Impact factor 5.49

Eurosurveillance

Europe's journal on infectious disease epidemiology, prevention and control

Vol. 19 | Weekly issue 4 | 30 January 2014

Rapid communications

Surveillance and outbreak reports

ecdc EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

www.eurosurveillance.org

Influenza season 2013/14 has started in Europe with influenza A(H1)pdm09 virus being the most prevalent subtype

C Adlhoch (Cornelia.Adlhoch@ecdc.europa.eu)[1], E Broberg[1], J Beauté[1], R Snacken[1], E Bancroft[1], P Zucs[1], P Penttinen[1], on behalf of the European Influenza Surveillance Network (EISN)
1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

Citation style for this article:
Adlhoch C, Broberg E, Beauté J, Snacken R, Bancroft E, Zucs P, Penttinen P, on behalf of the European Influenza Surveillance Network (EISN). Influenza season 2013/14 has started in Europe with influenza A(H1)pdm09 virus being the most prevalent subtype. Euro Surveill. 2014;19(4):pii=20686. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20686

Article submitted on 28 January 2014/ published on 30 January 2014

The 2013/14 influenza season has started in Europe. Four countries have reported medium intensity influenza activity, with children under 15 years being the most affected age group. A growing number of countries see increasing rates of influenza-like illness or acute respiratory infection and increasing proportions of specimens positive for influenza A(H1)pdm09 virus. In previous seasons, this subtype was associated with higher reported numbers of severe and fatal cases. Clinicians should offer influenza vaccination to unvaccinated persons belonging to risk groups.

We present an early descriptive analysis of the epidemiology and virology of the 2013/14 influenza season in Europe, following its recent start in some southern parts. We summarise current knowledge on the intensity of influenza-like illness (ILI) or acute respiratory infection (ARI) activity, circulating influenza viruses and the frequency and characteristics of severe cases for the benefit of decision-makers, public health experts and clinicians in European countries not yet affected by epidemic influenza this season.

Influenza causes substantial morbidity and mortality, has pandemic potential and is therefore under continuous global surveillance. In the European Union (EU) and European Economic Area (EEA), the European Influenza Surveillance Network (EISN) performs influenza surveillance [1,2]. Weekly epidemiological and virological influenza data are collected from 30 EU/EAA countries to determine the start, end, magnitude and severity of the season as well as the dominant circulating influenza viruses. Data collected include sentinel primary care consultations for ILI or ARI [3], the number of tested and influenza virus-positive specimens of sentinel patients, the results of typing, subtyping and antigenic and genetic characterisation of circulating influenza viruses [4,5]. In addition, 11 countries (Austria, Finland, France, Ireland, Malta, Portugal, Romania, Slovakia, Spain, Sweden and the United Kingdom (UK)) report hospitalised cases or severe acute respiratory infection (SARI) with laboratory-confirmed influenza, including cases with fatal outcome.

Epidemiological situation in primary healthcare

In the first three weeks of 2014 (week 1 started on 30 December 2013), both the ILI/ARI rates (number of cases per 100,000 population) and the percentage of influenza virus-positive sentinel specimens increased in 12 countries. Spain has reported medium intensity of influenza activity since week 1/2014, Bulgaria, Greece and Portugal since week 2/2014 (Table). Portugal and Spain have also indicated widespread geographical transmission. Indicators of influenza activity are described in [6].

In Bulgaria and Spain, ARI and ILI rates, respectively, in 2013/14 have exceeded those of the corresponding period (the first weeks after the start) of the 2012/13 season, but are comparable to the 2011/12 season, which were dominated by influenza B and A(H3) respectively. They have exceeded the rates in 2010/11 in Spain, but not in Bulgaria, when influenza A(H1) pdm09 accounted for the majority of circulating influenza viruses.

In Bulgaria and Spain, where ARI or ILI data from sentinel primary healthcare providers are reported by age group, children under 15 years of age have to date been affected the most (Figure 1). In Portugal, similar rates have been reported for people aged 5–14 years and those aged 15–64 years (Figure 1).

Virological situation in primary healthcare

The proportion of influenza virus-positive sentinel samples across Europe has increased steadily, from 4% in week 49/2013 to 34% in week 2/2014 (Figure 2). Since week 40/2013, 97% of sentinel specimens have tested positive for influenza type A virus and 3% for type B. Among subtyped influenza A viruses, A(H1)pdm09 and A(H3) were detected in almost equal proportions in

Table

Reported influenza intensity and dominant circulating influenza virus (sub)type (≥60% of (sub)type detections) by week, EU/EEA, weeks 40/2013–3/2014

Country	Week number, 2013													Week number, 2014		
	40	41	42	43	44	45	46	47	48	49	50	51	52	1	2	3
Austria																
Belgium												A				
Bulgaria														A(H1) pdm09	A(H1) pdm09	A(H1) pdm09
Croatia																
Cyprus																
Czech Republic																
Denmark													A	A	A	A
Estonia																
Finland																A
France													A	A	A	A
Germany																
Greece													A(H1) pdm09		A(H1) pdm09	A(H1) pdm09
Hungary																
Iceland																
Ireland																A
Italy									A(H3N2)	A(H3N2)	A(H3N2)	A(H3N2)		A(H3N2)	A	A
Latvia																
Lithuania																
Luxembourg																
Malta																A
Netherlands																
Norway									A	A	A	A	A	A	A	A
Poland																
Portugal													A	A	A	A(H1) pdm09
Romania																
Slovakia																
Slovenia																A(H3)
Spain									A(H1N1) & A(H3)	A(H1N1) & A(H3)	A(H3)		A(H1) pdm09	A(H1) pdm09	A(H1) pdm09	A(H1) pdm09
Sweden												A(H1) pdm09	A(H1) pdm09	A(H1) pdm09	A(H1) pdm09	A(H1) pdm09
UK (England)																
UK (Northern Ireland)																
UK (Scotland)														A(H1) pdm09	A(H1) pdm09	A(H1) pdm09
UK (Wales)												B		A(H1) & A(H3)	A	A

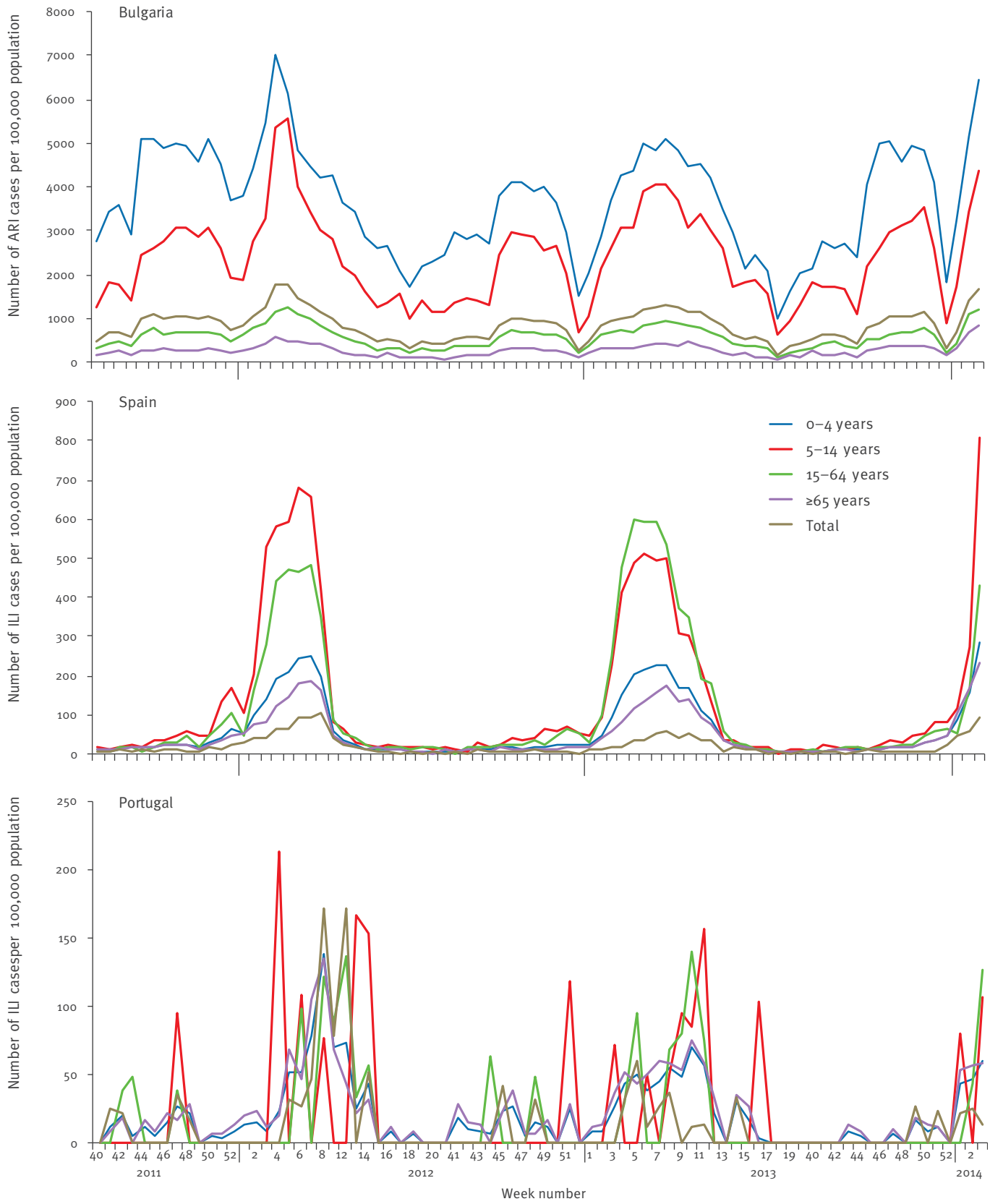
EEA: European Economic Area; EU: European Union; UK: United Kingdom.

Influenza intensity reported by country

- low intensity
- medium intensity
- no report

Figure 1

Influenza-like illness or acute respiratory infection rates[a] by age group, Bulgaria, Spain and Portugal[b], over the last three influenza seasons (weeks 40/2011–3/2014)



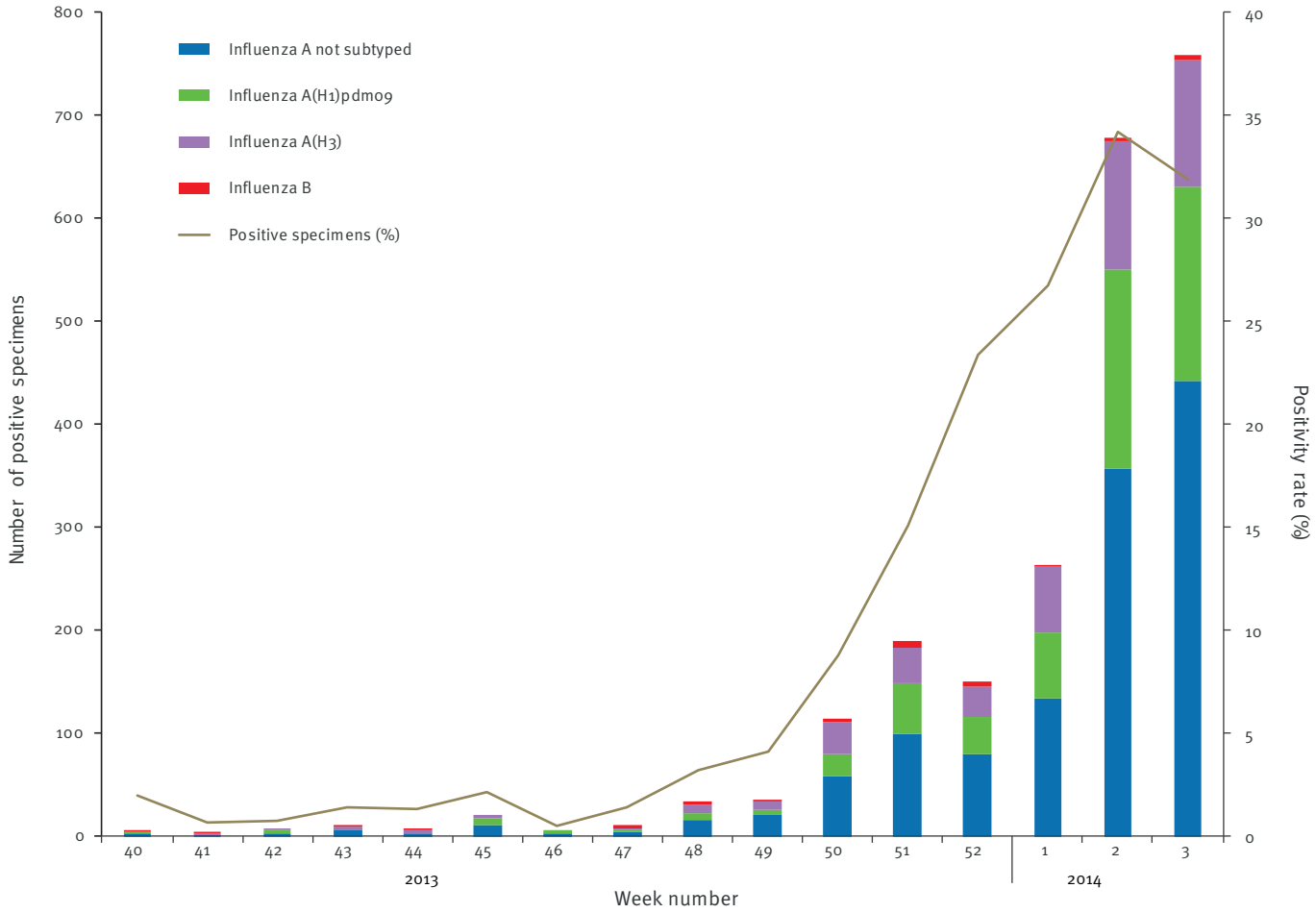
ARI: acute respiratory infection; ILI: influenza-like illness.

[a] Number of cases per 100,000 population.

[b] As of week 3/2014, the most affected countries of the 2013/14 season.

Figure 2

Number and percentage of influenza virus-positive sentinel specimens by (sub)type and week, EU/EEA, weeks 40/2013–3/2014



EEA: European Economic Area; EU: European Union.

week 1/2014; in weeks 2 and 3, the proportion of A(H1)pdm09 increased to 61% (Figure 2).

Based on specimens from sentinel and non-sentinel (e.g. specimens collected for diagnostic purposes in hospitals) sources, a total of 16 countries reported influenza A as the dominant circulating virus type at least for one week this season (Table). In week 3/2014, six countries (Bulgaria, Greece, Portugal, Spain, Sweden and the UK (Scotland) reported A(H1)pdm09 as dominant while Slovenia reported A(H3). The characterisation of the circulating viruses reported to date indicates a match with the current seasonal vaccine strains [7].

Epidemiological situation in hospitals

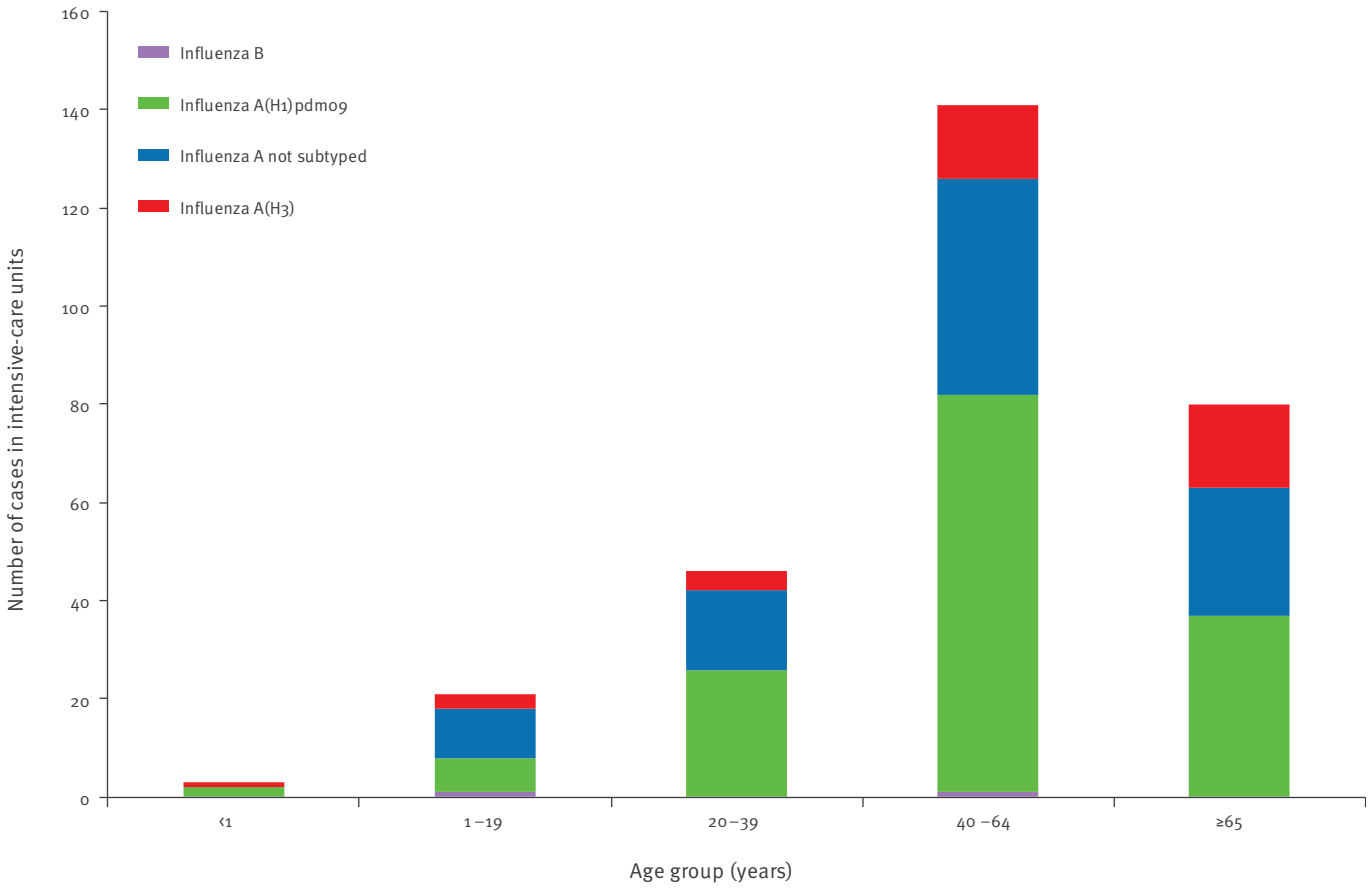
Since week 40/2013, France, Ireland, Romania, Spain, Sweden and the UK have reported a total of 409 cases admitted to intensive-care units (ICUs) with laboratory-confirmed influenza. These cases have mostly been 40–64 years of age and associated with influenza A(H1)pdm09 virus infection (Figure 3). The number of ICU cases reported from Spain during the beginning of this season has exceeded the numbers seen during the two previous seasons, but is lower than in the A(H1)pdm09-dominated season 2010/11 (in 2010/2011, there was a total of 596 cases in ICUs; in 2011/12, n=201; in 2012/13, n=202; in 2013/14, n=214).

France and Spain have reported 33 fatal cases in 2013/14, all due to influenza A virus infection. Of these, 19 were associated with A(H1)pdm09 infection, six with influenza A(H3) and in eight cases, only type A influenza was identified. Of the 19 cases with A(H1)pdm09, seven were between 40 and 64 years of age and nine were at least 65 years-old. Underlying risk factors for these cases are not systematically reported.

