Impact factor 6.15

Eurosurveillance

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

Vol. 18 | Weekly issue 15 | 11 April 2013

ecdc EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

www.eurosurveillance.org

A novel reassortant avian influenza A(H7N9) virus in China – what are the implications for Europe

A Nicoll (angus.nicoll@ecdc.europa.eu)[1], N Danielsson[1]
1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

Citation style for this article:
Nicoll A, Danielsson N. A novel reassortant avian influenza A(H7N9) virus in China – what are the implications for Europe. Euro Surveill. 2013;18(15):pii=20452. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2045

Article submitted on 10 April 2013 / published on 11 April 2013

As of 10 April 2013, 33 human cases infected with a novel influenza A(H7N9) virus have been laboratory confirmed in Shanghai, Anhui, Jiangsu and Zhejiang provinces in China (Figure1). This case count came after on 31 March 2013, the Chinese authorities had announced the identification of a novel influenza A virus, an A(H7H9) virus, in three people in Shanghai and Anhui province. Two men in Shanghai, 87 and 27 years old, respectively, had become ill with influenza-like (ILI) symptoms and progressed to severe lower respiratory tract infections within a week in mid to late February, and died from acute respiratory distress syndrome hereafter [1,2]. The two had no epidemiological link and no known exposure to evidently sick animals. One of them was a pork butcher. The third case was a 35-year-old woman from Anhui province, adjacent to Shanghai, who also became ill with ILI with symptom onset on 9 March followed by severe respiratory disease and death.

The detection of these cases was possible because of a well-functioning surveillance system with a laboratory component through which the initially non-subtypeable influenza A viruses were sent to the World Health Organization (WHO) Influenza Collaborating Centre at the Chinese Center for Disease Control and Prevention (CDC) in Beijing for sequencing. Upon laboratory identification of the new viruses, the responsible Chinese authorities notified the cases as required in the International Health Regulations (IHR) to WHO and other member states [3].

Moreover, researchers from the Chinese CDC posted the genetic information of the viruses on the publicly accessible GISAID website [4]. The viruses were not genetically identical, indicating they had been circulating for some time over a wide region [5]. The same type of viruses were reported by Chinese veterinary authorities from 4 April onward in different species of poultry and environmental samples from live bird markets in Shanghai [6]. The sequences of the veterinary and environmental specimens were also posted on the GISAID site by the Chinese national veterinary laboratory in Harbin [3].

Following the detection of the first cases, the Chinese CDC has rapidly made specific polymerase chain reaction (PCR) test kits for the new A(H7N9) viruses available to provincial and local laboratories across China to ensure timely testing of suspected cases. Since then individual human cases are being confirmed and made public daily by the Chinese authorities at provincial level in the four affected provinces. More cases are being detected with onset dates since late March (Figure 2). While this could simply reflect increasing awareness among clinicians and public health authorities and that testing became available more widely, close monitoring is necessary to detect changes in transmission patterns, especially human-to-human transmission and cases appearing in China beyond the four provinces.

