequently notified infections prone to causing outbreaks: the number of cases for various aggregations of the data can now easily be visualised. Moreover, the aberration detection tool for dynamic data queries on case counts is appreciated because it is not always straightforward to visually assess whether the numbers of a time series plot are higher than usual. The manual component of our system provides a statistically informed decision for this.

We developed a system that provides results that are easy to understand and use, while based on sound statistical methods, with disease- and user-specific adjustments. The system was the result of an interdisciplinary collaboration between computer scientists, statisticians and epidemiologists combining user-focused system design, correct treatment of uncertainty and infectious disease knowledge to obtain a decision support tool useful for everyday practice.

Although the system already produces valuable results for routine work at the RKI, a number of improvements are possible. We are working on the problem of comparing frequently incomplete first-version data (e.g. where a pathogen subtype and a possible travel history of the case may now be known yet) to historic, more complete, last-version data (e.g. where subtype and probably country of infection have been added); each version is automatically numbered by the system each time a change is made to a case report. Moreover, it may be possible to add specific detection algorithms for dealing with reporting delays [17,18]. Furthermore, we are currently only able to detect outbreaks when case numbers are above the threshold in at least one week, i.e. if an outbreak emerges very slowly over several weeks it might not be detected quickly. Here, cumulative sum (CUSUM)-oriented procedures could be better at picking up the signal [19] because they add evidence over several timepoints. On a geographical level, only a fixed set of regions is monitored: Germany as a whole, federal states, counties and each county with its adjacent neighbours (which may overlap state borders). Thus we are only able to geographically detect outbreaks that are visible in one of these predefined county clusters. However, the architecture of the system would allow us to include more sophisticated space–time methods into the surveillance process such as those used by Kulldorff, Tango et al. and Neill [20-22]. In addition, performing tests on any time series is a classical case of multiple testing and thus leads to false alarms. Currently, we offer the epidemiologists the linelist to delve deeper into the data generating the signals in order to better understand the context, so that they can easily navigate the different signals of a report. A framework for controlling overall false alarm rates individually for each user in combination with the signal abstractions could further improve user acceptance.

We see these accounts of successful implementation at public health institutions an important contribution because automatic detection systems are much needed in the current big data environments arising from routine surveillance data collection. Our aim here was to explain the RKI development strategy and user focus of the system. A more technical article describes the algorithmic functionality of the R *surveillance* package [17].

The amount of data held by public health institutes will certainly continue to grow. As a consequence, automatic outbreak detection systems such as the one presented here, will become increasingly important. At the same time, care is needed when integrating such a system into a workflow and taking further steps towards user acceptance. From an organisational point of view, a challenge is to design effective guidelines on how the generated signals are to be handled in a standardised way. This could range from considering signals only as an additional resource for surveillance to having each signal checked by an epidemiologist. Now that the system is in place, one could in the future tailor the detection even more to the needs of the users, e.g. by actively including user feedback in the statistical detection algorithms. Including user feedback could start by collecting appropriate data about the users' reaction to each signal. We think that our experience with an automatic surveillance system will motivate the development and maintenance of similar decision support tools in other European countries.

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Conflict of interest

None declared.

Authors' contributions

Based on the initial analysis and design by DS, MH and MS, DS implemented the system that HB now maintains and develops, while MS implemented the statistical algorithm in R.

DS, MS, MH drafted the first version of the manuscript. All authors critically revised the manuscript and approved the final version.

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Letter to the editor: The measles outbreak in Bulgaria, 2009-2011: an epidemiological assessment and lessons learnt - but not completely yet

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To the editor: The recent report by Muscat et al., on the 2009 to 2011 measles outbreak in Bulgaria, emphasised that this infectious disease still represents a health issue of concern that can re-emerge with a substantial magnitude in Europe [1]. Indeed, this epidemiological analysis recorded 24,364 cases in Bulgaria between April 2009 and December 2011 and, importantly, around 73% of those infected were children and adolescents‹15 years of age. According to a previous national survey, almost 30% of children aged 2 to 10 had not been immunised against measles [2]. Moreover, most cases occurred in the Roma ethnic group, where the immunisation rate was even lower than in the rest of the Bulgarian population [1].

The experience described illustrates that measles can still spread in European countries, if the vaccination coverage is not appropriate and, especially, if there are predisposing conditions, such as vulnerable ethnic groups, immigrants from endemic countries, as well as socially and economically disadvantaged people

In addition, the very recent report of a measles outbreak in a refugee settlement in Calais, France, between January and February 2016, reinforced the concept that the current measles threat does not necessarily result from individuals originating from outside Europe only, as the measles genotype identified in this outbreak is known to be one of the main measles strains circulating in Europe. Thus, the initial contamination might have come from a non-immunised European volunteer [3] and the refugee settlement with vulnerable individuals acted as an 'epidemiological amplifier' of this communicable infection. However, that was a small outbreak with limited number of cases.

The important lesson that Europe still needs to learn, or be reminded of, is that measles has not been eradicated yet and, that therefore, European countries cannot let down their guard, especially considering the current considerable migration of people from areas where the disease is endemic and where the vaccination programmes have been impacted by armed conflicts.

An important aspect of the Bulgarian outbreak is highlighted in the analysis of measles-related deaths and its age distribution. The authors recorded 24 fatal cases with 19 of them occurring in children ‹15 years. Importantly, 11 measles-related deaths were in the paediatric population aged‹1 year and another 5 cases were in the 1 to 4 years age group.

Indeed, in most developed countries, the measles, mumps, rubella (MMR) vaccine schedule consists of two doses: the first dose is usually administered between 12 and 15 months of life; the second dose is proposed at 5 to 6 years or 11 to 12 years of age, according to the respective national or regional plan. As a consequence, neonates and young infants are not actively immunised and their source of protection relies upon measles herd immunity and/or maternal antibody transmission, which anyway does not persist beyond the first 6 to 9 months of life [4]. Therefore, young infants represent the most susceptible population target of the infection and they are also the most vulnerable individuals to develop severe complications, as it is indicated by their case-fatality ratio during the Bulgarian outbreak (0.28%, compared with the overall rate of 0.1%) [1]. Moreover, measles infection early in life can predispose to later neurological complications, such as subacute sclerosing panencephalitis (SSPE); indeed, in infants ‹1 year, the prevalence is around 1 SSPE case per 5,000 infections vs 1 SSPE case per 25,000 in those infected overall [5,6].

According to current knowledge, herd immunity can be achieved through an immunisation rate›95% in the general population. Unfortunately, in the study by Andrews et al., evaluating measles susceptibility in

17 European countries, only seven met World Health Organization targets of immunisation against measles [2]. Recently, on 27 January 2016, the Italian Public Health Institute (Istituto Superiore di Sanita', ISS) published the assessment of the protection against measles in Italy which resulted in a national vaccination coverage of 82–83% with a great variability among Italian regions, ranging from 65% to 92%. Moreover, the vaccine coverage of the whole population was shown to decrease gradually over the years [7].

In conclusion, the mortality analysis during the measles outbreak can help us convey an important message to parents: establishing and maintaining an immunisation rate able to promote herd immunity is the only way to protect our offspring against measles in the first years of life, when their active immunisation process has not been completed yet. Communicating results from mortality analysis could reinforce vaccination campaigns for measles, by helping parents to recognise the importance of MMR vaccine, as nowadays a number of them refuse and/or underrate this means of protection.

Conflict of interest

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Authors' contributions

Dimitri Poddighe conceived and wrote the manuscript.

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