To date, of the 33 deaths this season, 29 have been reported from Spain, more than in the two previous seasons for the same period, but comparable to the 2010/11 season when 28 deaths were reported within the first three weeks of the season.

Figure 3

Laboratory-confirmed influenza cases admitted to intensive-care units, by age group and virus (sub)type, France, Ireland, Romania, Spain, Sweden and the United Kingdom, weeks 40/2013–3/2014 (n=409)



Discussion and recommendation

Influenza epidemics occur in Europe every winter, with their severity varying from one season to another. This can probably be largely explained by different circulating virus types and subtypes [8,9]. The 2013/14 influenza season, which still mostly affects southern Europe, has to date been characterised by an increasing proportion of the A(H1)pdm09 subtype, which now accounts for the majority of detected viruses, although A(H3) is co-circulating. As it is still early in the influenza season in Europe and many of the detected viruses have not yet been subtyped or further characterised, it may be too early to state anything definitive about the dominant subtype for this season. To date, influenza A(H1)pdm09 subtype appears to be associated with a higher number of ICU and fatal cases compared with the last two seasons, which were dominated by influenza A(H3) and type B viruses. Although ILI and ARI notification rates have been highest in the two youngest age groups (0–4 years and 5–14 years), most severe and fatal cases have been older than 40 years of age, which has been shown previously for A(H1)pdm09 infection [10]. This is only partly in line with current experience in the United States, where clinicians have recently been alerted about high numbers of severe cases reported this season, especially in young and middle-aged adults, due to circulation of influenza A(H1)pdm09 virus [11]. The European surveillance data collected during the first few weeks of the 2013/14 season provide no evidence of any similar excess numbers of severe influenza cases, but are comparable to the situation in the 2010/11 season. The data are, however, limited by the early time point in the season and the fact that only a few countries have reported influenza activity.

After influenza pandemics, seasonal excess mortality due to pneumonia and influenza or due to any cause is known to decrease over time, but remains at a relatively high level in subsequent years [12]. Similar to the situation in the United States, the influenza A(H1)pdm09 virus has continued to circulate in Europe after the 2009/10 pandemic [13]. Serological surveys have shown varying degrees of immunity against A(H1)pdm09 in different parts of the world and in different age groups: formerly unexposed parts of the European population can be expected to remain susceptible [14,15]. Only very little information is available about waning immunity and subsequent infection of people previously exposed to influenza A(H1)pdm09 virus.

On the basis of annual seroepidemiological studies conducted in Norway, it seems there is no waning immunity yet against A(H1)pdm09 virus, but rather an increased proportion of the population protected in all age groups [16]. As the 2013/14 influenza season in Europe has only just started, individuals belonging to risk groups, for whom influenza vaccination is recommended [17], can and should still be offered this season's influenza vaccine.

Acknowledgments

We thank all EISN members and European Reference Laboratory Network for Human Influenza (ERLI-Net) laboratories for their tireless commitment in collecting and providing high-quality European influenza surveillance data.

Conflict of interest

None declared.

Authors' contributions

C. Adlhoch: data analysis and draft of the manuscript; E. Broberg: virological surveillance and data analysis, review and revisions of the manuscript; J. Beauté: influenza surveillance of severe hospitalised and fatal cases, data maintenance and analysis, review of the manuscript; R. Snacken: surveillance data maintenance and analysis, review of the manuscript; E. Bancroft: critical review of the manuscript, data and literature review; P. Zucs: surveillance and concept of the data analysis as well as review manuscript; P. Penttinen: Surveillance strategy and data collection, critical review of the manuscript.

References

1. Paget J, Marquet R, Meijer A, van der Velden K. Influenza activity in Europe during eight seasons (1999-2007): an evaluation of the indicators used to measure activity and an assessment of the timing, length and course of peak activity (spread) across Europe. BMC infect Dis. 2007;7:141. http://dx.doi.org/10.1186/1471-2334-7-141
2. Meijer A, Valette M, Manuguerra JC, Pérez-Bre-a P, Paget J, Brown C, et al. Implementation of the community network of reference laboratories for human influenza in Europe. J Clin Virol. 2005;34(2):87-96. http://dx.doi.org/10.1016/j.jcv.2005.02.005
3. European Centre for Disease Prevention and Control (ECDC). Influenza case definitions. Stockholm: ECDC. [Accessed 29 Jan 2014]. Available from: http://ecdc.europa.eu/en/activities/surveillance/eisn/surveillance/pages/influenza_case_definitions.aspx
4. Meijer A, Brown C, Hungnes O, Schweiger B, Valette M, van der Werf S, et al. Programme of the Community Network of Reference Laboratories for Human Influenza to improve Influenza Surveillance in Europe. Vaccine. 2006;24(44-46):6717-23. http://dx.doi.org/10.1016/j.vaccine.2006.05.068
5. Meijer A, Paget WJ, Meerhoff TJ, Brown CS, Meuwissen LE, van der Velden J, et al. Epidemiological and virological assessment of influenza activity in Europe, during the 2004-2005 winter. Euro Surveill. 2006;11(5):pii=623. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=623
6. European Centre for Disease Prevention and Control (ECDC). Indicators of influenza activity. Stockholm: ECDC. [Accessed 29 Jan 2014]. Available from: http://ecdc.europa.eu/en/activities/surveillance/EISN/surveillance/Pages/indicators_influenza.aspx
7. European Centre for Disease Prevention and Control (ECDC). Weekly influenza surveillance overview. Main surveillance developments in week 3/2014 (13–19 January 2014). Stockholm: ECDC; 2014. Available from: http://ecdc.europa.eu/en/publications/Publications/influenza-surveillance-overview-24-jan-2014.pdf
8. Kaji M, Watanabe A, Aizawa H. Differences in clinical features between influenza A H1N1, A H3N2, and B in adult patients. Respirology. 2003;8(2):231-3. http://dx.doi.org/10.1046/j.1440-1843.2003.00457.x
9. Wie SH, So BH, Song JY, Cheong HJ, Seo YB, Choi SH, et al. A comparison of the clinical and epidemiological characteristics of adult patients with laboratory-confirmed influenza A or B during the 2011-2012 influenza season in Korea: a multi-center study. PloS One. 2013;8(5):e62685. http://dx.doi.org/10.1371/journal.pone.0062685
10. Karageorgopoulos DE, Vouloumanou EK, Korbila IP, Kapaskelis A, Falagas ME. Age distribution of cases of 2009 (H1N1) pandemic influenza in comparison with seasonal influenza. PloS One. 2011;6(7):e21690. http://dx.doi.org/10.1371/journal.pone.0021690
11. Centers for Disease Control and Prevention (CDC). Notice to clinicians: early reports of pH1N1-associated illnesses for the 2013-14 influenza season. Atlanta, GA: CDC; 2013. Available from: http://www.bt.cdc.gov/HAN/han00359.asp
12. Simonsen L, Clarke MJ, Schonberger LB, Arden NH, Cox NJ, Fukuda K. Pandemic versus epidemic influenza mortality: a pattern of changing age distribution. J Infect Dis. 1998;178(1):53-60. http://dx.doi.org/10.1086/515616
13. Beauté J, Broberg E, Plata F, Bonmarin I, O'Donnell J, Delgado C, et al. Overrepresentation of influenza A(H1N1)pdm09 virus among severe influenza cases in the 2011/12 season in four European countries. Euro Surveill. 2012;17(9):pii=20105. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20105
14. Uyeki TM. Preventing and controlling influenza with available interventions. N Engl J Med. 2014 Jan 22. [Epub ahead of print]. http://dx.doi.org/10.1056/NEJMp1400034
15. Broberg E, Nicoll A, Amato-Gauci A. Seroprevalence to influenza A(H1N1) 2009 virus--where are we? Clin Vaccine Immunol. 2011;18(8):1205-12. http://dx.doi.org/10.1128/CVI.05072-11
16. Folkehelseinstituttet. Rapport om scenarier for influensautbrudd i 2014. [Report on scenarios for influenza outbreaks in 2014]. Oslo: Folkehelseinstituttet. [Accessed 29 Jan 2014]. Norwegian. Available from: http://www.fhi.no/dokumenter/ode112b750.pdf
17. European Council. Council recommendation of 22 December 2009 on seasonal influenza vaccination. Official Journal of the European Union. Luxembourg: Publications Office of the European Union. 29.12.2009:L 348. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:348:0071:0072:EN:PDF

Two cases of Zika fever imported from French Polynesia to Japan, December 2013 to January 2014

S Kutsuna (sonare.since1192@gmail.com)[1], Y Kato[1], T Takasaki[2], M L Moi[2], A Kotaki[2], H Uemura[1], T Matono[1], Y Fujiya[1], M Mawatari[1], N Takeshita[1], K Hayakawa[1], S Kanagawa[1], N Ohmagari[1]

1. National Center for Global health and Medicine, Disease Control and Prevention Center, Tokyo, Japan
2. Department of Virology 1, National Institute of Infectious Diseases, Shinjukuku, Tokyo, Japan

Citation style for this article:
Kutsuna S, Kato Y, Takasaki T, Moi ML, Kotaki A, Uemura H, Matono T, Fujiya Y, Mawatari M, Takeshita N, Hayakawa K, Kanagawa S, Ohmagari N. Two cases of Zika fever imported from French Polynesia to Japan, December 2013 to January 2014 . Euro Surveill. 2014;19(4):pii=20683. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20683

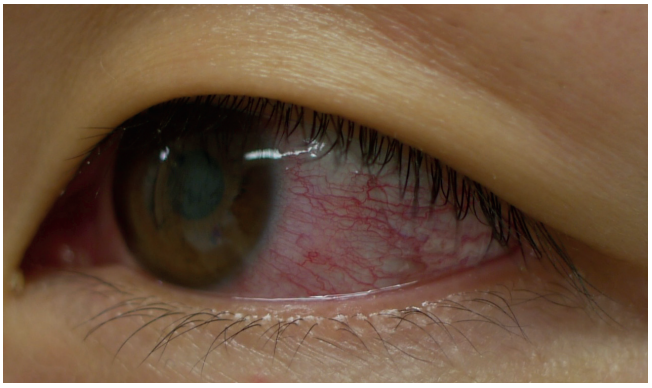
Article submitted on 19 January 2014 / published on 30 January 2014

We present two cases of imported Zika fever to Japan, in travellers returning from French Polynesia, where an outbreak due to Zika virus (ZIKV) is ongoing since week 41 of 2013. This report serves to raise awareness among healthcare professionals, that the differential diagnosis of febrile and subfebrile patients with rash should include ZIKV infection, especially in patients returning from areas affected by this virus.

We report two cases of Zika fever in Japan, which were imported from French Polynesia, where on 6 November 2013 public health authorities reported an outbreak of subfebrile illness with rash due to Zika virus (ZIKV). The epidemic started spreading across the archipelago beginning in week 41 of 2013 [1]. During weeks 42 to 52, the syndromic surveillance network reported 6,630 suspected ZIKV infection cases to the Bureau de Veille Sanitaire. About 500 of these cases were tested at the Institute Louis Malarde laboratory in Papeete for confirmation; 333 were confirmed by real-time reverse transcription-polymerase chain reaction (RT-PCR) as ZIKV infections [2]. The outbreak is currently ongoing and as of 13 January 2014, 361 laboratory-confirmed cases have been reported [3]. Symptoms of most ZIKV infection cases are mild and self-limited (mean duration of symptoms is 3–6 days). No hospitalisations for acute infection have been reported.

Figure 1

Conjunctivitis in a case of imported Zika virus infection from French Polynesia, Japan, January 2014



Although the patient was afebrile upon examination, both bulbar conjunctivas appeared congested.

Case 1

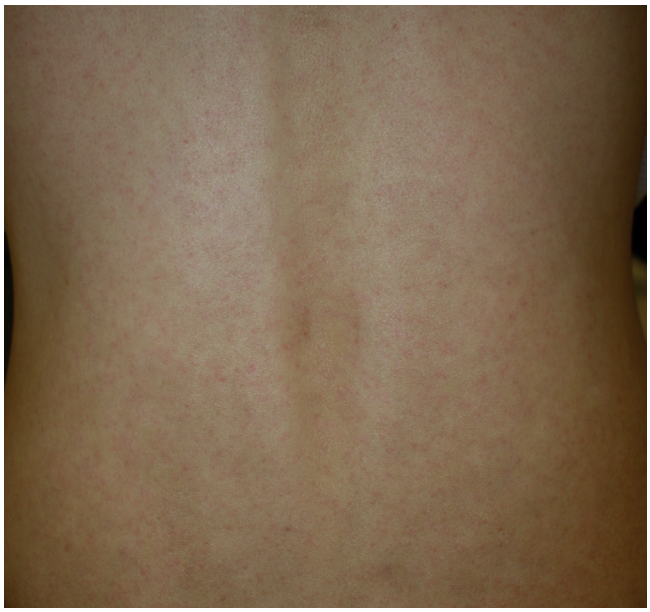
A previously healthy Japanese man in his mid-20s presented to our hospital in mid-December 2013 after four days of fever (self-reported), headache, and arthralgia and one day of rash. He had visited Bora Bora in French Polynesia, in the first week of December 2013 for six days for sightseeing with his partner. He did not use insect repellent during the trip. Upon examination, his body temperature was 37.2°C (99°F) and he had maculopapular rash on his face, trunk, and extremities. Other clinical examination results were normal. Laboratory tests revealed leucopenia (3,300 $\times 10^6$/L; norm: 3,500–8,500$\times 10^6$/L) and thrombocytopenia (14,900$\times 10^6$ /L; norm: 15,000–35,000$\times 10^6$ /L). ZIKV RNA was detected in serum using real-time RT-PCR performed at the National Institute of Infectious Diseases in Japan with primer-probe sets previously described [4]; thus, we diagnosed the patient with Zika fever. His fever and other symptoms subsided a day after first presentation and his rash disappeared over the next few days.

Case 2

A previously healthy Japanese woman in her early 30s presented to our hospital in the beginning of January 2014 for retro-orbital pain, slight fever (self-reported), rash, and itches. Her retro-orbital pain and mild fever had appeared five days prior to her visit at our hospital, while the rash and itches appeared on the day before the visit. She had travelled to Bora Bora where she stayed for 10 days starting mid-December 2013 for sightseeing with a companion. The first symptoms occurred six days after this journey. She had used insect repellent during her travels, but reported mosquito bites. She was afebrile and in good general condition at the first presentation to the hospital. On examination, both bulbar conjunctivas appeared

Figure 2

Maculopapular rash on the back in a case of imported Zika virus infection from French Polynesia, Japan, January 2014



congested (Figure 1). She had maculopapular rash on her face, trunk, and extremities (Figure 2).

Laboratory tests on the day of first presentation at the hospital revealed leucopenia ($3{,}500\times10^6$/L; norm: $3{,}500–8{,}500\times10^6$/L) and thrombocytopenia ($14{,}400\times10^6$/L; norm: $15{,}000–35{,}000\times10^6$/L). Real-time RT-PCR assays, performed at the National Institute of Infectious Diseases, gave negative results for ZIKV RNA in serum but presence of the virus was detected in urine. The patient was diagnosed with Zika fever. Her leucocyte and platelet levels returned to the normal range 12 days after first presentation at the hospital. The positive versus negative ratios (P/N ratio) of Zika-specific IgM antibodies were positive in two serum samples collected on the first day at the hospital and five days later (P/N ratios = 2.4 and 9.8, respectively; ratios were considered positive when greater than or equal to 2.0). The neutralising antibody titres of the serum in these two consecutive samples were $PRNT_{50}$=1:20 and $PRNT_{50}$=1:1,280, respectively.

Background

Zika fever is a febrile or subfebrile illness caused by ZIKV, which mainly spreads through the bite of infected mosquitoes. ZIKV is a member of the family Flaviviridae, which includes dengue viruses, West Nile, and yellow fever viruses [5]. The most common symptoms reported in confirmed ZIKV infections are fever, headache, malaise, maculopapular rash, fatigue or myalgia, and arthritis and arthralgia [6].

ZIKV was first isolated from the blood of a sentinel rhesus monkey from the Zika Forest in Uganda [7]. Serological studies and isolation of ZIKV strains have subsequently demonstrated that the virus has a wide geographical distribution, including eastern and western Africa, south and south-east Asia, and Micronesia [8], where in 2007, an outbreak of Zika fever was reported on Yap Island [9].

Phylogenetic analysis of the Zika virus sequence retrieved from case 2

Phylogenetic analysis of the partial ZIKV E-protein genome sequence (470 bp, GenBank accession number: AB908162*) obtained from the urine sample of case 2, shows that this sequence has 99.1% identity with the sequence of a ZIKV strain isolated from Cambodia in 2010 (GenBank accession number: JN860885), and 97.9% identity with the sequence of a ZIKV strain isolated in Yap islands in 2007 (GenBank accession number: EU545988) (Figure 3). The sequence from case 2 sample was also similar to previously identified ZIKV sequences of strains in Asia and Micronesia [8]. In the phylogenetic tree, these sequences formed a distinct cluster from that of sequences from Zika viruses of African origin. Further studies using full-length genome of the ZIKV will address the similarity between virus strains of the African and Asian clusters.

Discussion and conclusion

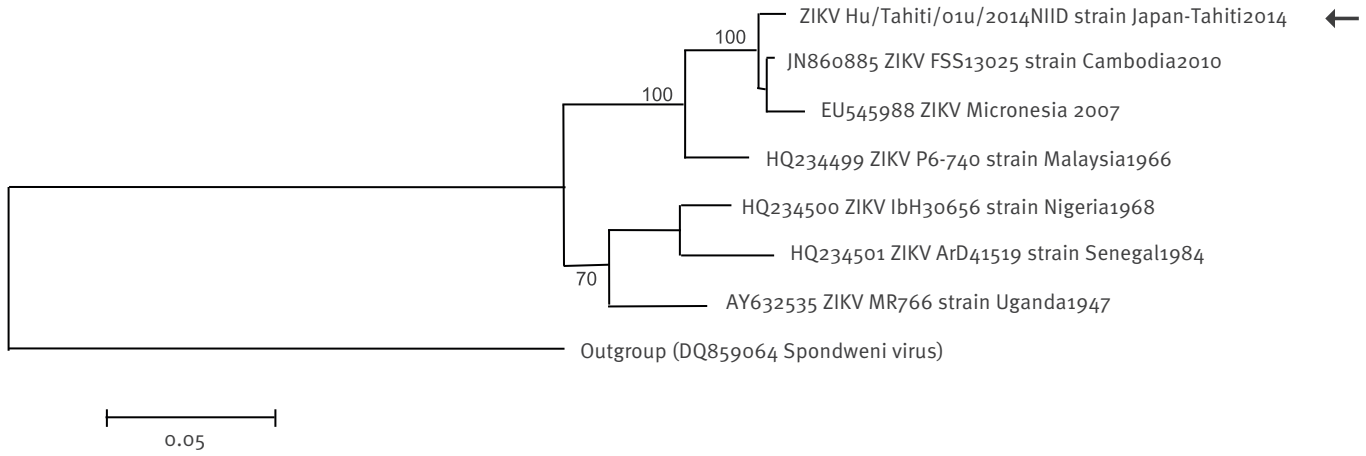
Our two cases are among the first imported cases found linked to the recent outbreak in French Polynesia starting in 2013. They occur shortly after 26 imported cases into New Caledonia from the same outbreak, as well as the report of one indigenous case [10]. Aside from cases related to French Polynesia, imported Zika fever cases have been previously identified in travellers returning from Africa and south-east Asia. These include a case of sexually transmitted Zika fever following two imported cases from Senegal into the United States, and an imported case of Zika fever from Indonesia to Australia [11,12]. Two imported cases from Thailand, one to Canada [13] and one to Germany [14] have also recently been reported.