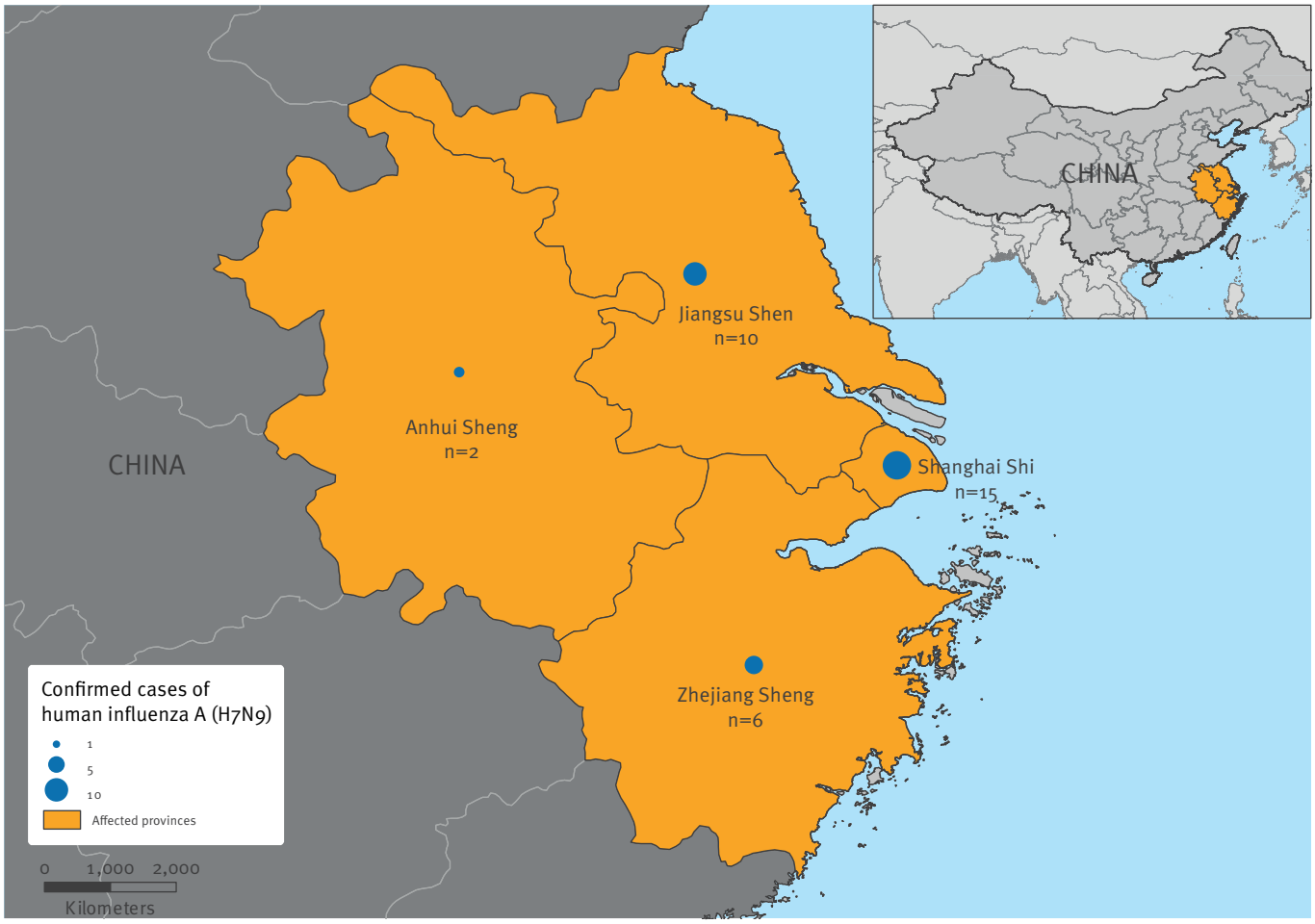
While the novel A(H7N9) virus has been detected in birds and environmental specimens at a bird markets in Shanghai and the other affected provinces, the source of infection in most of the cases still remains to be determined [6]. It is equally unclear how the virus is introduced into the markets. Nevertheless, China has stepped up vigilance and intensified human and animal surveillance [7]. It has also implemented public health measures that include the closure of some live poultry and bird markets and culling of birds [8].

A striking feature is that human cases are sporadic and very few possible clusters have been detected. They are being investigated by the Chinese authorities. So far, there has been no documented sustained human-to-human transmission and there is no clear indication of such transmission even though the virus has genetic markers that are known to be associated with improved replication of avian influenza viruses in mammals [4,5].

When compared with A(H5N1) viruses, animal-to-human transmissibility seems to be higher for influenza A(H7N9). It is noteworthy that the timeframe during which cases have been identified is very different from that of human cases of influenza A(H5N1) detected in China of late. Between January 2010 and March 2013, only seven human A(H5N1) cases were reported, five

Figure 1

Laboratory-confirmed cases of human influenza A(H7N9) in China as of 10 April 2013 (n=33)



Source: European Centre for Disease Prevention and Control (ECDC).

of which are known to have died [9]. Few human cases due to infection with avian influenza A(H7) viruses have been described in the literature, possibly because the symptoms are usually mild in humans and of low pathogenicity in poultry [10]. A well described outbreak involving humans was that of a highly pathogenic avian influenza A(H7N7) among poultry in the Netherlands in 2003. It resulted in 86 mild infections, mainly conjunctivitis, among poultry workers, three cases of non-sustained human-to-human transmission among their household contacts, but only one fatality [11,12].

Only careful serological surveys in China can reveal if there were such transmissions and these investigations are underway. Of the detected 33 human A(H7N9) cases as of 10 April, 30 developed severe illness with nine fatalities while three presented with mild symptoms (Figure 2). It can be expected that surveillance activities will lead to detection of additional cases in the coming weeks, but so far no cases have been identified outside the four Chinese Provinces.