Although the numbers of imported cases described so far are limited, the possibilities of ZIKV infections to be underdiagnosed and underreported are high due to generally mild symptoms and self-limited disease. Additionally, due to the similarity of ZIKV disease symptoms to those of dengue and chikungunya, differential diagnosis is required to define the extent of ZIKV epidemic. Importantly, as dengue virus (DENV) outbreaks also occur in French Polynesia [2], differential diagnosis between ZIKV infection and dengue is required in cases related to this area. Because of the ongoing dengue epidemic in Bora Bora, DENV infection was excluded in both cases in this study, by confirming that the serum samples were negative for both dengue virus nonstructural glycoprotein-1 (NS1) antigen and IgM/IgG antibodies, using rapid diagnostic kits (SD Bioline Dengue Duo Combo, Alere Medical, Inc.).

In this study, the two cases of ZIKV infection had not only leucopenia but also mild thrombocytopenia.

Figure 3

Phylogenetic analysis of a Zika virus sequence derived from a case of imported Zika virus infection from French Polynesia, Japan, January 2014



The phylogenetic tree was based on partial E-protein nucleotide sequences and compiled using the neighbour joining method (Genetyx, Japan). The sequence of the Spondweni virus (GenBank accession number DQ859064) was used as an outgroup. Bootstrap percentages based on 1,000 replicates are shown on the tree nodes. The sequence of the case of imported Zika virus infection from French Polynesia to Japan in January 2014 is indicated with an arrow. Scale bar (0.05) indicates nucleotide substitutions per site.

Previous investigators reported leucopenia, but not thrombocytopenia in patients with ZIKV infection [12]. Our two cases suggest that ZIKV infection can be associated with clinical features including thrombocytopenia and leucopenia, and shares similar clinical features to those of dengue fever and yellow fever.

In the second case identified in this study, viral RNA was negative in the serum sample but was positive in the urine sample. To our knowledge, this is the first case diagnosed by detection of Zika viral particles in urine. Detection of DENV genome in urine after disappearance of the viral genome in serum samples by real-time RT-PCR has been a useful laboratory diagnostic method [15]. Our case suggests that detection of Zika virus genome in urine by real-time RT-PCR is useful to confirm ZIKV infection, particularly after disappearance of viraemia in serum.

Phylogenetic analysis revealed that the ZIKV genome sequences of case 2, had a high sequence homology with recent strains from Asia and Micronesia, including those detected in Cambodia in 2010, but sequence homology was low with a strain isolated in 1947, the Ugandan prototype MR766 strain [4].

The ongoing ZIKV outbreaks in French Polynesia and the confirmation of ZIKV viraemic travellers in our study suggests that in addition to enhanced and continued surveillance efforts, awareness among healthcare professionals should be raised that ZIKV infection ought to be considered as differential diagnosis in febrile patients with rash returning from areas affected by this virus. Further prevention measures, such as offering advice on the use of insect repellents during travel to regions with outbreaks, would be important for ZIKV disease control.

Acknowledgments

This work was supported by funding from Research on Emerging and Re-emerging Infectious Diseases by the Ministry of Health, Labor and Welfare, Japan (H24-shinkou-ippan-013, H23-shinkou-ippan-010).

Conflict of interest

None declared.

Authors' contributions

Satoshi Kutsuna collected the data and drafted the manuscript; Yasuyuki Kato participated in the coordination and concept of the manuscript and edited the manuscript and helped with the draft of the manuscript; Tomohiko Takasaki, Meng Ling Moi, Akira Kotaki performed real-time RT-PCR and performed the phylogenetic analysis; Haruka Uemura, Takashi Matono, Yoshihiro Fujiya, Momoko Mawatari, Nozomi Takeshita, Kayoko Hayakawa collected the data and participated in the concept of the manuscript; Shuzo Kanagawa, Norio Ohmagari revised the article for intellectual content. All authors read and critically revised the first as well as the subsequent and final drafts of this manuscript.

* Addendum:

The GenBank accession number of the partial Zika virus nucleotide sequence derived from a sample obtained from case 2 was added on 07 February 2014.

* Erratum:

The title of this manuscript was initially wrong at the time of publication: 'Two cases of Zika fever imported from French Polynesia to Japan, December to January 2013'. The mistake was corrected on 31 January 2014.

References

1. Institut de Veille Sanitaire (InVS). Bulletin hebdomadaire international du 30 octobre au 5 novembre 2013. N°424. Bulletin hebdomadaire international. 2013; 30-oct to 5-nov 2013; 424. French. Available from: http://www.invs.sante.fr/Publications-et-outils/Bulletin-hebdomadaire-international/Tous-les-numeros/2013/Bulletin-hebdomadaire-international-du-30-octobre-au-5-novembre-2013.-N-424
2. Zika and dengue: the Epidemic. Tahiti info. [Accessed 30 Jan 2014]. French. Available from: http://www.tahiti-infos.com/Dengue-et-Zika-le-point-sur-l-epidemie_a91663.html
3. ProMED-mail. Zika virus - French Polynesia (03). Archive Number: 20140123.2227452. 23 January 2014. Available from: http://www.promedmail.org/direct.php?id=2227452
4. Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, et al. Genetic and Serologic Properties of Zika Virus Associated with an Epidemic, Yap State, Micronesia, 2007. Emerg Infect Dis. 2008;14(8):1232-9. http://dx.doi.org/10.3201/eid1408.080287
5. Kuno G, Chang GJ, Tsuchiya KR, Karabatsos N, Cropp CB. Phylogeny of the genus Flavivirus. J Virol. 1998;72(1):73-83.
6. Heang V, Yasuda CY, Sovann L, Haddow AD, Travassos da Rosa AP, Tesh RB, et al. Zika virus infection, Cambodia, 2010. Emerg Infect Dis. 2012;18(2):349-51. http://dx.doi.org/10.3201/eid1802.111224
7. Dick GW, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity.Trans R Soc Trop Med Hyg. 1952;46(5):509-20. http://dx.doi.org/10.1016/0035-9203(52)90042-4
8. Haddow AD, Schuh AJ, Yasuda CY, Kasper MR, Heang V, Huy R, et al. Genetic characterization of Zika virus strains: geographic expansion of the Asian lineage. PLoS Negl Trop Dis. 2012;6(2):e1477. http://dx.doi.org/10.1371/journal.pntd.0001477
9. Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med. 2009;360(24):2536-43. http://dx.doi.org/10.1056/NEJMoa0805715
10. ProMED-mail. ZIKA VIRUS - NEW CALEDONIA. Archive Number: 20140122.2224823. 22 January 2014. Available from: http://www.promedmail.org/direct.php?id=2224823
11. Foy BD, Kobylinski KC, Chilson Foy JL, Blitvich BJ, Travassos da Rosa A, Haddow AD, et al. Probable non-vector-borne transmission of Zika virus, Colorado, USA. Emerg Infect Dis. 2011;17(5):880-2. http://dx.doi.org/10.3201/eid1705.101939
12. Kwong JC, Druce JD, Leder K. Zika virus infection acquired during brief travel to Indonesia. Am J Trop Med Hyg. 2013;89(3):516-7. http://dx.doi.org/10.4269/ajtmh.13-0029
13. ProMED-mail. ZIKA VIRUS - CANADA ex THAILAND. Archive Number: 20130529.1744108. 29 March 2013. Available from: http://www.promedmail.org/direct.php?id=1744108
14. Tappe D, Rissland J, Gabriel M, Emmerich P, Günther S, Held G, Smola S, Schmidt-Chanasit J. First case of laboratory-confirmed Zika virus infection imported into Europe, November 2013. Euro Surveill. 2014;19(4):pii=20685. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20685
15. Hirayama T, Mizuno Y, Takeshita N, Kotaki A, Tajima S, Omatsu T, et al. Detection of dengue virus genome in urine by real-time reverse transcriptase PCR: a laboratory diagnostic method useful after disappearance of the genome in serum. J Clin Microbiol. 2012;50(6):2047-52. http://dx.doi.org/10.1128/JCM.06557-11

First case of laboratory-confirmed Zika virus infection imported into Europe, November 2013

D Tappe[1,2], J Rissland[2,3], M Gabriel[1], P Emmerich[1], S Günther[1], G Held[4], S Smola[2,3], J Schmidt-Chanasit (jonassi@gmx.de)[1,2,5]
1. Bernhard Nocht Institute for Tropical Medicine, WHO Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research, Hamburg, Germany
2. These authors contributed equally to this work
3. Institute of Virology, Saarland University Medical Center, Homburg/Saar, Germany
4. Internal Medicine I, Saarland University Medical Center, Homburg/Saar, Germany
5. German Centre for Infection Research (DZIF), partner site Hamburg-Luebeck-Borstel, Hamburg, Germany

Citation style for this article:
Tappe D, Rissland J, Gabriel M, Emmerich P, Günther S, Held G, Smola S, Schmidt-Chanasit J. First case of laboratory-confirmed Zika virus infection imported into Europe, November 2013. Euro Surveill. 2014;19(4):pii=20685. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20685

Article submitted on 27 January 2014 / published on 30 January 2014

In November 2013, an acute Zika virus (ZIKV) infection was diagnosed in a German traveller returning from Thailand. The patient reported a clinical picture resembling dengue fever. Serological investigations revealed anti-ZIKV-IgM and -IgG, as well as ZIKV-specific neutralising antibodies in the patient's blood. In Europe, viraemic travellers may become a source of local transmission of ZIKV, because *Aedes albopictus* (Skuse) and *Ae. aegypti* (Linnaeus) are invasive mosquitoes and competent vectors for ZIKV.

We report the clinical and laboratory findings of a Zika virus (ZIKV) infection imported into Europe by a German traveller from Thailand, in winter of 2013.

Case description

A previously healthy German traveller in his early 50s was seen at a tertiary hospital, Germany, on 22 November 2013, after returning from a vacation in Thailand. During the patient's three-week round trip (in early November) which included visits to Phuket, Krabi, Ko Jum, and Ko Lanta, he developed joint pain and swelling of his left ankle and foot on 12 days after entering the country. Pain and swelling was followed by a maculopapular rash on his back and chest that later spread to the face, arms, and legs over a period of four days before fading. Concomitantly, the patient suffered from malaise, fever (self-reported), and chills. Fever and shivering were treated by self-medication with non-steroidal anti-inflammatory drugs and only lasted for one day. The patient had noted several mosquito bites previously, despite using insect repellents regularly. He had sought pre-travel advice and his travel partner did not have any symptoms and also did not develop any.

Upon return to Germany, the patient was asymptomatic except for the subjective complaint of ongoing exhaustion. Physical examination was normal and no particular treatment was initiated. Laboratory parameters 10 days after disease onset revealed a slightly increased C-reactive protein level (5.9 mg/L; normal value ‹5.0), a normal leucocyte count of 8,200 g/µL (45% lymphocytes, 5% monocytes, and a mildly decreased relative neutrophil count of 47% (normal range: 50–75%)). Platelet count was normal with 238,000 g/µL. Lactate dehydrogenase levels were elevated (311 U/L; normal ‹262 U/L), with an increased plasma fibrinogen concentration (422 mg/dL; normal range: 180–400 mg/dL) and serum ferritin concentration (486 ng/mL; normal range: 30–400). Serum electrophoresis, clotting tests, kidney and liver function tests were normal except for an increased gamma-glutamyltransferase activity of 81 U/L (normal ‹60 U/L).

A serum sample from the same day (10 days after symptom onset) showed a positive result for anti-dengue virus (DENV)-IgM in both the indirect immunofluorescence assay (IIFA), according to [1-3]) and rapid test (SD BIOLINE Dengue Duo NS1 Ag + Ab Combo). However, anti-DENV-IgG was not detected in either test. Testing for DENV nonstructural protein-1 (NS1) antigen (tested by enzyme-linked immunosorbent assay (ELISA): Bio-Rad Platelia Dengue NS1 Ag) and rapid test (SD BIOLINE Dengue Duo NS1 Ag + Ab Combo) were also negative. The detection of isolated anti-DENV-IgM prompted us to investigate a probable flavivirus etiology other than DENV of the patient's illness. Serological tests for Japanese encephalitis virus (JEV), West Nile virus (WNV), yellow fever virus (YFV), tick-borne encephalitis virus (TBEV), and ZIKV were performed according to [1-3] and the IIFAs showed only positive results for anti-ZIKV-IgM and -IgG antibodies (Table), demonstrating an acute or recent ZIKV-infection of the patient. Serological tests for chikungunya virus (CHIKV) were negative (Table).

ZIKV-specific real-time reverse transcription-polymerase chain reaction (RT-PCR) (in-house) with primers ZIKAf (5'-TGGAGATGAGTACATGTATG-3'), ZIKAr (5'-GGTAGATGTTGTCAAGAAG-3'), probe – labeled with 6- carboxyfluorescein (FAM) and black hole quencher 1

Table

Serological results of a case of Zika virus infection from Thailand imported into Germany, November 2013

Antibody or antigen tested	Serum samples taken after symptom onset (days)		
	10	31	67
Anti-ZIKV-IgG[a]	1:5,120	1:2,560	1:2,560
Anti-ZIKV-IgM[a]	1:10,240	1:2,560	1:320
Anti-DENV-IgG[a]	<1:20	1:80	1:160
Anti-DENV-IgM[a]	1:40	<1:20	<1:20
DENV NS1[b]	Negative (0.1 arbitrary units)	Negative (0.2 arbitrary units)	Negative (0.1 arbitrary units)
Anti-JEV-IgG[a]	<1:20	1:40	1:20
Anti-JEV-IgM[a]	<1:20	<1:20	<1:20
Anti-WNV-IgG[a]	<1:20	1:20	1:80
Anti-WNV-IgM[a]	<1:20	<1:20	<1:20
Anti-YFV-IgG[a]	<1:20	<1:20	1:20
Anti-YFV-IgM[a]	<1:20	<1:20	<1:20
Anti-CHIKV-IgG[a]	<1:20	<1:20	<1:20
Anti-CHIKV-IgM[a]	<1:20	<1:20	<1:20

CHIKV: chikungunya virus; DENV: dengue virus; JEV: Japanese encephalitis virus; NS1: nonstructural protein-1; WNV: West Nile virus; YFV: yellow fever virus; ZIKV: Zika virus.

[a] Indirect immunofluorescence assay (IIFA) titres <1:20 for serum were considered negative [1-3].

[b] SD BIOLINE Dengue Duo NS1 Ag + Ab Combo and Bio-Rad Platelia Dengue NS1 Ag.

(BHQ-1) – ZIKAp (5'-FAM-CTGATGAAGGCCATGCACACTG-BHQ1-3`) was negative on serum. Generic flavivirus real-time RT-PCR [4] was negative as well on serum. A significant 5-fold anti-ZIKV-IgM titre decrease in the IIFA was demonstrated in the third serum sample collected 67 days after disease onset (Table). The presence of ZIKV-specific neutralising antibodies in the third serum sample was confirmed by a virus neutralisation assay. No laboratory investigation was conducted with the travel partner.

Background

ZIKV is a mosquito-borne RNA virus of the *Flaviviridae* family causing a dengue fever -like syndrome in humans. The virus was first isolated in 1947 from a febrile sentinel rhesus monkey in the Zika Forest of Uganda [5]. ZIKV virus is thought to be maintained in a sylvatic cycle involving non-human primates and several *Aedes* species (*Ae. africanus*, *Ae. aegypti*, and others) as mosquito vectors [6-8]. Human infection is acquired after an infective mosquito bite in endemic countries. However, the possibility of a secondary sexual transmission has been reported recently [9]. The virus is endemic in Africa and south-east Asia [8], and phylogenetic analysis suggested that African and Asian strains emerged as two distinct lineages [10-11]. ZIKV has caused an outbreak involving 49 confirmed and 59 probable cases on Yap Island, Federated States of Micronesia, in 2007 [12]. This outbreak highlighted the potential of the virus as an emerging pathogen [9], and epidemiological and phylogenetic studies provided evidence that the outbreak strain has been introduced from south-east Asia [10].

The most common signs and symptoms of ZIKV infection are rash, fever, arthralgia, myalgia, headache, and conjunctivitis. The rash is most often maculopapular. Occasionally, oedema, sore throat, cough, vomiting, and loose bowels are reported [11-13]. ZIKV infection can easily be confused with dengue and might be misdiagnosed during local dengue outbreaks [8]. ZIKV-associated illness may thus be underreported or misdiagnosed [9].

In contrast to acute dengue cases, our patient neither showed elevated aspartate amino transferase (AST) or alanine amino transferase (ALT) levels, nor thrombocytopenia. It is unclear whether these test results may help in differentiating ZIKV from dengue cases, as information about laboratory data during ZIKV infection is very scarce. An Australian case [11] did not show thrombocytopenia or elevated liver function tests either. It was reported recently that a low platelet count is a key variable distinguishing between dengue versus chikungunya [14], the latter being another mosquito-borne virus infection with similar clinical presentation and geographical distribution. Chikungunya is thus also an important differential diagnosis for ZIKV disease and future studies might address this issue for ZIKV.

Despite the virus endemicity in many geographical areas and its potential to cause outbreaks, imported cases to non-endemic areas are rarely reported. In 2013, one imported case from Indonesia to Australia and one imported case from Thailand to Canada were diagnosed in travellers [11,15]. Also in the Australian and Canadian cases, anti-DENV-IgM was positive and DENV NS1 antigen testing was negative. In both cases, ZIKV infection was diagnosed after sequencing of a positive generic flavivirus RT-PCR amplicon. Four further cases of imported ZIKV to temperate regions have been reported in American scientists who had returned from Senegal and in Japanese travellers who returned from French Polynesia, where a ZIKV outbreak is currently ongoing [16,17]. A secondary infection in the wife of one of the American patients was assumed to be due to sexual contact [9]. The ZIKV outbreak in French Polynesia so far comprises more than 361 laboratory-confirmed cases [18]. The first indigenous infection in New Caledonia was recently reported suggesting the spread of ZIKV, as 26 imported cases of ZIKV infection from French Polynesia have been observed in this territory [19].

Conclusions

This report constitutes, to the best of our knowledge, the first laboratory-confirmed case of a ZIKV infection imported into Europe. The case highlights that unusual DENV serology results might be caused by a flavivirus different than DENV despite a similar clinical picture. A serological study after the Yap outbreak indicated that ZIKV-infected patients can be positive in anti-DENV-IgM assays [20], as also experienced in our case. This cross-reaction in the Yap outbreak was seen especially if ZIKV was a secondary flavivirus infection. These findings underscore the importance of a careful diagnostic investigation in travellers suspected with dengue, and the well-known serological cross-reactions in the flavivirus group. Thus, the rate at which seemingly imported dengue cases among travellers from endemic areas in the recent years were actually ZIKV infections remains a question.

In all published cases of imported ZIKV infections, in outbreak and sporadic endemic cases, the symptoms were dengue-like. Clinicians, virologists, and public health authorities should thus be aware of this emerging flavivirus infection. As the local transmission of DENV by previously introduced competent vectors in non-endemic countries has recently been reported from Croatia, France and Madeira [2,21,22], there might be the risk of a similar establishment in Europe of ZIKV, after import by viraemic travellers, in particular in areas where ZIKV competent vectors *Ae. albopictus* and *Ae.aegypti* are present.

Acknowledgments

We would like to thank Insa Bonow, Corinna Thomé and Birgit Hüsing for technical assistance.

Conflict of interest

None declared.

Authors' contributions

Wrote the manuscript: JSC, SG, DT, JR, SS, GH; performed laboratory or epidemiological investigations: JSC, PE, MG, JR, GH, DT; performed data analysis: JSC, PE, JR, GH.

References

1. Tappe D, Schmidt-Chanasit J, Ries A, Ziegler U, Müller A, Stich A. Ross River virus infection in a traveller returning from northern Australia. Med Microbiol Immunol. 2009;198(4):271-3. http://dx.doi.org/10.1007/s00430-009-0122-9
2. Schmidt-Chanasit J, Haditsch M, Schöneberg I, Günther S, Stark K, Frank C. Dengue virus infection in a traveller returning from Croatia to Germany. Euro Surveill. 2010;15(40):pii: 19677. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19677
3. Schmidt-Chanasit J, Emmerich P, Tappe D, Günther S, Schmidt S, Wolff D, et al. Autochthonous dengue virus infection in Japan imported into Germany, September 2013. Euro Surveill. 2014;19(3):pii=20681. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20681
4. Chao DY, Davis BS, Chang GJ. Development of multiplex real-time reverse transcriptase PCR assays for detecting eight medically important flaviviruses in mosquitoes. J Clin Microbiol. 2007;45(2):584-9. http://dx.doi.org/10.1128/JCM.00842-06
5. Dick GW, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity. Trans R Soc Trop Med Hyg. 1952;46(5):509-20. http://dx.doi.org/10.1016/0035-9203(52)90042-4
6. Haddow AJ, Williams MC, Woodall JP, Simpson DI, Goma LK. Twelve isolations of Zika virus from Aedes (Stegomyia) africanus (Theobald) taken in and above Uganda forest. Bull World Health Organ. 1964;31:57-69.
7. Marchette NJ, Garcia R, Rudnick A. Isolation of Zika virus from Aedes aegypti mosquitoes in Malaysia. Am J Trop Med Hyg. 1969;18(3):411-5.
8. Hayes EB. Zika virus outside Africa. Emerg Infect Dis. 2009;15(9):1347-50. http://dx.doi.org/10.3201/eid1509.090442
9. Foy BD, Kobylinski KC, Chilson Foy JL, Blitvich BJ, Travassos da Rosa A, Haddow AD, et al. Probable non-vector-borne transmission of Zika virus, Colorado, USA. Emerg Infect Dis. 2011;17(5):880-2. http://dx.doi.org/10.3201/eid1705.101939
10. Haddow AD, Schuh AJ, Yasuda CY, Kasper MR, Heang V, Huy R, et al. Genetic characterization of Zika virus strains: geographic expansion of the Asian lineage. PLoS Negl Trop Dis. 2012;6(2):e1477. http://dx.doi.org/10.1371/journal.pntd.0001477
11. Kwong JC, Druce JD, Leder K. Zika virus infection acquired during brief travel to Indonesia. Am J Trop Med Hyg. 2013;89(3):516-7. http://dx.doi.org/10.4269/ajtmh.13-0029
12. Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med. 2009;360(24):2536-43. http://dx.doi.org/10.1056/NEJMoa0805715
13. Heang V, Yasuda CY, Sovann L, Haddow AD, Travassos da Rosa AP, Tesh RB, et al. Zika virus infection, Cambodia, 2010. Emerg Infect Dis. 2012;18(2):349-51. http://dx.doi.org/10.3201/eid1802.111224
14. Lee VJ, Chow A, Zheng X, Carrasco LR, Cook AR, Lye DC, et al. Simple clinical and laboratory predictors of Chikungunya versus dengue infections in adults. PLoS Negl Trop Dis. 2012;6(9):e1786.
15. ProMED-mail. Zika virus - Canada ex Thailand. Archive Number: 20130529.1744108. Available from: http://www.promedmail.org/direct.php?id=1744108
16. ProMED-mail. Zika virus - Japan: ex French Polynesia. Archive Number: 20131219.2126046. Available from: http://www.promedmail.org/direct.php?id=2126046
17. Kutsuna S, Kato Y, Takasaki T, Moi ML, Kotaki A, Uemura H, Matono T, Fujiya Y, Mawatari M, Takeshita N, Hayakawa K, Kanagawa S, Ohmagari N. Two cases of Zika fever imported from French Polynesia to Japan, December to January 2013 . Euro Surveill. 2014;19(4):pii=20683. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20683

18. ProMED-mail. Zika virus - French Polynesia (03). Archive Number: 20140123.2227452. Available from: http://www.promedmail.org/direct.php?id=2227452
19. ProMED-mail. Zika virus - New Caledonia. Archive Number: 20140122.2224823. Available from: http://www.promedmail.org/direct.php?id=2224823
20. Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerg Infect Dis. 2008;14(8):1232-9. http://dx.doi.org/10.3201/eid1408.080287
21. La Ruche G, Souarès Y, Armengaud A, Peloux-Petiot F, Delaunay P, Desprès P, et al. First two autochthonous dengue virus infections in metropolitan France, September 2010. Euro Surveill. 2010;15(39):pii=119676. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19676
22. Frank C, Höhle M, Stark K, Lawrence J. More reasons to dread rain on vacation? Dengue fever in 42 German and United Kingdom Madeira tourists during autumn 2012. Euro Surveill. 2013;18(14):pii=20446. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20446

Internet-based syndromic monitoring of acute respiratory illness in the general population of Germany, weeks 35/2011 to 34/2012

C Bayer (bayerc@rki.de)[1,2], C Remschmidt[1], M an der Heiden[1], K Tolksdorf[1], M Herzhoff[1], S Kaersten[1], S Buda[1], W Haas[1], U Buchholz[1]

1. Robert Koch Institute, Department for Infectious Disease Epidemiology, Berlin, Germany
2. Postgraduate Training for Applied Epidemiology (PAE, German FETP) and European Programme for Intervention Epidemiology Training (EPIET)

Citation style for this article:
Bayer C, Remschmidt C, an der Heiden M, Tolksdorf K, Herzhoff M, Kaersten S, Buda S, Haas W, Buchholz U. Internet-based syndromic monitoring of acute respiratory illness in the general population of Germany, weeks 35/2011 to 34/2012 . Euro Surveill. 2014;19(4):pii=20684. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20684

Article submitted on 05 May 2013 / published on 30 January 2014

In March 2011, the German sentinel surveillance system for influenza (Arbeitsgemeinschaft Influenza (AGI)) was complemented by an Internet-based syndromic monitoring system (GrippeWeb) for acute respiratory infections (ARI) and influenza-like-illness (ILI). To assess representativeness of GrippeWeb participants, key demographic variables and lifetime prevalence of asthma and diabetes were compared with data from the general population of Germany. To 'validate' GrippeWeb, we compared weekly ARI and medically attended ARI (MAARI) rates, generated between weeks 35/2011 and 34/2012, with AGI MAARI rates and overlaid GrippeWeb ILI rates with the number of positive influenza samples obtained by the AGI. GrippeWeb had high weekly participation rates (62% of participants reported in ≥90% of possible weeks). Although it varied by age group, participants reported a mean of between 1.3 and 6.0 ARI episodes and between 0.1 and 2.4 ILI episodes during the study year. Estimated GrippeWeb MAARI incidence was very similar to the AGI MAARI incidence and influenza circulation was reflected well in the GrippeWeb ILI rates. GrippeWeb became a reliable monitoring system shortly after implementation, capturing the burden of ARI and ILI at general population level. The high degree of agreement between GrippeWeb's and AGI's MAARI data lends support to the validity of both systems.

Introduction

In Europe, surveillance for influenza is traditionally based on sentinel systems of primary care physicians who collect syndromic data on patients presenting with influenza-like illness (ILI) or acute respiratory infection (ARI) [1]. To this end, the Working Group for Influenza (Arbeitsgemeinschaft Influenza (AGI)) in Germany was founded in 1992 [2]. It is the German member of the European influenza surveillance network (EISN) coordinated by the European Centre for Disease Prevention and Control (ECDC) and the respective system of the World Health Organization Regional Office for Europe (EuroFlu) [3]. While most sentinel systems in Europe collect ILI data, the AGI collects ARI data. Because this type of surveillance focuses on illnesses of patients who seek healthcare, several countries have added Internet-based monitoring systems, in which data are collected from the population directly [4-10]. In March 2011, the Robert Koch Institute (RKI) launched an Internet-based syndromic monitoring system for ARI and ILI in Germany, named GrippeWeb [11]. Experiences from similar projects in other countries have shown that continuous participation of registered individuals is essential for data quality [4,5,7,10,12], but some systems have reported difficulties in achieving sustained participation [9,13]. Compared with the levels of ILI in the sentinel systems, the rise, peak and fall of ILI activity in the Internet-based and sentinel systems of Belgium, the Netherlands and Portugal, respectively, occurred at similar times [7-10]. However, a direct, quantitative comparison of medically attended illness rates of the two systems has not been published. Representativeness of the Internet-based systems has been reported to be good in terms of age and co-morbidity [7,8], but there were difficulties in reaching minors as well as elderly people [7-10].

GrippeWeb [14] runs throughout the year. Every person residing in Germany who is at least 14 years-old can register. Parents need to register separately, but one parent can report for their children aged 13 years or younger. Upon registration, participants answer 10 questions on demographic variables, lifetime physician-diagnosed chronic conditions, smoking, household size, daily occupation and main mode of transportation. Since the launch of the system in March 2011, participants have been recruited through paper-based or online media reports, during public events where GrippeWeb was presented and by word of mouth. In addition, public institutions, such as

county health departments, were provided with leaflets for further distribution to popularise GrippeWeb. GrippeWeb is carried out according to the German legislation on data protection. The GrippeWeb procedures were approved by the German Federal Commissioner for Data Protection and Freedom of Information.

Every Monday morning, participants receive an email summarising the GrippeWeb results published on the system's website and inviting them to complete their weekly questionnaire. In this questionnaire, participants are asked whether they have experienced the onset of a new respiratory illness during the previous week (Monday to Sunday). If the participant has had no respiratory illness with new symptom onset, no further questions need to be answered. In the alternative case, participants are asked to report date of onset, select symptoms from a short list (cough, sore throat, fever, runny nose), if they have consulted a physician because of the illness and whether they have been able to continue their usual daily occupation. Influenza vaccination is recorded weekly during the winter season as a separate item on the questionnaire. If a participant has missed filling in the questionnaire of a particular week, they can report weekly answers up to the previous four weeks. GrippeWeb defines an ARI as a subjectively reported respiratory illness with new onset of fever (subjective) or cough or sore throat. ILI is defined as a subjectively reported respiratory illness with a new onset of fever and cough or sore throat.

Individual results are fed back to each participant in a diary function; aggregated results are published in weekly reports on the website. To motivate participants aged 18 years or older to report as regularly as possible, they have the option to take part in a prize draw where a number of technical devices, such as a notebook or digital camera, can be won. The chance of winning can be increased by accumulating points with every report during a one-year period between August and July, after which winners are identified. Staff and family of the authoring institute (RKI) are excluded from participating in the draw.

Here we describe the characteristics of GrippeWeb and analyse the representativeness of its participants compared with the general population of Germany. We present ARI and ILI data from the first year of GrippeWeb, including the influenza season 2011/12, and compare these data with those generated by the AGI.

Methods

We analysed GrippeWeb data collected from week 35/2011 to week 34/2012 (52 weeks). To calculate the proportion of weekly reports submitted after registration, we divided the participants in two groups: (i) those already registered at week 35/2011 (the beginning of the analysed period); and (ii) those who registered after the period of observation had started (for example, in week 40/2011). To determine the denominator of the maximum number of possible reports between weeks 35/2011 and 34/2012 (the end of the period of analysis), we used as the denominator the total number of weeks between week 35/2011 and 34/2012 (first group) or the number of weeks from the beginning of registration until week 34/2012 (second group). For the numerator, we calculated for both groups the number of weekly reports submitted.

Representativeness

We calculated the proportional age distribution of GrippeWeb participants as of week 34/2012 for six age groups (0–4, 5–14, 15–24, 25–34, 35–59 and ›59 years), in alignment with the AGI age groups, the distribution by sex and the geographical distribution by federal state and compared these with the population of Germany as of 31 December 2011 using data provided by the German Federal Statistical Office (Destatis) [14]. In addition, we compared the age-adjusted lifetime prevalence of asthma and diabetes among GrippeWeb participants aged 18 years or above with those obtained in a representative survey of the population of Germany in 2010 (GEDA) [15,16].

Impact of the prize draw

We investigated whether participants enrolled in the prize draw differed from those not enrolled and whether participation in the draw skewed responses. We compared the age and sex distribution, response rates and ARI/ILI rates in the two groups. Because the minimum age to take part in the prize draw was 18 years, we restricted these comparisons for participants aged at least 18 years.

Calculation of ARI and ILI estimates

Weekly ARI/ILI rates were calculated by dividing the number of participants with ARI/ILI in a particular week by the total number of reporting participants in the same week. To calculate the mean number of ARI (ILI) during the one year study period, we used only the cohort of participants that were registered already on week 35/2011 and submitted a report in more than 46 (90%) of the 52 weeks in the following year. To estimate ARI/ILI rates for the general population of Germany, the sample was weighted according to the sex and age distribution based on the 2011 data of DESTATIS, the Federal Statistical Office [14]. We assigned each individual a weight according to the following formula [9]:

$W_i = P_{i\ Germany} / P_{i\ GrippeWeb}$

W_i = weight of individual GrippeWeb participant

$P_{i\ Germany}$ = proportion of the general population of Germany in the same age and sex group as the individual i

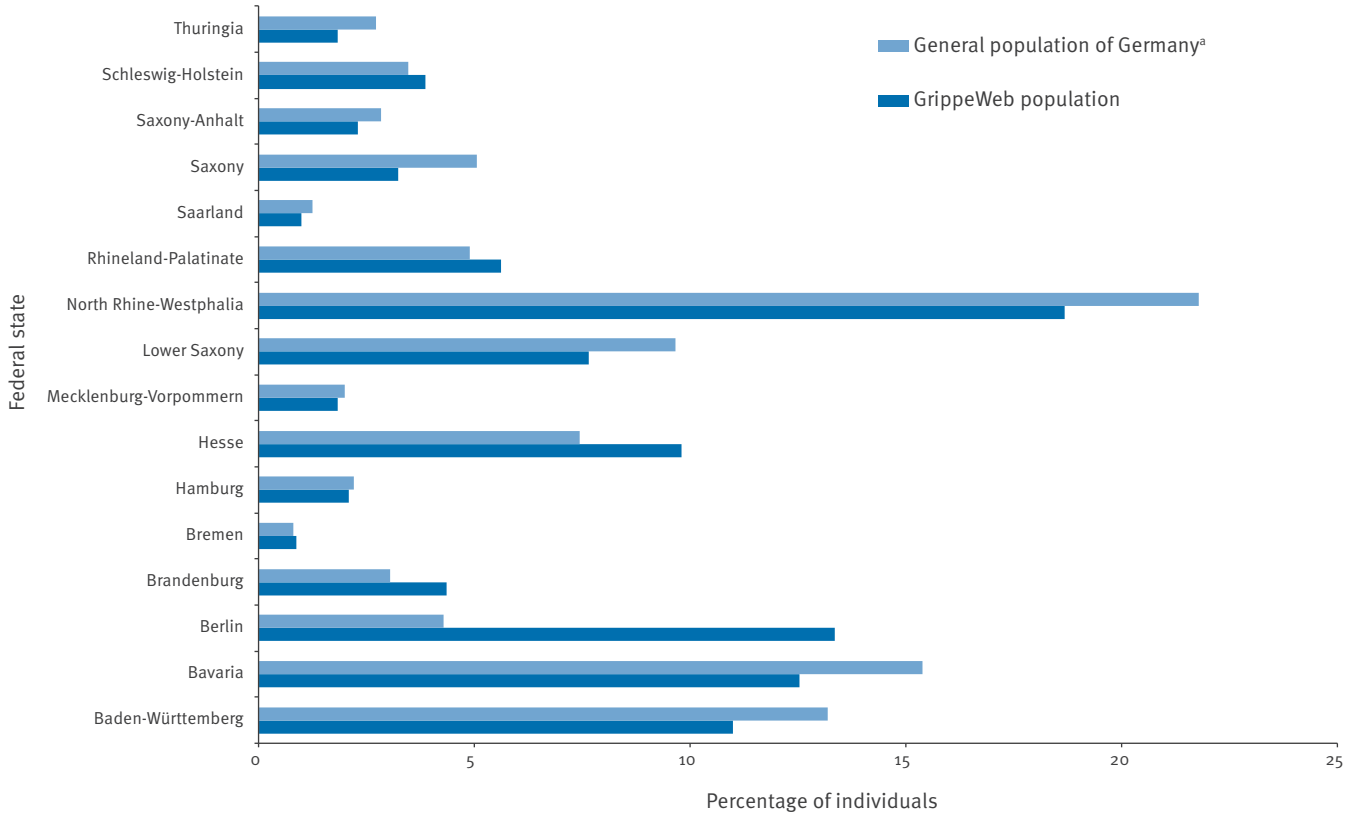
$P_{i\ GrippeWeb}$ = proportion of the GrippeWeb population in the same age and sex group as the individual i;

To reduce the effect of individuals who register as a response to an acute illness and because participants

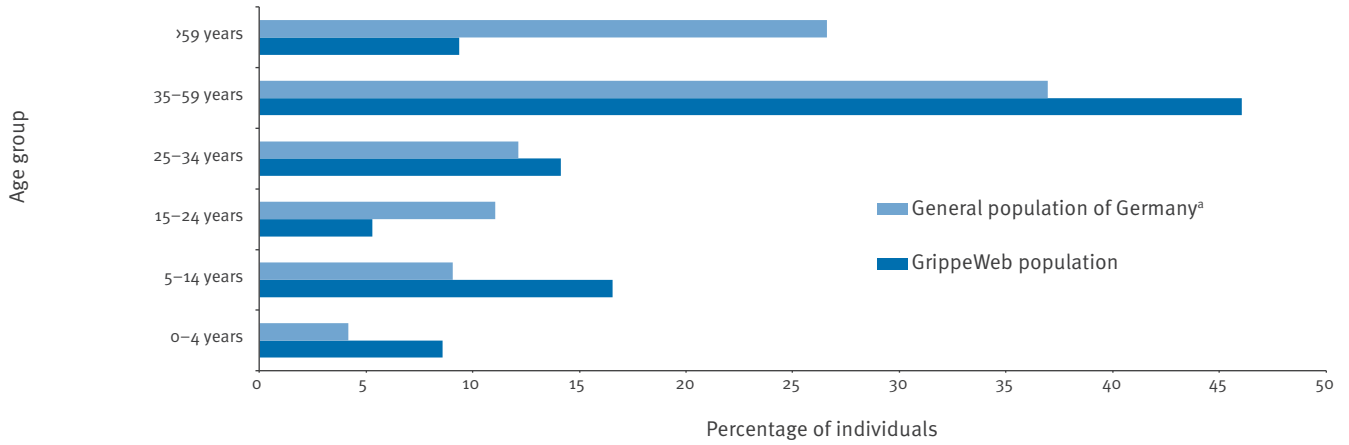
Figure 1

GrippeWeb participants (week 34/2011) and the general population of Germany (as of 31 December 2011) by federal state (A) and age group (B), Germany

A Participation by federal state



B Participation by age group



[a] Data from DESTATIS (Federal Statistical Office).

can report up to four weeks backwards, we restricted our dataset to participants' fifth and subsequent reports to calculate weekly ARI/ILI rates. Recurrent episodes of ARI and ILI of a participant were only counted if they did not report an ARI or ILI for at least one week after the last ARI/ILI.