A limited number of scenarios that could follow from the emergence of this novel virus are possible. The one that explains the current human and animal epidemiological situation best, based on available clinical and virological analyses, is that of the emergence of a novel reassortant avian influenza virus of low pathogenicity to birds but of significant pathogenicity to humans. This virus has probably spread undetected among poultry in parts of eastern China. When this started is unclear. It only came to light because some people infected through contact with birds or environmental exposure, became severely ill. Even though the viruses were found in poultry and the environment in live bird markets in Shanghai, the species introducing the infection into the markets has not been identified. The various species reported as being infected may have only become infected at the markets.

The speed, transparency and intensity of the work performed in respect to the novel A(H7N9) virus in China and by the Chinese CDC and veterinary authorities is

impressive and deserves full credit [13]. It also has to be acknowledged that there is tremendous value for all those concerned with public health in that the WHO Collaborating Centre for Influenza at the Chinese Center for Disease Control and Prevention has shared the viruses and that the molecular data have been published on the publicly accessible GISAID database. This data sharing platform has been important for scientists to gain important insight into the molecular virus characteristics and the origins of the virus as well as for public health experts to assess the current situation.

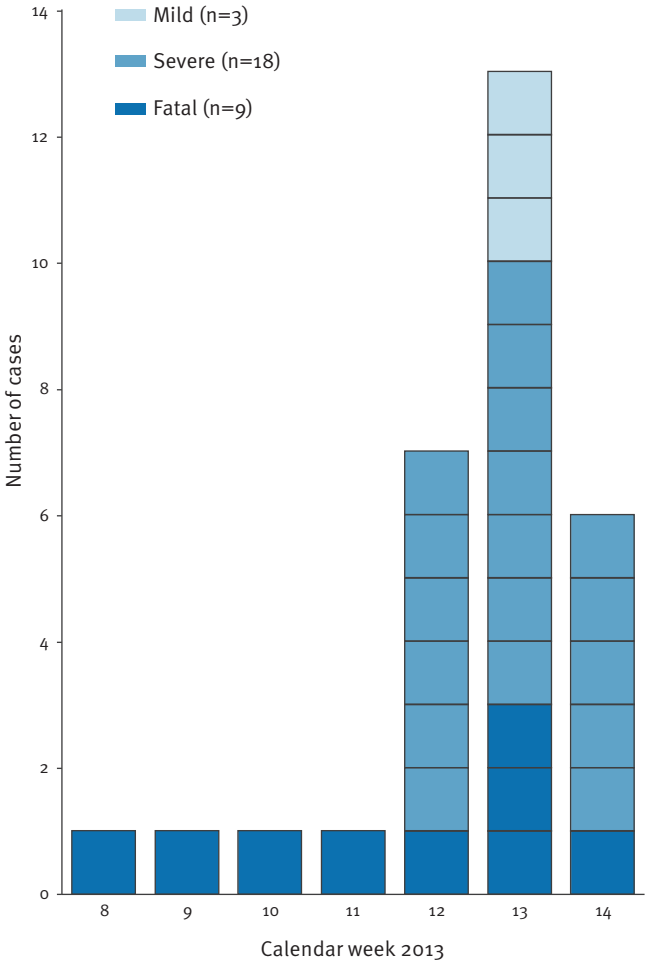
However, the tasks lying ahead, namely analysing, describing and especially controlling the virus cannot be underestimated. The extent of distribution of this A(H7N9) virus in domestic poultry in China and possibly other countries is unclear and surveillance and control of a low pathogenicity avian influenza virus in countries with complex mixes of informal and formal poultry sectors will be challenging. The markers of poultry die-offs seen with high pathogenicity avian influenza A viruses such as H5N1 and H7N7, will not signal the presence of the new A(H7N9) virus. In such situations, animal surveillance on the basis of sampling of live birds, including wild birds, such as done in Hong Kong and in European Union (EU) countries will be essential [14,15].

What are the possible implications of the current situation for Europe and European citizens and which actions should the EU take and which ones have been taken already? The European Centre for Disease Prevention and Control (ECDC) published its first risk assessment on 3 April and is providing updated assessments and short reports on the epidemiology as new information emerges [16]. Several guidance documents on prevention of infections, infection control and case management developed earlier for influenza A(H1N5) by ECDC, WHO and Member States are, with some modifications, applicable to the current situation [16-18]. Visitors to China and other countries where avian influenzas have caused severe human disease of late [9], should avoid visiting bird markets and follow basic hygienic measures. Persons returning from China who develop severe respiratory infection within 10 days should be evaluated and tested for the new virus to rule out such infection [17], though most likely another infection will be detected. Case management and infection control guidelines for A(H5N1) apply in the short term. This will include antiviral treatment given that the Chinese CDC promptly established that the A(H7N9)viruses are susceptible to neuraminidase inhibitors [4,5].