Comparison between ARI/ILI rates in GrippeWeb and the sentinel system of the AGI

The AGI defines ARI as a physician-diagnosed acute pharyngitis or bronchitis or pneumonia with or without fever [2]. To estimate the activity of medically attended ARI (MAARI), the AGI calculates the incidence of ARI in persons who consulted a physician because of it

(MAARI incidence) [17]. The AGI complements syndromic surveillance with virological data from samples taken by a subgroup of all sentinel physicians [18].

We conducted two 'validation' procedures of GrippeWeb data using AGI data. Firstly, to compare MAARI incidence of the AGI (AGI MAARI incidence) with data obtained by GrippeWeb (GrippeWeb MAARI incidence), we multiplied the weekly ARI rate with the weekly proportion of ARI patients who had indicated that they had consulted a physician due to their illness. Secondly, to investigate if the influenza wave of the 2011/12 season was reflected in GrippeWeb data, we overlaid ILI rates obtained by GrippeWeb (because ILI is more specific for influenza than ARI) with the number of samples positive for influenza A(H3N2) and B, the two circulating virus (sub)types in Germany during the 2011/12 season.

Statistical analyses

Data analyses were performed using Stata version 12 (Stata Corporation, United States). For comparisons of two proportions, we used a chi-squared test, for comparisons of numerical values between two groups, we used the Mann–Whitney U test or Student's t-test. To compare the values of the GrippeWeb MAARI incidence and the GrippeWeb ARI incidence, respectively, with the AGI MAARI incidence and to compare ARI/ILI rates in GrippeWeb participants enrolled and not enrolled in the prize draw, we calculated the Pearson correlation coefficient r or Spearman's rho. To measure similarity of pairs of time series as a function of time lag, we calculated cross-correlations. All p values were calculated using two-sided tests. P values of less than 0.05 were considered statistically significant. Weekly GrippeWeb ARI/ILI incidences were calculated as a three-week moving average.

Results

The number of registered participants rose from 1,385 in week 35/2011 to 3,803 in week 34/2012. The total cohort of participants who were registered at any time between week 35/2011 and week 34/2012 consisted of 4,102 participants. During the study period, 3,933 participants (96%) contributed reports. The major source that led participants to find out about GrippeWeb was the Internet (56%, 1,616 of 2,902 who answered the question). During the period analysed, participants contributed 125,393 reports to our dataset. For the analysis of ARI and ILI rates, 113,919 and 115,016 reports respectively were included, after exclusion of the first four reports submitted by participants and after exclusion of recurrent ARI and ILI episodes from one week to another.

During the period analysed, more than half of the participants (2,144/4,102) reported to GrippeWeb in more than 96% of the possible weeks, 62% (2,553/4,102) in at least 90% and 68% (2,805/4,102) of participants reported in at least 80%.

Representativeness

Participants from all 16 German federal states registered for GrippeWeb. While the number of GrippeWeb participants by state correlated well overall with the number of residents of the respective state (rho = 0.90, $p<0.001$), there were differences between individual states. GrippeWeb participants were over-represented in several states (n=4, particularly the federal state of Berlin), and under-represented in Bavaria, Baden-Württemberg, Lower Saxony, North Rhine-Westphalia, Saxony and Thuringia (Figure 1A).

The age-adjusted proportion of female GrippeWeb participants was higher than in the general population of Germany (52% vs 51% , chi-squared test: $p<0.001$).

All age groups were represented in GrippeWeb. People aged 35–59 years constituted the largest portion in both GrippeWeb and the general population of Germany (Figure 1B). The proportion of 0–4, 5–14 and 35-59 year-old GrippeWeb participants was significantly higher, whereas the proportion of 15–24 and >59 year-old participants was significantly lower compared with the proportions in the general population of Germany.

The lifetime prevalence of asthma in GrippeWeb participants aged 18 and older was lower than the lifetime prevalence in the adult population of Germany (8.1% vs 9.7%, chi-squared test: $p<0.001$). GrippeWeb participants had also a lower lifetime prevalence of diabetes compared with that of the general population of Germany (5.3% vs 8.8%, chi-squared test: $p<0.001$).

Impact of the prize draw

Among participants aged 18 and older, 80% (n=2,411) of 3,018 participants had signed up for the prize draw. Compared with GrippeWeb participants who had not enrolled in the draw, those who had enrolled did not differ by age (mean age 44.1 years vs 44.1 years, Mann–Whitney U test: $p=0.70$) and sex (chi-squared test: $p=0.60$). Weekly ARI and ILI rates were similar in both groups (for ARI, $r= 0.90$, $p<0.001$, 95% confidence interval (CI): 0.83–0.94 and for ILI, $r= 0.42$, $p<0.002$, 95% CI: 0.17–0.62) (Figure 2A).

Regarding the reporting rate, those enrolled in the draw reported more consistently throughout the study period. For example, 67% (1,607/2,411) of those enrolled vs 55% (332/607) of those not enrolled submitted at least 90% of the possible number of reports (Figure 2B; Mann–Whitney U test: $p <0.001$).

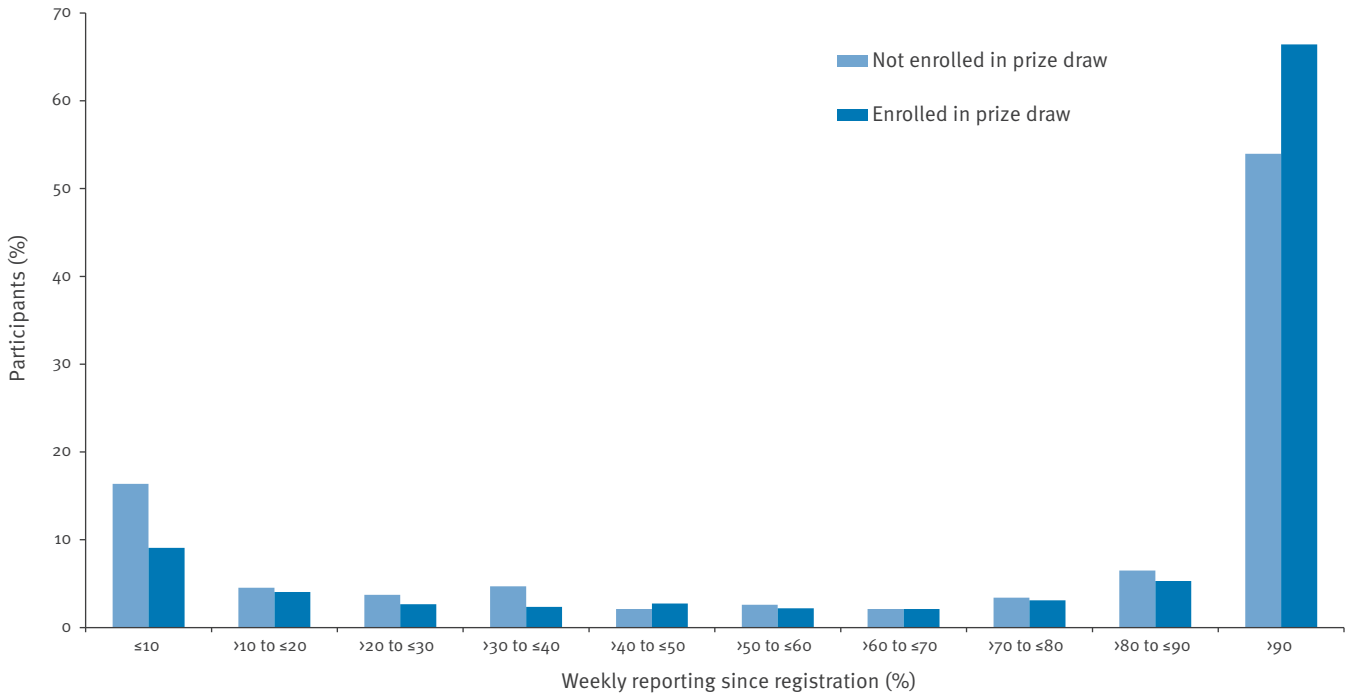
Estimates of ARI and ILI among GrippeWeb participants

During the observed time period, estimated weekly ARI rates ranged between 3.0% (95% CI: 2.3–3.7) and 8.4% (95% CI: 6.7–10.1) for all ages, between 4.9% (95% CI: 3.2–6.7) and 14.1% (95% CI: 10.8–17.5) for children (aged 14 years or younger) and between 2.7% (95% CI: 1.9–3.5) and 8.2% (95% CI: 6.4–10.2) for participants

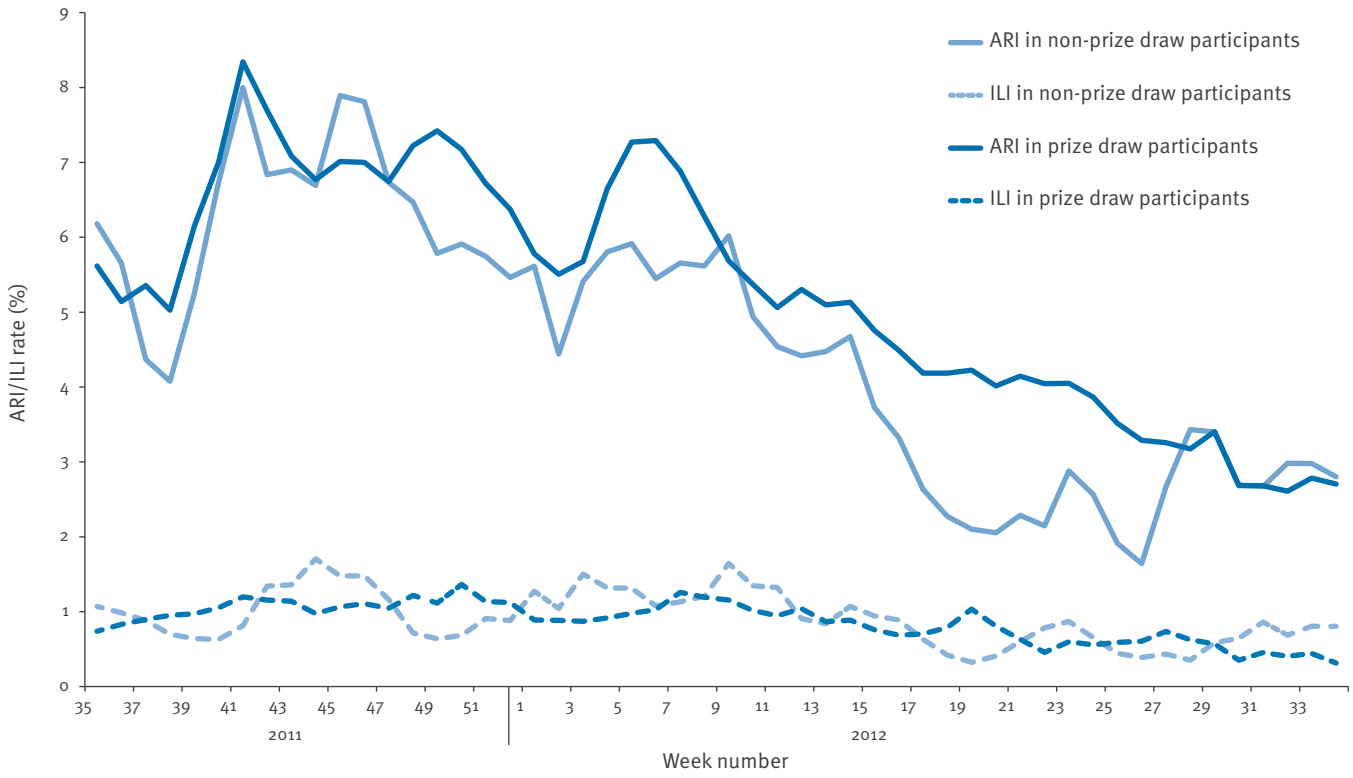
Figure 2

GrippeWeb participants enrolled and not enrolled in the prize draw: proportion of possible weekly reports after registration (A) and three-week moving average of reported acute respiratory illness and influenza-like illness (B), Germany, weeks 35/2011–34/2012

A Weekly reporting



B ARI and ILI

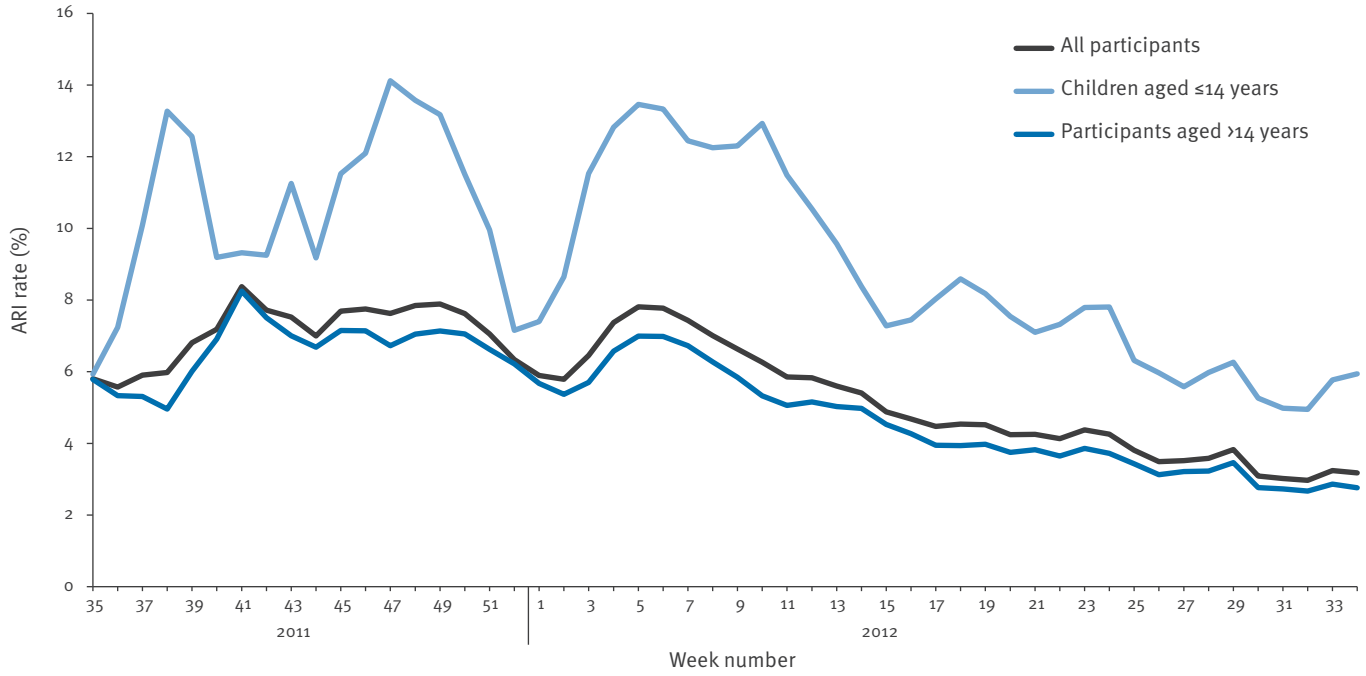


ARI: acute respiratory illness; ILI: influenza-like illness.

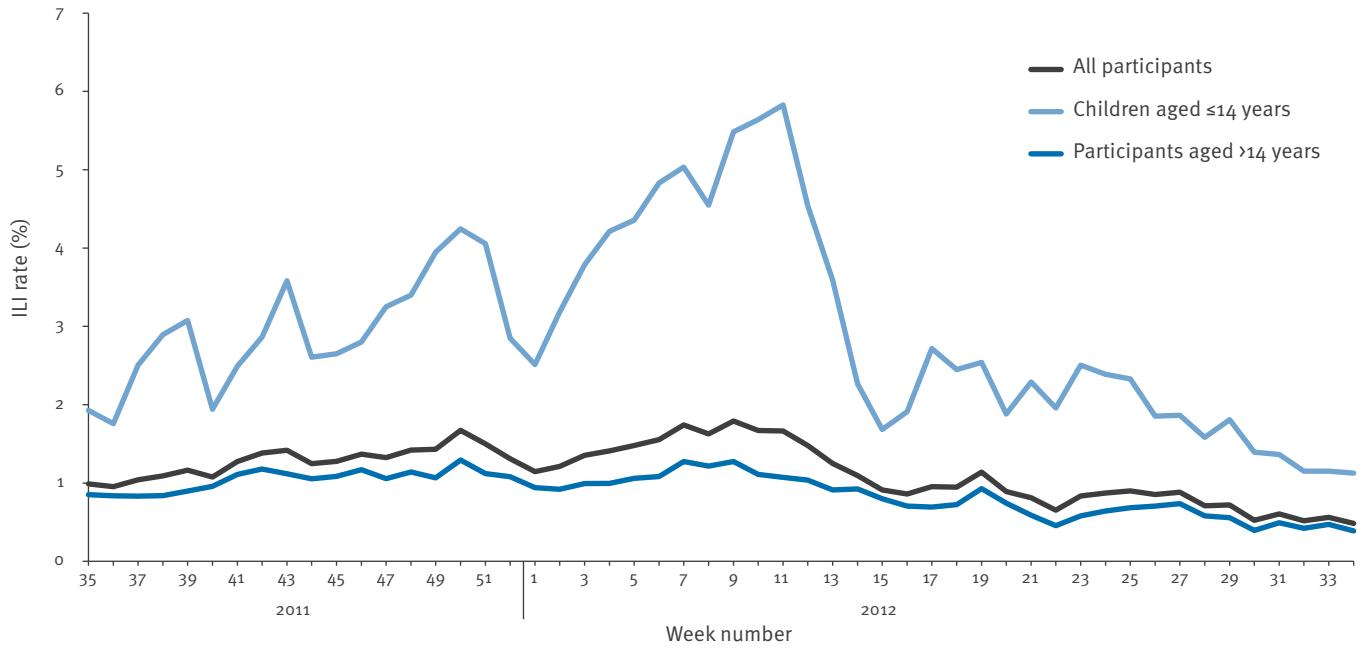
Figure 3

Three-week moving average for children ≤14 years, participants >14 years and all age groups measured by GrippeWeb for acute respiratory illness (A) and influenza-like illness (B), Germany, weeks 35/2011–34/2012

A ARI



B ILI



ARI: acute respiratory illness; ILI: influenza-like illness.

Table

Number of acute respiratory illness and influenza-like illness reports of GrippeWeb participants, Germany, during a one-year period (weeks 35/2011–34/2012)

Age group in years[a]	Number of participants[b]	Number of ARI reports		Number of ILI reports	
		Mean	Median (25% percentile; 75% percentile)	Mean	Median (25% percentile; 75% percentile)
≤4	38	6.0	6 (4; 8)	2.4	2 (1; 3)
5–14	115	3.4	3 (2; 4)	0.9	1 (0; 1)
15–34	125	3.2	3 (2; 4)	0.5	0 (0; 1)
35–59	438	2.3	2 (1; 3)	0.4	0 (0; 1)
≥60	64	1.3	1 (0; 2)	0.1	0 (0; 0)

ARI: acute respiratory illness; ILI: influenza-like-illness.

[a] Participants were included in the calculation only if they were already registered in week 35/2011 and reported to GrippeWeb a minimum 47 weeks out of the possible 52 weeks during weeks 35/2011 to 34/2012.

[b] One parent can report for children aged 13 years or younger.

aged >14 years (Figure 3A). ILI rates ranged between 0.5% (95% CI: 0.2–0.8) and 1.8% (95% CI: 1.3–2.3) for all ages, between 1.1% (95% CI: 0.3–2.0) and 5.8% (95% CI: 3.9–7.7) for children ≤14 years and between 0.4% (95% CI: 0.1–0.7) and 1.3% (95% CI: 0.7–1.9) for participants aged >14 years (Figure 3B). Rates of ARI and ILI reports dropped around weeks 40–42/2011, 01/2012 and 14/2012, particularly in children aged 0–14 years, coinciding with the autumn, Christmas and Easter holiday periods. ILI rates peaked in weeks 7–9/2012 in participants aged >14 years and in week 11/2012 in children aged ≤14 years. In an average week, GrippeWeb received 46 ARI and 15 ILI reports among children (aged 0–14 years) and 88 ARI and 16 ILI reports of participants aged >14 years. Mean weekly ARI rates in children were between 1.0 and 2.7 times higher than those in participants aged >14 years, while the mean weekly ILI rates in children were between 2.0 and 5.4 times higher.

During the period analysed, the mean number of ARI and ILI reports was strongly age dependent, varying from 6.0 in children aged 0–4 years to 1.3 in participants aged 60 years or older for ARI; for ILI, it varied from 2.4 in the 0–4 year-olds to 0.1 in those aged ≥60 years (Table).

Overall, participants consulted a physician in 18% and 42% of reported ARI and ILI episodes, respectively, due to their illness. After stratification by age, a physician was consulted most frequently for children aged 0–4 years (for ARI in 25% of episodes and for ILI in 49%) and participants aged 15–34 years consulted least frequently for ARI (15%) and adults aged 35–59 years least frequently for ILI (39%).

Regarding school or work absenteeism, participants reported in 30% of ARI and in 68% of ILI episodes that they refrained from their usual daily activity (day care, school, work, etc.) due to their illness.

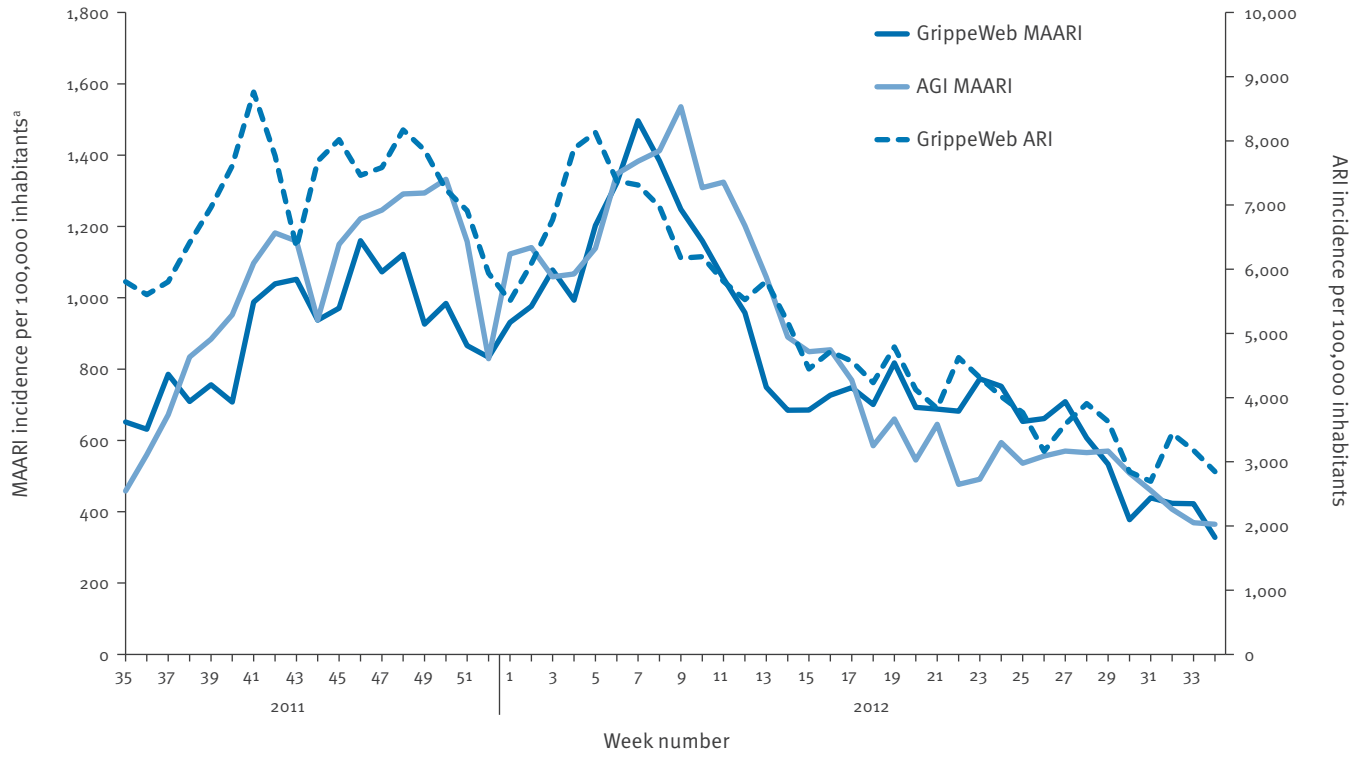
Comparison of GrippeWeb ARI/ ILI rates with data from the AGI

The weekly GrippeWeb ARI incidence and MAARI incidence curves show the same trends as the AGI's consultation incidence curve (Figure 4). Peaks of incidence curves occurred a little earlier for GrippeWeb ARI (week 5/2012) and GrippeWeb MAARI (week 7/2012) compared with AGI MAARI (week 9/2012). The weekly GrippeWeb ARI incidences were about 4.8–10.8 times higher than the AGI MAARI incidence. Over the whole period analysed, the weekly GrippeWeb MAARI incidences differed by a factor 0.6–1.4 from the AGI MAARI incidences, and by a factor of only 0.9–1.4 (GrippeWeb MAARI/AGI MAARI) when considering only weeks 6–16/2012, which were retrospectively defined by the AGI as the time when the influenza epidemic occurred in Germany [2] (Figure 4). The GrippeWeb ARI incidence and MAARI incidence correlated significantly with the AGI MAARI incidence ($r = 0.80$ $p<0.001$, 95% CI: 0.68–0.88 (GrippeWeb ARI) and $r = 0.89$, $p<0.001$, 95% CI: 0.82–0.94 (GrippeWeb MAARI)). The correlation could be improved up to 0.89 by using a lag of two weeks for the correlation of GrippeWeb ARI incidence and AGI MAARI incidence.

Superimposing GrippeWeb ILI rates with the number of samples positive for influenza A(H3N2) and influenza B virus identified by the AGI demonstrated that the occurrence of the influenza wave was reflected in the ILI rates of children, but was less obvious among adults (Figure 5). Circulation of influenza A(H3N2) virus reached its peak in week 9/2012, preceding that of influenza B in week 12. During the period when influenza virus circulated most, ILI rates among adults peaked during weeks 7–9/2012, while among children they peaked during weeks 9–12/2012.

Figure 4

Incidence of acute respiratory illnesses and medically attended acute respiratory illness (MAARI) measured by GrippeWeb and MAARI incidence measured by the German sentinel surveillance system for influenza, weeks 35/2011–34/2012



AGI: Arbeitsgemeinschaft Influenza, German sentinel surveillance system for influenza; ARI: acute respiratory illnesses; MAARI: medically attended acute respiratory illness.

[a] Three-week moving average.

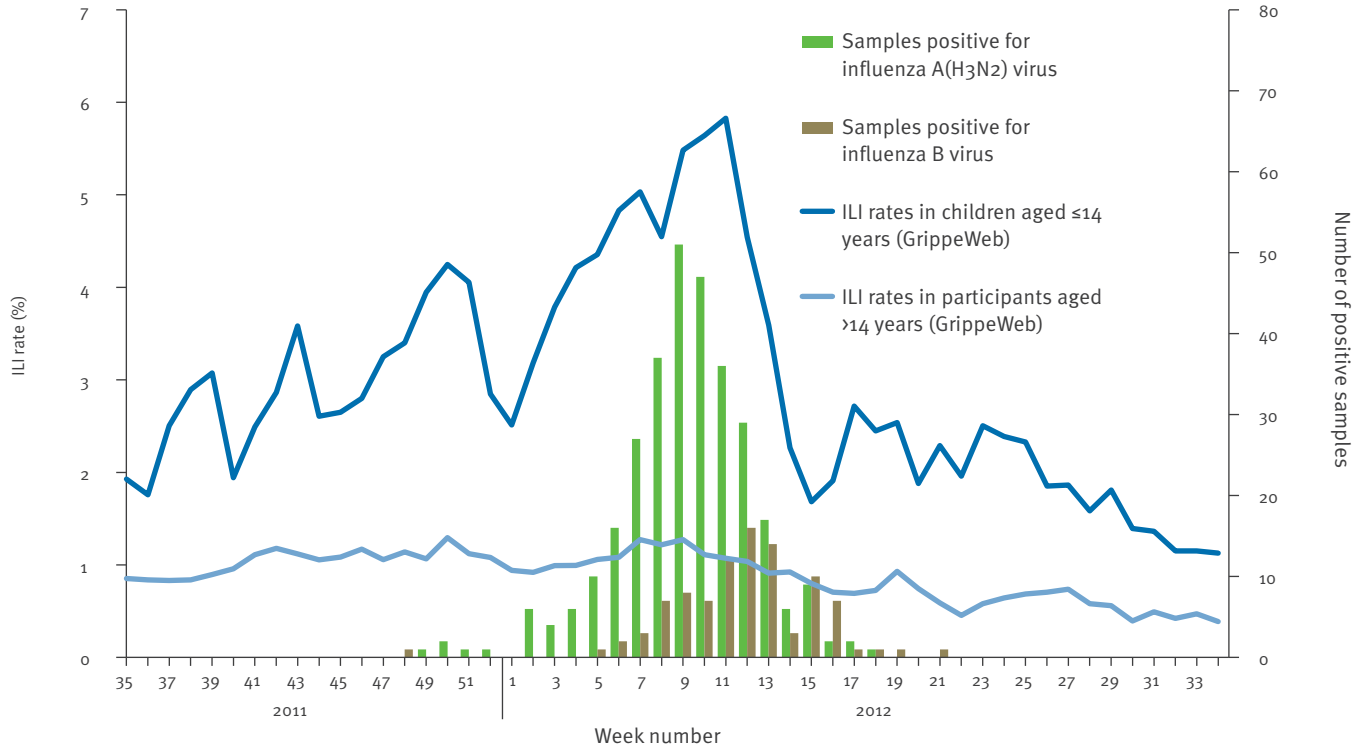
Discussion

During the one-year study period, at the start of GrippeWeb's existence, the system experienced a constantly growing number of participants, with very high weekly reporting rates throughout the year. Participants came from all German federal states and all age groups were represented. Signing up to the prize draw did not seem to affect validity of reporting, but enhanced reporting rates. Estimated GrippeWeb MAARI incidence was in the same range as the MAARI incidence measured by the physician-based AGI system and influenza circulation was reflected by the GrippeWeb ILI rates, particularly among children.

Start-up systems running with voluntary participation, such as GrippeWeb, always need to reach a minimum number of participants to be able to generate reasonably precise and reliable data [19]. During the period analysed, we were able to almost triple the number of GrippeWeb participants. Although statistically significant differences of the GrippeWeb participants existed when compared to the general population in Germany, these may have resulted because of the large numbers compared. Overall, the geographical (with the exception of Berlin in particular) and sex distribution of participants were reasonably similar to that of the German population, but the age distribution could be improved. However, while other European Internet-based monitoring systems had reported under-representation of children [7,8,10], the two age groups of children in GrippeWeb (0–4 years and 5–14 years) were not under-represented, perhaps due to the simplicity and rapidity with which parents can report for their children. Nevertheless, similar to other Internet-based systems [7,8,10], the oldest age group (60 years and above) was under-represented in GrippeWeb, probably due to the lack of familiarity with the internet in this age group. In 2012, only 36% of persons living in Germany aged 65 years or older were Internet users [20]. One practical consequence of this under-representation is that other means of promoting the GrippeWeb system to elderly people need to be considered. The under-representation of the 15–24 year-old age group was at first surprising, but might be linked to the fact that parents can no longer report for their children when they turn 14 years. In addition, health-related topics might be of less interest to young people in this age group and might result in a lower willingness to sign up for GrippeWeb. Furthermore, this age group might tend to prefer the use of smartphone apps and social media such as Facebook instead of 'classic' Internet

Figure 5

GrippeWeb influenza-like illness rates for children aged ≤14 years and participants aged >14 years compared with the number of samples positive for influenza A(H3N2) and B viruses[a], Germany, weeks 35/2011–34/2012



ILI: influenza-like illness.

[a] Identified by the German sentinel surveillance system for influenza.

and email communication. Unavailability of GrippeWeb as a smartphone app and existing strict privacy regulations (prohibiting a link with Facebook) might lower the attractiveness of the system to those aged 15–24 years.

Another way to assess representativeness is to compare the proportion of participants with certain chronic diseases. We found a statistically significant difference between the proportion of GrippeWeb participants with asthma and diabetes compared to the proportion in the general population of Germany, with the participants having a lower prevalence. Data of the general population showed a negative association of diabetes mellitus lifetime prevalence and level of education [15]. Hence GrippeWeb might attract individuals with a higher educational background, who might have a more health-conscious behaviour and lower rates of diabetes in turn.

It is very encouraging to observe the good adherence of GrippeWeb participants, demonstrated by the fact that 62% of participants reported in at least 90% of all possible weeks during the period under study. This rate is very high considering that other Internet-based monitoring systems in Europe reported for the 2011/12 influenza season that at most 25% of participants reported at least 90% of weeks [19]. The very high participation rate in GrippeWeb might be related to the following: (i) the personal, individualised feedback that is automatically given to participants in the form of a diary whenever they log in; (ii) the fact that delivering the weekly report is simple and takes only a few seconds when reporting no new onset of an ARI and up to, at most, a couple of minutes when reporting a respiratory illness with new onset and answering the related questions; and (iii) the prize draw might have attracted individuals who would otherwise not have participated. The way prizes are drawn (increased chance to win with continuous participation) may have fostered the willingness of those eligible to report frequently.

Because of the constancy of our participants, we were able to quantify the mean number of ARI and ILI people in different age groups had during one year of observation. While this number may differ to a certain extent from year to year, the magnitude and degree of difference between adults and children was interesting and declined steadily from the very young to the very old. Data like this are important and might be used for

calculations of burden of disease due to respiratory infections.

The proportion of participants sick with ARI or ILI consulting a physician is also an important parameter and we see clear differences by severity of disease (ARI vs ILI) and age (children vs adults), with highest proportions among 0–4 year-olds who have ILI (49%). In the same season, other European countries, such as France, Italy and Belgium, reported similar proportions between children and adults whereas the Netherlands and the United Kingdom reported rather lower proportions [21]. Data on physician consultations may be heavily influenced by societal factors, for example, at which point in time employees are required to present a medical certificate when they become ill.

Because the AGI system collects ARI data, we compared them with GrippeWeb ARI data. The two case definitions are similar: while the AGI defines an ARI as acute onset of pharyngitis, bronchitis or pneumonia with or without fever, an ARI in GrippeWeb is defined as a subjectively reported new onset of a respiratory illness with fever or cough or sore throat. It was reassuring that the course of GrippeWeb ARI rates was similar in its dynamic compared with the AGI MAARI rates, where the difference in magnitude reflects the rate at which patients seek professional medical advice. This concurs with the experience from other European systems [7-10]. The improved correlation coefficient (of 0.80 to 0.89, when a lag of two weeks is allowed for) suggests that GrippeWeb ARI rates might detect substantial changes in the population perhaps one or two weeks earlier than the AGI system.

It is novel to compare directly and quantitatively MAARI rates of a sentinel-based surveillance system (that of AGI) with those estimated by an Internet-based monitoring system (GrippeWeb). It is remarkable that the two, entirely independent systems with different data sources, sampling schemes, geographical distribution and extrapolation procedures to the whole population agree not only in their weekly patterns throughout the year, but also estimate very similar numerical values (illustrated by the large correlation coefficient of 0.89). This agreement even holds, albeit to a lesser degree, after stratification into age groups (data not shown). We regard this as a sort of 'mutual validation' of the two systems. We were also pleased to see that the actual influenza circulation, as measured by the virological surveillance of the AGI, was also reflected in our ILI data. However, it also shows that syndromic data must always be interpreted in the context of virological surveillance. It would be even more helpful to have virological information (on a broader range of agents) from samples coming directly from participants in the GrippeWeb system, for example, as done in [22].

Strengths of GrippeWeb are that the system could be relatively easily extended or adapted according to, for example, acute needs in an epidemic or even pandemic and it could include other symptoms, such as diarrhoea/nausea/vomiting. Data are gathered in a timely manner: individual (not aggregated) data on demographic variables, lifestyle and underlying health conditions of participants might allow the identification of risk factors to an extent that is hardly possible by physician-based sentinel systems. The data allow us to assess influenza vaccination uptake and estimate influenza vaccination effectiveness (to protect from ILI). The costs of GrippeWeb are limited when compared with those of a sentinel surveillance network: after gathering data from further seasons, modelling should be capable of estimating the burden of disease in the population due to influenza (or other viruses, if data become available).

GrippeWeb has the following limitations. Participants are Internet-users and may have an interest in health topics, which may result in a cohort with a behaviour that is more health conscious than that of the general population. We do not believe, however, that this specifically affects ARI and ILI rates, otherwise the comparison with AGI data would be substantially worse. The number of participants during the reported period was small, resulting sometimes in very small numbers, for example when examining age or other strata. Lastly self-reporting may lead to a tendency to report only when illness occurs. However, by including data of all participants only after they have reported four times for calculation of ARI, ILI and MAARI rates, we have controlled for the 'starter bias'; moreover, because GrippeWeb participants reported very regularly, we feel that it is justified to have a high degree of confidence in the data. Nevertheless, as GrippeWeb is a very young system, it is possible that participants' motivation will decrease over time and participation rate will drop.

Conclusion

Already in its second year after implementation, GrippeWeb has become a reliable tool to estimate ARI and ILI in the general population. It proved to be a valuable complement to the physician-based sentinel system of the AGI. Both systems report their data in parallel. The constant increase of registered participants in GrippeWeb, adequate representativeness, remarkably high continuity of participation and excellent agreement with an independent data source (AGI) provide good and an increasing amount of data. The inclusion of an incentive system for regular participation has shown to be effective. Future strategic steps include a further increase of GrippeWeb subscribers and the collection of samples directly from GrippeWeb participants, for example, by using a self-swabbing approach.

Acknowledgments

The authors thank all participants who contribute to GrippeWeb. We would also like to thank the AGI for their support and provision of data. The authors are grateful to Dr

Cornelia Müller for providing us with the GEDA data and Dr Katharina Alpers (PAE coordinator, RKI) for her valuable comments on the study. The launch of GrippeWeb was supported by grants from the German Federal Ministry of Health.