There is a standing procedure in place in Europe to send all non-subtypeable influenza A viruses isolated from humans promptly to the WHO Collaborating Center in London for further analysis. Notwithstanding this, ECDC, the WHO Regional Office for Europe, the WHO Influenza Collaborating Centre, the University of Bonn and the Community Network Reference Laboratories are working in together to make testing for A(H7N9) possible in all National Influenza Centres in Europe as soon as possible.

Figure 2

Laboratory-confirmed cases of human influenza A(H7N9) by week of symptom onset and severity as of 10 April, China March–April 2013 (n=30)



Three patients with unknown dates of symptom onset not included.
Source: European Centre for Disease Prevention and Control (ECDC).

Some candidate H7 and H9 vaccines viruses already exist under WHO's strain selection system for the eventuality of an emerging virus [19]. They may not be effective against the new influenza A(H7N9) virus and once the regulatory laboratories have obtained the novel virus, WHO and presumably EU authorities will now need to consider if they wish to proceed with the very early stages of vaccine development as has been done for the candidate H7 and H9 viruses.

Overall, how concerned Europe should be cannot yet be determined. The new virus is a reassortant virus based on an haemaglutinin antigen A(H7) to which

most humans will not have been exposed. Therefore, if human-to-human transmission starts, and that is only an 'if', population immunity cannot be presumed. It would have to be assessed now by determining age-specific sero-reactivity of human sera to this influenza A(H7N9) virus as a priority. Immunity, or lack of it, in the human population are key data required for assessing pandemic risk. As stated above, they needed to come from field investigations in China as well as seroepidemiological studies in Europe based on protocols developed precisely for such situations [20].

At this very moment it cannot be ruled out that there are some human-to-human transmissions causing mild or asymptomatic infections as happened in the Netherlands in 2003. It also remains unclear to what extent the predominance of severe disease may represent a bias because mainly people with severe disease are tested. Investigations of patients' contacts including serological studies, will clarify this point. Such investigations orchestrated by the Chinese CDC are underway.

There will be many other calls for research and it will be important and difficult to prioritise. Fortunately a framework exists for making decisions on priorities. The Influenza Risk Assessment Tool (IRAT) has been developed since 2011 for this purpose by the United States (US) Centers for Disease Control and Prevention with some international partners [21,22]. It looks at 10 parameters bundled into three families: properties of the virus, attributes of the population, ecology and epidemiology. It has already been deployed to inform US decisions on the A(H3N2)v vaccines. It does not predict pandemic risk or make decisions but it informs decisions. Though the IRAT is still being evaluated as a tool it will certainly indicate what should be some of the most important public health research priorities for A(H7N9).

It is also important that the sequence and virological analyses are considered in combination with the epidemiological findings. Despite the virological markers described in the recent report from the WHO Collaborating Centres [5] it should not be seen as inevitable on the longer term that this reassortant A(H7N9) will develop efficient human-to-human transmissibility or become established in Europe, though both should be kept in mind as possibilities. Neither has happened for the highly pathogenic influenza A(H5N1) virus in the decade and a half since its emergence in China in 1996 [23]. Despite multiple detections of the A(H5N1)virus in wild birds and some outbreaks in domestic poultry flocks in Europe, the high levels of biosafety in the EU have not permitted A(H5N1) viruses to become established in European domestic poultry. It is fortunate that the European Commission and the Member States have since 2007 established surveillance for low pathogenicity avian influenza in domestic and wild birds in Europe [14]. The recent events have underlined the importance of this system.

Acknowledgements

The authors acknowledge that some of these analyses have been possible using the virological and genetic molecular data provided in the publicly accessible GISAID database by the WHO Collaborating Centre for Influenza at the Chinese Center for Disease Control and Prevention.

References

1. Chinese Center for Disease Control and Prevention (CDC). Questions and Answers about human infection with A(H7N9) avian influenza virus. Beijing: CDC; 31 Mar 2013. Available from: http://www.chinacdc.cn/en/ne/201303/t20130331_79282.html
2. World Health Organization (WHO). Global Alert and Response (GAR). Disease Outbreak News. Human infection with influenza A(H7N9) virus in China. Geneva: WHO; 1 Apr 2013. Available from: http://www.who.int/csr/don/2013_04_01/en/index.html
3. World Health Organization (WHO). International Health Regulations 2005 second edition. Geneva: WHO; 2008. Available from: http://whqlibdoc.who.int/publications/2008/9789241580410_eng.pdf
4. Global Initiative on Sharing All Influenza Data (GISAID). GISAID EpiFlu Database. USA: GISAID Foundation. [Accessed 10 Apr 2013]. Available from: http://platform.gisaid.org/epi3/frontend#307300
5. World Health Organization Regional Office for Europe (WHO/Europe). Public health relevant virological features of Influenza A(H7N9) causing human infection in China. Copenhagen: WHO/Europe. 2013. Available from: http://www.euro.who.int/__data/assets/pdf_file/0008/186677/050413-H7N9-influenza-viruses-Virologic-features_update.pdf
6. World Organisation for Animal Health (OIE). Low pathogenic avian influenza (poultry), China (People's Rep. of). Paris: OIE. [Accessed 10 Apr 2013]. Available from: http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=13238
7. Food and Agriculture Organization of the United Nations (FAO). Strong biosecurity measures required in response to influenza A(H7N9) virus. Rome: FAO; 5 Apr 2013. Available from: http://www.fao.org/news/story/en/item/173655/icode/
8. European Centre for Disease Prevention and Control (ECDC). Epidemiological update of 11 April: novel influenza A virus A(H7N9) in China. Stockholm: ECDC; 11 Apr 2013. Available from: http://www.ecdc.europa.eu/en/press/news/Lists/News/ECDC_DispForm.aspx?List=32e43ee8-e230-4424-a783-8574212402 9a&ID=888&RootFolder=%2Fen%2Fpress%2Fnews%2FLists%2FNews
9. World Health Organization (WHO). Cumulative number of confirmed human cases for avian influenza A(H5N1) reported to WHO. Geneva: WHO; Mar 2013. Available from: http://www.who.int/influenza/human_animal_interface/H5N1_cumulative_table_archives/en/
10. Belser JA, Bridges CB, Katz JM, Tumpey TM. Past, present, and possible future human infection with influenza virus A subtype H7. Emerg Infect Dis. 2009;15(6):859-65. http://dx.doi.org/10.3201/eid1506.090072. PMid:19523282. PMCid:2727350.
11. Koopmans M, Wilbrink B, Conyn M, Natrop G, van der Nat H, Vennema H, et al. Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. Lancet. 2004;363(9409):587-93. http://dx.doi.org/10.1016/S0140-6736(04)15589-X
12. Du Ry van Beest Holle M, Meijer A, Koopmans M, de Jager CM. Human-to-human transmission of avian influenza A/H7N7, The Netherlands, 2003. Euro Surveill. 2005;10(12):pii=584. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=584. PMid:16371696.
13. Heymann DL, Mackenzie JS, Peiris M. SARS legacy: outbreak reporting is expected and respected. Lancet. 2013;381(9869):779-81. http://dx.doi.org/10.1016/S0140-6736(13)60185-3
14. Leung YH, Zhang LJ, Chow CK, Tsang CL, Ng CF, Wong CK, et al. Poultry drinking water used for avian influenza surveillance. Emerg Infect Dis. 2007;13(9):1380–2. http://dx.doi.org/10.3201/eid1309.070517. PMid:18252115. PMCid:2857302.
15. European Commission (EC) DG Health and Consumers. Animals. Avian influenza. Surveillance. Surveillance for Health Influenza. Brussels: EC. Available from: http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/eu_resp_surveillance_en.htm
16. European Centre for Disease Prevention and Control (ECDC). Rapid risk assessment. Severe respiratory disease associated with a novel influenza A virus, A(H7N9) – China. Stockholm:

ECDC; 3 Apr 2013. Available from: http://www.ecdc.europa.eu/en/publications/Publications/AH7N9-China-rapid-risk-assessment.pdf