Conflict of interest

None declared.

Authors' contributions

UB, CR, SK, SB and WH developed the design of the study; UB, CB, CR, MH, KT, SK and MH were involved in data management and analyses; CB drafted the manuscript; all co-authors reviewed and assisted in the editing of the final version of the manuscript.

References

1. European Centre for Disease Prevention and Control (ECDC). About European Surveillance Network (EISN). Stockholm: ECDC; 2012. [Accessed 1 Mar 2013]. Available from: http://ecdc.europa.eu/en/activities/surveillance/EISN/about_EISN/Pages/About_network.aspx
2. Robert Koch Institute (RKI). Bericht zur Epidemiologie der Influenza in Deutschland Saison 2011/12. [Report - the epidemiology of influenza in Germany, season 2011/12]. Berlin: RKI; 2012. German. Available from: http://influenza.rki.de/Saisonberichte/2011.pdf
3. Snacken R, Zucs P, Brown C, Jorgensen P, Mott JA, Amato-Gauci A. Influenza surveillance in Europe. Eur J Public Health. 2011;21(5):674-5. http://dx.doi.org/10.1093/eurpub/ckq185
4. Brooks-Pollock E, Tilston N, Edmunds WJ, Eames KT. Using an online survey of healthcare-seeking behaviour to estimate the magnitude and severity of the 2009 H1N1v influenza epidemic in England. BMC infect Dis. 2011;11:68. http://dx.doi.org/10.1186/1471-2334-11-68
5. Dalton C, Durrheim D, Fejsa J, Francis L, Carlson S, d'Espaignet ET, et al. Flutracking: a weekly Australian community online survey of influenza-like illness in 2006, 2007 and 2008. Commun Diseases Intell Q Rep. 2009;33(3):316-22.
6. Eames KT, Brooks-Pollock E, Paolotti D, Perosa M, Gioannini C, Edmunds WJ. Rapid assessment of influenza vaccine effectiveness: analysis of an internet-based cohort. Epidemiol Infect. 2012;140(7):1309-15. http://dx.doi.org/10.1017/S0950268811001804
7. Friesema IH, Koppeschaar CE, Donker GA, Dijkstra F, van Noort SP, Smallenburg R, et al. Internet-based monitoring of influenza-like illness in the general population: experience of five influenza seasons in the Netherlands. Vaccine. 2009;27(45):6353-7. http://dx.doi.org/10.1016/j.vaccine.2009.05.042
8. Marquet RL, Bartelds AI, van Noort SP, Koppeschaar CE, Paget J, Schellevis FG, et al. Internet-based monitoring of influenza-like illness (ILI) in the general population of the Netherlands during the 2003-2004 influenza season. BMC Public Health. 2006;6:242. http://dx.doi.org/10.1186/1471-2458-6-242
9. Tilston NL, Eames KT, Paolotti D, Ealden T, Edmunds WJ. Internet-based surveillance of Influenza-like-illness in the UK during the 2009 H1N1 influenza pandemic. BMC Public Health. 2010;10:650. http://dx.doi.org/10.1186/1471-2458-10-650
10. van Noort SP, Muehlen M, Rebelo de Andrade H, Koppeschaar C, Lima Lourenço JM, Gomes MG. Gripenet: an internet-based system to monitor influenza-like illness uniformly across Europe. Euro Surveill. 2007;12(7):pii=722. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=722
11. Robert Koch Institute (RKI). Syndromische Surveillance akuter Atemwegserkrankungen unter direkter Mitarbeit der Allgemeinbevölkerung. [Syndromic surveillance of acute resporatory dieases by direct participation of the gerneral population]. Berlin: RKI; 2012. Epidemiologisches Bulletin. 40;8 Oct 2012. German. Available from: http://www.rki.de/DE/Content/Infekt/EpidBull/Archiv/2012/Ausgaben/40_12.pdf?__blob=publicationFile
12. Hulth A, Rydevik G. Web query-based surveillance in Sweden during the influenza A(H1N1)2009 pandemic, April 2009 to February 2010. Euro Surveill. 2011;16(18):pii=19856. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19856
13. Influenzanet. de GroteGriepMeting.nl. 2011 - 2012 (all countries) – ILI. Amsterdam: Influenzanet. [Accessed 12 Mar 2013]. Available from: https://www.degrotegriepmeting.nl/nl/results/?page=results&group=overview&country=compare&season=2011&casedef=ilif&cold=cold&baseline=none&lang=en&type=compare__eiss
14. Robert Koch Institute (RKI). GrippeWeb. Berlin: RKI. Updated 16 Jan 2014. [Accessed 21 Jan 2014]. German. Available from: http://grippeweb.rki.de
15. Robert Koch Institute (RKI). Beiträge zur Gesundheitsberichterstattung des Bundes Daten und Fakten: Ergebnisse der Studie »Gesundheit in Deutschland aktuell 2010« [Data and facts: results of the study 'Health in Germany in 2010']. Berlin: RKI; 2012. German. Available from: http://www.rki.de/DE/Content/Gesundheitsmonitoring/Gesundheitsberichterstattung/GBEDownloadsB/GEDA2010.pdf?__blob=publicationFile
16. Statistisches Bundesamt. GENESIS-Online Datenbank. [GENESIS online database]. Wiesbaden: Statistisches Bundesamt. [Accessed 1 Mar 2013]. German. Available from: https://www-genesis.destatis.de/genesis/online
17. der Heiden MA, Köpke K, Buda S, Buchholz U, Haas W. Estimates of excess medically attended acute respiratory infections in periods of seasonal and pandemic influenza in Germany from 2001/02 to 2010/11. PLoS One. 2013;8(7):e64593. http://dx.doi.org/10.1371/journal.pone.0064593
18. Robert Koch Institute (RKI). Bericht zur Epidemiologie der Influenza in Deutschland Saison 2012/13. [Report - the epidemiology of influenza in Germany, season 2012/13]. Berlin: RKI; 2013. German. Available from: http://influenza.rki.de/Saisonberichte/2012.pdf
19. Lwanga SK, Lemeshow S. Sample size determination in health studies : a practical manual. Geneva: World Health Organization; 1991. Available at: http://www.who.int/iris/handle/10665/40062
20. Statistisches Bundesamt. Private Haushalte in der Informationsgesellschaft Nutzung von Informations- und Kommunikations-technologien. [Private households in the the information society - use of information and communication technologies]. Wiesbaden: Statistisches Bundesamt; 2012. German. Available from: https://www.destatis.de/DE/Publikationen/Thematisch/EinkommenKonsumLebensbedingungen/PrivateHaushalte/PrivateHaushalteIKT2150400127004.pdf?__blob=publicationFile
21. Influenzanet. Influenzanet results. Amsterdam: Influenzanet. [Accessed 1 Mar 2013]. Available from: http://www.influenzanet.eu/en/results/
22. Akmatov MK, Krebs S, Preusse M, Gatzemeier A, Frischmann U, Schughart K, et al. E-mail-based symptomatic surveillance combined with self-collection of nasal swabs: a new tool for acute respiratory infection epidemiology. Int J Infect Dis.2011;15(11):e799-803. http://dx.doi.org/10.1016/j.ijid.2011.07.005

Laboratory preparedness in EU/EEA countries for detection of novel avian influenza A(H7N9) virus, May 2013

E Broberg (eeva.broberg@ecdc.europa.eu)[1], D Pereyaslov[2], M Struelens[3], D Palm[3], A Meijer[4], J Ellis[5], M Zambon[5], J McCauley[6], R Daniels[6]

1. Surveillance and Response Support, European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
2. World Health Organization Regional Office for Europe (WHO/Euro), Copenhagen, Denmark
3. Microbiology Coordination section, European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
4. Division of Virology, Centre for Infectious Disease Research, Diagnostics and Screening, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands
5. Public Health England (PHE) Microbiology Services - Colindale, London, United Kingdom
6. World Health Organization (WHO) Collaborating Centre for Reference and Research on Influenza, Medical Research Council (MRC) National Institute for Medical Research (NIMR), Mill Hill, London , United Kingdom

Citation style for this article:
Broberg E, Pereyaslov D, Struelens M, Palm D, Meijer A, Ellis J, Zambon M, McCauley J, Daniels R. Laboratory preparedness in EU/EEA countries for detection of novel avian influenza A(H7N9) virus, May 2013. Euro Surveill. 2014;19(4):pii=20682. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20682

Article submitted on 29 August 2013 / published on 30 January 2014

Following human infections with novel avian influenza A(H7N9) viruses in China, the European Centre for Disease Prevention and Control, the World Health Organization (WHO) Regional Office for Europe and the European Reference Laboratory Network for Human Influenza (ERLI-Net) rapidly posted relevant information, including real-time RT-PCR protocols. An influenza RNA sequence-based computational assessment of detection capabilities for this virus was conducted in 32 national influenza reference laboratories in 29 countries, mostly WHO National Influenza Centres participating in the WHO Global Influenza Surveillance and Response System (GISRS). Twenty-seven countries considered their generic influenza A virus detection assay to be appropriate for the novel A(H7N9) viruses. Twenty-two countries reported having containment facilities suitable for its isolation and propagation. Laboratories in 27 countries had applied specific H7 real-time RT-PCR assays and 20 countries had N9 assays in place. Positive control virus RNA was provided by the WHO Collaborating Centre in London to 34 laboratories in 22 countries to allow evaluation of their assays. Performance of the generic influenza A virus detection and H7 and N9 subtyping assays was good in 24 laboratories in 19 countries. The survey showed that ERLI-Net laboratories had rapidly developed and verified good capability to detect the novel A(H7N9) influenza viruses.

Introduction

On 31 March 2013, Chinese authorities announced the identification of a novel reassortant A(H7N9) influenza virus isolated from three unlinked fatal cases of severe respiratory disease in eastern China. A few small clusters had been detected but no sustained human-to-human transmission had been observed [1]. The Chinese Center for Disease Control and Prevention (CCDC) subtyped and sequenced the novel viruses and showed them to be low-pathogenic viruses of avian origin [2]. This is the first time that human infection with avian influenza A(H7N9) virus and human deaths due to a low-pathogenicity avian influenza virus have been identified [3]. As of 24 January 2014, 225 laboratory-confirmed human cases including 55 deaths had been reported from eight neighbouring provinces, two municipalities, the Hong Kong Special Administrative Region and Taiwan [4].

Detailed genetic sequence data from human, avian and environmental specimens and isolates of the novel avian influenza A(H7N9) viruses have been made available through the Global Initiative on Sharing All Influenza Data (GISAID) EpiFlu database and the International Nucleotide Sequence Database Collaboration (INSDC). These data suggest that multiple reassortment events have taken place, potentially involving wild birds [2,5-7]. The six RNA segments encoding the internal proteins of the outbreak virus are closely related to avian A(H9N2) viruses recently isolated from poultry in China, while, the segment encoding haemagglutinin (HA) belongs to the Eurasian A(H7) avian influenza virus lineage, and the segment for neuraminidase (NA) is most similar to those present in avian A(H11N9) and A(H7N9) viruses [2,5-7]. However, the nearest matches found for HA and NA are considerably less related than the nearest matches found for the six RNA segments encoding the internal proteins. This distinguishes the outbreak viruses from previously isolated avian influenza A(H7N9) viruses, including those reported in birds in Europe. The sequence diversity observed between different isolates of the novel influenza A(H7N9) virus, compared with historical data, suggests circulation of

Table 1

Laboratories participating in the sequence-based survey exercise for detection of the novel avian influenza A(H7N9) virus, May 2013 (n=32 laboratories in 29 countries)

Organisation name and city	Country
Medical University Vienna, Vienna	Austria[a,b]
Scientific Institute of Public Health, Brussels	Belgium[a,b]
National Centre of Infectious and Parasitic Diseases, Sofia	Bulgaria
National Institute of Public Health, Prague	Czech Republic[a,b]
Nicosia General Hospital, Nicosia	Cyprus
Statens Serum Institut, Copenhagen	Denmark[a,b]
Health Board, Tallinn	Estonia
National Institute for Health and Welfare, Helsinki	Finland[a,b]
Centre Hospitalier Lyon Sud, Lyon	France[a,b]
Pasteur Institute of Paris, Paris	France[a,b]
Robert Koch Institute, Berlin	Germany[a,b]
Hellenic Pasteur Institute, Athens	Greece[a,b]
National Centre for Epidemiology, Budapest	Hungary
National University Hospital of Iceland, Reykjavík	Iceland[a]
University College Dublin, Dublin	Ireland[a,b]
Istituto Superiore di Sanità, Rome	Italy[a,b]
State Agency Infectology Centre of Latvia, Riga	Latvia
Centre for Communicable Diseases and AIDS, Vilnius	Lithuania[a,b]
Laboratoire National de Santé, Luxembourg	Luxembourg[a,b]
National Institute for Public Health and the Environment (RIVM), Bilthoven	The Netherlands[a,b]
Norwegian Institute of Public Health, Oslo	Norway[a,b]
Pathology laboratory, Sptar Mater Dei, Msida	Malta
National Influenza Center, Warsaw	Poland[a]
National Institute of Health Dr Ricardo Jorge, Lisbon	Portugal[a,b]
National Institute of Research and Development for Microbiology and Immunology Cantacuzino, Bucharest	Romania
Public Health Authority of the Slovak Republic, Bratislava	Slovakia[a]
Institute for Public Health, Ljubljana	Slovenia[a,b]
National Centre for Microbiology, Barcelona	Spain
National Centre for Microbiology, Madrid	Spain[a,b]
Swedish Institute for Communicable Disease Control, Solna	Sweden[a,b]
Public Health England, Colindale	United Kingdom-England [a,b]
Specialist Virology Centre for Wales, Cardiff	United Kingdom-Wales

[a] Laboratories in these 22 countries received A/Anhui/1/2013 positive control materials from the World Health Organization Collaborating Centre (WHO CC) in London.

[b] Laboratories based in these 19 countries returned real-time RT-PCR results based on the novel avian influenza A(H7N9) vRNA standard dispatched by WHO CC London and, in addition, the West of Scotland Specialist Virology Centre, Glasgow, Scotland (UK) provided experimental RT-PCR results.

the virus in birds before recent multiple introductions to humans [8]. The reservoir for this novel infection remains unknown, but the virus has been detected in domestic birds at live markets in eastern China [9].

It is recognised that real-time RT-PCR assays are at the forefront of influenza virus detection, with generic assays based on the matrix (M) gene for identification of influenza A, and specific assays for the HA and NA genes for identification of the different subtypes [10]. According to a survey conducted in July 2011, the majority of European national influenza reference laboratories (31 laboratories in 25 countries) are using generic RT-PCR tests based on the influenza A virus M gene [11], which have the potential to detect also the novel A(H7N9) viruses.

To assist European laboratories in verifying and ensuring their diagnostic capability to detect and identify the novel avian influenza A(H7N9) viruses, the

Table 2

Results of the survey on detection of the novel avian influenza A(H7N9) virus, May 2013 (n=32 laboratories in 29 countries)

Capability	Countries n	Laboratories n
Generic detection assay for influenza A is predicted to detect influenza A(H7N9)	27	30
Have BSL3 laboratory facilities that they can use for culture of this virus	27	29
Have isolation capability	22	24
Influenza A(H7) subtyping available	27	29
Influenza A(N9) subtyping available	20	22

BSL: biosafety level.

European Centre for Disease Prevention and Control (ECDC), jointly with the World Health Organization (WHO) Collaborating Centre for Reference and Research on Influenza (WHO CC) in London, other members of the European Reference Laboratory Network for Human Influenza (ERLI-Net) and the WHO Regional Office for Europe (WHO/Europe), has released a technical briefing note on diagnostic preparedness in Europe for detection of the novel avian influenza A(H7N9) viruses [12]. The briefing note provides a list of considerations to ensure European-wide diagnostic capability, an update on currently available methods used for molecular detection of human infection with the novel avian influenza A(H7N9) virus by real-time RT-PCR, a table of validation criteria for A(H7) HA molecular assays, and information on positive controls for RT-PCR assays.

To complement the technical briefing note, we conducted a questionnaire-based survey with the objective of assessing the capability (but not assessing the detection capacity in terms of numbers over time) of EU/EEA countries to detect and subtype the novel avian influenza A(H7N9) viruses, given the possibility of their spread to Europe. Subsequently, all influenza reference laboratories were offered positive control material by WHO CC London to verify and to report on the experimental sensitivity of their RT-PCR assays.

Methods

The survey questionnaire was developed jointly by ECDC, the ERLI-Net coordination team and WHO/Europe upon request of the European Commission. It was distributed to all ECDC influenza surveillance laboratory contact points (ERLI-Net laboratories) in May 2013. The questionnaire asked questions related to the current capability of the countries to detect the novel A(H7N9) viruses and their prediction, based on influenza RNA sequence analysis, regarding the need to update their detection primer sets. It also included questions asking for details of the primer sets in use and for the countries' capability to isolate and propagate influenza A(H7N9) viruses.

The WHO CC Beijing supplied A/Anhui/1/2013(H7N9) to the WHO CC London as a virus stock that had been passaged twice in embryonated hens' eggs (E2) in compliance with the WHO Pandemic Influenza Preparedness (PIP) framework [13]. A single preparation of viral RNA (vRNA) was made from a virus stock that had been passaged once more in eggs (E2/E1) and grown to an HA titre of 256/512 as assessed with turkey red blood cells. This virus stock yielded a concentration of $2x10^9$ plaque forming units (PFU)/mL on Madin-Darby canine kidney (MDCK) cells. vRNA was extracted with a QIAamp vRNA extraction kit (Qiagen, catalogue no. #52906), and each influenza reference laboratory was supplied with 40 µL of an undiluted vRNA standard via dry-ice shipment. From the above data, it was calculated that 5 µL (the amount commonly used in a 25 µL real-time RT-PCR assay) of a 10^{-8} dilution of the vRNA standard would contain between 2.4 and 24 vRNA copies, assuming that 1 PFU equates to 10–100 virus particles. Laboratories were asked to share with WHO CC London the results they generated with the vRNA standard in their existing or recently implemented real-time RT-PCR assays for generic influenza A virus detection and H7N9 subtyping (including information on primer sets, RT-PCR kits and thermocycler platforms/cycling parameters, and dilution(s) of vRNA tested).

Results

Sequence-based analysis questionnaire

Thirty-two of 36 laboratories in 29 Member States of the European Union and European Economic Area (EU/EEA) responded to the questionnaire within a month (Table 1).

Thirty laboratories in 27 countries predicted that their M gene-based generic detection assay for influenza A virus would also detect the novel A(H7N9) viruses (Table 2). One laboratory considered their generic influenza A RT-PCR detection assay inappropriate for detecting the novel virus. One laboratory indicated use of a commercial influenza A detection assay, but not

Table 3

Testing results with the novel avian influenza A(H7N9) vRNA standard in real-time RT-PCR assays, May 2013 (n=24 laboratories in 19 countries)

Positive control (vRNA) dispatch		Number of laboratories		Countries represented
Acknowledged receipt of vRNA		32		22
Tested primer/probe protocols with the vRNA		24		19
Number of laboratories reporting (number of assays carried out)	Number of countries	Number of different assays	Product size range	Number of reports on end point titrations (range of end points reported)[a]
M gene (generic influenza A assay)				
19[b]	16	8	77–205	6 (10^{-7}–10^{-9})
H7-HA gene				
24 (33)[c]	19	16	52–254	10 (10^{-7}–10^{-9})
N9-NA gene				
16 (17)[d]	15	6	107–153	4 (10^{-6}–10^{-7})

HA: haemagglutinin; M: matrix protein; NA: neuraminidase.

[a] The number of reports that included end point titrations is given, with the titration range for the assays in brackets).

[b] The most commonly used assay (six of 19 reports) was the InfA primer set from the United States Centers for Disease Control and Prevention (US CDC) in Atlanta [17]. The other assays were developed locally.

[c] Seven laboratories in different countries reported using more than one primer set: Austria (n=2), Belgium (n=2), Germany (n=4), Italy (n=2), Luxembourg (n=2), the Netherlands (n=2), and Norway (n=2), generating 33 reports.

[d] Germany tested two primer sets, and 12 of the 17 reports were for the CCDC [15] N9 primer set.

knowing the primer sequences, was unable to predict its diagnostic capability.

It is recommended by WHO that the novel A(H7N9) viruses be propagated in biosafety level (BSL) 3 facilities [14]; 29 laboratories in 27 countries reported having such facilities. Seven countries (Bulgaria, Cyprus, Estonia, Ireland, Malta, Romania and Slovenia) and Wales (United Kingdom (UK)), indicated that they would not propagate the novel A(H7N9) virus in their laboratories because they lacked capability in their BSL3 facilities.

Twenty-nine influenza reference laboratories in 27 countries indicated having a real-time RT-PCR assay for H7 subtyping in place. Nine laboratories in eight countries had implemented subtyping assays based on H7 primers and probes developed by CCDC [15] or Corman et al. [16] or their own primer/probe sets based on sequence alignments. Fourteen laboratories in 13 countries had more than one H7 subtyping assay in place which showed some variation (within 1 log; cycle threshold (Ct): 3.2) in the sensitivity of detection of the novel avian influenza A(H7N9) viruses. Eight laboratories in eight countries had implemented the complete protocol of the United States Centers for Disease Control and Prevention (US CDC) [17]. Four were using other alternative assay protocols than the ones listed here, for example those of Slomka et al. [18].

Twenty-two laboratories in 20 countries had set up N9 subtyping at the time of the survey (May 2013). Ten laboratories in nine countries indicated that they had not yet tested their protocol or did not have this test. Thirteen countries had chosen to use the CCDC [15] assay with the primers and probes for N9 from that protocol. Seven laboratories in six countries had developed their own assay, and three laboratories used the primers and probes described by Corman et al. [16]. One country indicated that they would sequence the NA gene instead of setting up a specific subtyping real-time RT-PCR assay for N9.

To share viruses with a WHO CC and the WHO GISRS, 22 laboratories in 19 countries reported use of the WHO shipment fund for shipments to WHO CC London in the influenza season and during emerging outbreaks. Four countries used their own budgets with additional WHO shipment funding, and eight laboratories from eight countries used only their own budget. Three countries and laboratories indicated use of Quality Assurance Exercises and Networking on the Detection of Highly Infectious Pathogens (http://www.quandhip.info/) for shipments, in addition to the WHO shipment fund, and none of the responding laboratories indicated further need for financial support for sample shipment.

Use of positive control and sensitivity of RT-PCR assays

Having been alerted to the availability of A/Anhui/1/2013-derived positive controls for their detection assays, 35 laboratories in 22 countries requested and received material from the WHO CC London. Twenty-one of these laboratories, three of which

participate in the OFFLU OIE/FAO network of expertise on animal influenza (one each in Germany, Italy and the UK), received live virus and vRNA, 11 laboratories received vRNA only, and three laboratories received inactivated virus. Three further countries confirmed that they would receive or had received the US CDC kit including the corresponding positive controls (catalogue no. 1257 and 1258, available from the Influenza Reagent Resource, https://www.influenzareagentresource.org/). Two laboratories indicated that they needed assistance in setting up relevant assays, and they received individual support from WHO CC London.

The 24 laboratories (19 countries) that subsequently reported their results on detecting the novel influenza A(H7N9) vRNA standard in their real-time RT-PCR protocols, had correctly predicted their capability to detect the novel virus (Table 3). Nineteen laboratories reported on generic influenza A virus detection, all used real-time RT-PCR assays based on the M gene. Eight different assays were employed that generated product sizes in the range 77–205 nt, and six of 19 reports employed the US CDC InfA primer set (106 nt PCR product). The six end point titration results all showed good sensitivity (five were positive for dilutions in the range 10^{-7}–10^{-8} and one laboratory showed positivity up to 10^{-9}), five of them with the US CDC InfA primer set.

H7 detection was reported by all 24 laboratories (Table 3). Sixteen different H7 assays were employed, generating PCR fragments in the range 52–254 nt. The primer sets spanned five different regions in the HA gene, two in HA1, one spanning the HA1/2 cleavage site, and two in HA2. Seven laboratories in different countries reported on more than one primer set, resulting in 33 individual primer set reports, 19 of which employed the following primer sets: CCDC HA1 [15] (n=10), Slomka et al. HA2 [18] (n=4), Corman et al. HA2 [16] (n=3), and US CDC HA2 [17] (n=2). The remaining reports used primers developed in-house or modifications of the primers listed here above, making them more specific for the novel avian influenza A(H7N9). The 10 H7 end point titrations, all of which showed good sensitivity (nine were positive for dilutions in the range 10^{-7}–10^{-8} and one laboratory showed positivity up to 10^{-9}), were using among others the CCDC HA1 [15] (n=3), Slomka et al. HA2 [18] (n=2), Corman et al. HA2 [16] (n=2) and the US CDC HA2 [17] (n=1) primer sets.

Sixteen laboratories reported on N9 detection (Table 3). Six assays were employed (PCR product size range: 107–153 nt), two of which were the initial and modified versions of the CCDC assay. Twelve reports employed one of the CCDC N9 primer sets. Of the four N9 end point titrations, three employed a CCDC primer set (PCR product size 107 nt), while the fourth employed an assay developed in-house (RIVM, the Netherlands, product size: 125 nt); both N9 assays were less sensitive than the generic (M gene) and H7 assays, with end points at dilutions in the range 10^{-6}–10^{-7}. One laboratory (NIC, Norway) obtained good sensitivity through end point titration (10^{-8}–10^{-9} for M and H7 but still only 10^{-6}–10^{-7} for N9) with real-time RT-PCRs set up using an AgPath-1D One-Step RT-PCR kit (Life Technologies) and run on Rotor-Gene 3000 or 6000 thermocyclers (Qiagen); this may be due to dilution of the vRNA standard with water containing carrier RNA as supplied with the QIAamp vRNA extraction kit (Qiagen).

A wide variety of RT-PCR kits and thermocycler platforms were used across the reporting laboratories for detection and subtyping of A(H7N9) viruses. Most laboratories used one-step RT-PCR kits from Life Technologies (TaqMan Fast Virus 1-Step, SuperScript III Platinum One Step qRT-PCR, AgPath-1D One-Step RT-PCR) or Qiagen (One-Step RT-PCR, Quantifast Probe RT-PCR), while some laboratories employed two-step systems (e.g. QuantiTect Reverse Transcription kit, Qiagen) developed in-house, and one laboratory reported on generic influenza A detection as part of a multiplex assay. The various assays were implemented on Stratagene (MX3005), ABI (7300, 7500, 7500 FAST, 7900 HT), Rotor-Gene/Qiagen (Q, 3000, 6000), Roche (LC480) and Bio-Rad (CFX96) thermocyclers.

Discussion

ERLI-Net laboratories had built up detection capability for the novel influenza A(H7N9) viruses within approximately two months from the first reports of this virus. Most ERLI-Net laboratories had developed or applied specific H7 and/or N9 real-time RT-PCR assays to identify the novel A(H7N9) viruses: 27 countries have an H7 assay and 21 an N9 assay in place in their influenza reference laboratories. Overall, 28 of 31 laboratories in 27 countries reported an ability to subtype A(H7) viruses, with the remaining three laboratories proposing to send their non-subtypeable viruses to WHO CC London.

Overall, laboratories in EU/EEA countries appear to be well prepared for the detection and identification of the novel avian A(H7N9) influenza virus, because they either can detect the viruses themselves or, if not, have a mechanism in place to forward the viruses to WHO CC for characterisation. Furthermore, it is likely that the H7-specific HA2 primer set from the US CDC will be adopted by more laboratories, as it is now available through the Influenza Reagent Resource (https://www.influenzareagentresource.org #FR-1258). Due to the high genetic diversity in the HA of influenza viruses of the A(H7) subtype, it has not been possible to design a universal primer/probe set of the required specificity and sensitivity to detect all avian influenza A(H7) viruses. However, both the US CDC H7-HA2 primer set [17] and the set by Slomka et al. [18] have been evaluated and are capable of detecting Eurasian H7 avian influenza viruses typically infecting poultry in Europe that have the potential to cause zoonoses.

From the survey responses and results reported on the use of an A/Anhui/1/2013(H7N9) vRNA standard, it is apparent that ERLI-Net laboratories across EU/

EEA countries have a range of assays available that are suitable for detecting the M, HA and NA genes of the novel A(H7N9) influenza virus. With current capabilities, these novel avian influenza A(H7N9) viruses would be detected in the majority of EU/EEA countries on submission of a sample to a national influenza reference laboratory for characterisation. However, as these A(H7N9) viruses are likely to evolve, sequence-based comparison of primer/probe sets with circulating H7N9 viruses should be part of a continuous monitoring practice in all influenza reference laboratories, with modification of set(s) as required. Despite such monitoring it is clear that, even when the same detection algorithm, equipment and laboratory protocols are used, the human factor plays a role in laboratory detection, and assay performance can only be verified through external quality assessment (EQA) and clinical validation [19]. ERLI-Net undertook an EQA in autumn 2013 that included an A(H7N9) virus in the panel; the results are pending.

Conclusions

This capability assessment would not have been possible without the prompt actions of the Chinese authorities and the CCDC who rapidly deposited sequence data in the GISAID database and provided virus for culture and RNA extraction to WHO CC London. Feedback from ERLI-Net laboratories indicates that EU/EEA countries have good detection capabilities for these novel avian influenza A(H7N9) viruses. Generally, this study illustrates the importance of having a coordinated laboratory network such as ERLI-Net, with a direct link to the WHO GISRS for virus and reagent sharing, and the usefulness of timely responses to sequence-based analysis surveys as well as testing of performance and proficiency to inform a regional risk management response.

A large diversity of assays and platforms for influenza detection and diagnosis are available in the European health sector, reflecting prevailing local conditions. Nevertheless, good technical performance can be achieved, even though a lack of detailed knowledge of primer and probe binding sites in commercial kits makes it difficult to predict their match with the viral target genes. The mechanism described here (a survey including sequence-based analysis followed by practical assessment) is likely to be necessary every time a new variant of influenza virus with pandemic potential emerges. During such surveys, clear technical communication channels both within and between countries, ERLI-Net/ECDC, WHO/Europe and WHO CC London, are a crucial part of preparedness and response.

In influenza reference laboratory networks, the existing pathways for specimen referral to the WHO CCs and annual EQAs have proven useful tools in ensuring good seasonal influenza surveillance. The same pathways can be used in an emergency. For EU/EEA countries, an additional element in an emergency response is enhanced communication between ECDC, WHO/Europe, WHO CC London and other ERLI-Net virology experts, to ensure high quality and rapid technical support for the ERLI-Net laboratories. The network benefits from WHO CC functions through the distribution of positive controls for RT-PCR and support in the validation of protocols. Larger network laboratories, such as Public Health England Colindale, can support the network in the clinical validation of the detection assays. However, a sequence-based computational assessment of the detection platforms is not enough, and EQA of the assays is crucial to ensure the field validation of primers and reagents. This ERLI-Net model could be applied to other dedicated communicable disease networks to assess the performance of their schemes for sample referral, setup of detection assays and validation of the assays for emerging infectious disease events.

The current response to the emergence of influenza A(H7N9) has demonstrated a good preparedness in European influenza reference laboratories. Nevertheless, a number of areas can be improved: (i) how best to assess the detection assays used in the primary diagnostic laboratories, (ii) how well evaluated and clinically validated a detection protocol should be before it is shared with the network laboratories, (iii) where to post all technical material so that it can be easily found, (iv) how to speed up the distribution of positive controls, (v) the best way to communicate rapidly within the network, (vi) how the questionnaire results are followed up with the laboratories, and (vii) how the required training, based on EQA results, is delivered. Overall, there is a need to decide on a standard operation procedure for emergency responses in the network, so that all parties know their role(s) and timeline(s) for response and what is expected from them when a novel virus emerges. As the influenza A(H7N9) situation is still evolving, there is no reason for complacency, and preparedness at the European level will continue to be monitored. Gene sequences from the most recent influenza A(H7N9) zoonotic infections available in GISAID, as of 24 January 2014, indicate that the low levels of genetic drift observed are unlikely to adversely affect the detection capabilities that have been developed in EU/EEA countries .

Authors' contributions

EB, RD, JMcC were responsible for the analyses of returned data and drafting of the manuscript. These authors and the others (JE, AM, DPa, DPe, MS and MZ) took part in discussions relating to the development and drafting of guidance for ERLI-Net/NIC laboratories and the sequence-based questionnaire, and critical review of the present manuscript.

Conflict of interest

None declared.

Acknowledgements

The authors thank all persons in EU/EEA countries and their human influenza reference laboratories that responded to the sequence-based analysis questionnaire and the request for feedback on the use of positive control vRNA from a representative novel avian influenza A(H7N9) virus shared with GISRS by WHO CC Beijing. The work of the WHO CC was supported by MRC programme U117512723.

References

1. Qi X, Qian YH, Bao CJ, Guo XL, Cui LB, Tang FY, et al. Probable person to person transmission of novel avian influenza A (H7N9) virus in Eastern China, 2013: epidemiological investigation. BMJ. 2013;347:f4752. http://dx.doi.org/10.1136/bmj.f4752
2. Gao R, Cao B, Hu Y, Feng Z, Wang D, Hu W, et al. Human infection with a novel avian-origin influenza A (H7N9) virus. N Engl J Med. 2013;368(20):1888-97. http://dx.doi.org/10.1056/NEJMoa1304459
3. European Centre for Disease Prevention and Control (ECDC). Human infection with a novel avian influenza virus, A(H7N9) – China. Stockholm: ECDC; 2013. Available from: http://www.ecdc.europa.eu/en/publications/Publications/influenza-A%28H7N9%29-China-rapid-risk-assessment-8-may-2013.pdf
4. European Centre for Disease Prevention and Control (ECDC). Communicable disease threats. Stockholm: ECDC; 2014. Available from: http://www.ecdc.europa.eu/en/publications/Publications/communicable-disease-threats-report-25-jan-2014.pdf
5. Kageyama T, Fujisaki S, Takashita E, Xu H, Yamada S, Uchida Y, et al. Genetic analysis of novel avian A(H7N9) influenza viruses isolated from patients in China, February to April 2013. Euro Surveill. 2013;18(15):20453. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20453
6. World Health Organization (WHO). Overview of the emergence and characteristics of the avian influenza A(H7N9) virus. Geneva: WHO; 2013. Available from: http://www.who.int/influenza/human_animal_interface/influenza_h7n9/WHO_H7N9_review_31May13.pdf
7. Wu A, Su C, Wang D, Peng Y, Liu M, Hua S, et al. Sequential reassortments underlie diverse influenza H7N9 genotypes in China. Cell Host Microbe. 2013;14(4):446-52. http://dx.doi.org/10.1016/j.chom.2013.09.001
8. Jonges M, Meijer A, Fouchier RA, Koch G, Li J, Pan JC, et al. Guiding outbreak management by the use of influenza A(H7Nx) virus sequence analysis. Euro Surveill. 2013;18(16):20460. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20460
9. Shi JZ, Deng GH, Liu PH, Zhou JP, Guan LZ, Li WH, et al. Isolation and characterization of H7N9 viruses from live poultry markets—Implication of the source of current H7N9 infection in humans. Chin Sci Bull. 2013; 58(16). doi: 10.1007/s11434-013-5873-4. http://dx.doi.org/10.1007/s11434-013-5873-4
10. Zhang WD, Evans DH. Detection and identification of human influenza viruses by the polymerase chain reaction. J Virol Methods. 1991 Jun;33(1-2):165-89. http://dx.doi.org/10.1016/0166-0934(91)90017-T
11. European Centre for Disease Prevention and Control (ECDC). CNRL in silico exercise to determine the capabilities of network laboratories to detect triple reassortant swine origin influenza A(H3N2) viruses. Stockholm: ECDC; 2012. Available from: http://ecdc.europa.eu/en/publications/Publications/1204-TER-CNRL-Capability-Exercise.pdf
12. European Centre for Disease Prevention and Control (ECDC), Community Network of Reference Laboratories for Human Influenza in Europe, World Health Organization Regional Office for Europe. Diagnostic preparedness in Europe for detection of avian influenza A(H7N9) viruses. Stockholm: ECDC; 2013. Available from: http://ecdc.europa.eu/en/publications/Publications/Forms/ECDC_DispForm.aspx?ID=1103
13. World Health Organization (WHO). Pandemic influenza preparedness framework for the sharing of influenza viruses and access to vaccines and other benefits. Geneva: WHO; 2011 Available from: http://whqlibdoc.who.int/publications/2011/9789241503082_eng.pdf
14. World Health Organization (WHO). Laboratory biorisk management for laboratories handling human specimens suspected or confirmed to contain avian influenza A(H7N9) virus causing human disease. Interim recommendations. Geneva: WHO; 2013. Available from: http://www.who.int/influenza/human_animal_interface/influenza_h7n9/InterimRecLaboratoryBioriskManagementH7N9_10May13.pdf
15. World Health Organization (WHO) Collaborating Center for Reference and Research on Influenza at the Chinese National Influenza Center B, China. Real-time RT-PCR Protocol for the Detection of Avian Influenza A(H7N9) Virus. Geneva: WHO; 2013. Available from: http://www.who.int/influenza/gisrs_laboratory/cnic_realtime_rt_pcr_protocol_a_h7n9.pdf
16. Corman VM, Eickmann M, Landt O, Bleicker T, Brunink S, Eschbach-Bludau M, et al. Specific detection by real-time reverse-transcription PCR assays of a novel avian influenza A(H7N9) strain associated with human spillover infections in China. Euro Surveill. 2013;18(16):20461. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20461
17. Centers for Disease Control and Prevention (CDC). Seasonal Influenza, Diagnostics for Detecting H7N9 Using rRT-PCR. Atlanta: CDC. [Accessed latest 22 January 2014]. Available from: http://www.cdc.gov/flu/avianflu/h7n9/detecting-diagnostics.htm
18. Slomka MJ, Pavlidis T, Coward VJ, Voermans J, Koch G, Hanna A, et al. Validated RealTime reverse transcriptase PCR methods for the diagnosis and pathotyping of Eurasian H7 avian influenza viruses. Influenza Other Respi Viruses. 2009;3(4):151-64. http://dx.doi.org/10.1111/j.1750-2659.2009.00083.x
19. Meijer A, Beerens A, Claas E, Hermans M, de Jong A, Molenkamp R, et al. Preparing the outbreak assistance laboratory network in the Netherlands for the detection of the influenza virus A(H1N1) variant. J Clin Virol. 2009;45(3):179-84. http://dx.doi.org/10.1016/j.jcv.2009.06.003