17. European Centre for Disease Prevention and Control (ECDC). Technical report. Avian influenza portfolio. Collected risk assessments, technical guidance to public health authorities and advice to the general public Stockholm, June 2006. Stockholm: ECDC. Jun 2006. Available from: http://ecdc.europa.eu/en/publications/Publications/0606_TER_Avian_Influenza_Portafolio.pdf
18. World Health Organization (WHO). Influenza. Influenza at the Human-Animal Interface. Geneva: WHO. [Accessed 10 Apr 2013]. Available from: http://www.who.int/influenza/human_animal_interface/en/
19. World Health Organization (WHO). Influenza. WHO recommended candidate viruses for vaccine development and production for the northern hemisphere 2013-2014 influenza season. All available candidate vaccine viruses and potency reagents. Geneva: WHO. [Accessed 10 Apr 2013]. http://www.who.int/influenza/vaccines/virus/candidates_reagents/home/en/
20. Van Kerkhove MD, Broberg E, Engelhardt OG, Wood J, Nicoll A; The CONSISE steering committee. The consortium for the standardization of influenza seroepidemiology (CONSISE): a global partnership to standardize influenza seroepidemiology and develop influenza investigation protocols to inform public health policy. Influenza Other Respi Viruses. 2012. [Epub ahead of print].
21. Trock SC, Burke SA, Cox NJ. Development of an influenza virologic risk assessment tool. Avian Dis. 2012;56(4 Suppl):1058-61. http://dx.doi.org/10.1637/10204-041412-ResNote.1. PMid:23402136.
22. Centers for Disease Control and Prevention (CDC). Influenza Risk Assessment Tool (IRAT). Atlanta: CDC. Available from: http://www.cdc.gov/flu/pandemic-resources/tools/risk-assessment.htm
23. World Health Organization (WHO). H5N1 avian influenza: Timeline of major events. Geneva: WHO; 17 Dec 2012. Available from: http://www.who.int/influenza/H5N1_avian_influenza_update_20121217b.pdf

Genetic analysis of novel avian A(H7N9) influenza viruses isolated from patients in China, February to April 2013

T Kageyama[1,2], S Fujisaki[1,2], E Takashita[1], H Xu[1], S Yamada[3], Y Uchida[4], G Neumann[5], T Saito[4,6], Y Kawaoka[3,5,7,8], M Tashiro (mtashiro@nih.go.jp)[1]

1. Influenza Virus Research Center, National Institute of Infectious Diseases, Tokyo, Japan
2. These authors contributed equally to this work
3. Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Tokyo, Japan
4. Influenza and Prion Disease Research Center, National Institute of Animal Health, National Agriculture and Food Research Organization, Ibaraki, Japan
5. Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, United States
6. The United Graduate School of Veterinary Sciences, Gifu University, Gifu, Japan
7. ERATO Infection-Induced Host Responses Project, Japan Science and Technology Agency, Saitama, Japan
8. Department of Special Pathogens, International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo, Tokyo, Japan

Citation style for this article:
Kageyama T, Fujisaki S, Takashita E, Xu H, Yamada S, Uchida Y, Neumann G, Saito T, Kawaoka Y, Tashiro M. Genetic analysis of novel avian A(H7N9) influenza viruses isolated from patients in China, February to April 2013. Euro Surveill. 2013;18(15):pii=20453. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20453

Article submited on 08 April 2013 / published on 12 April 2013

Novel influenza viruses of the H7N9 subtype have infected 33 and killed nine people in China as of 10 April 2013. Their haemagglutinin (HA) and neuraminidase genes probably originated from Eurasian avian influenza viruses; the remaining genes are closely related to avian H9N2 influenza viruses. Several characteristic amino acid changes in HA and the PB2 RNA polymerase subunit probably facilitate binding to human-type receptors and efficient replication in mammals, respectively, highlighting the pandemic potential of the novel viruses.

Humans are rarely infected with avian influenza viruses, with the exception of highly pathogenic avian influenza A(H5N1) viruses, which have caused 634 infections and 371 deaths as of 12 March 2013 [1]. A few isolated cases of human infection with viruses of the H7N2, H7N3, and H7N5 subtypes have been reported, but none were fatal [2-11]. In 2003, in the Netherlands, 89 people were infected with an influenza virus of the H7N7 subtype that caused conjunctivitis and one fatality [5,7].

On 19 February 2013, an 87 year-old man in Shanghai developed a respiratory infection and died on 4 March, and on 27 February 2013, a 27 year-old pork seller in a Shanghai market became ill and died on 10 March. A 35 year-old woman in Chuzhou City in Anhui province (west of Shanghai), who had contact with poultry, became ill on 15 March 2013, and remains hospitalised in critical condition. There is no known epidemiological relationship among these three cases. A 38 year-old man in Hangzhou (Zhejiang province, south of Shanghai) became ill on 7 March 2013 and died on 27 March. All four cases presented with respiratory infections that progressed to severe pneumonia and breathing difficulties.

On 31 March 2013, the Chinese Centre for Disease Control and Prevention announced the isolation in embryonated eggs of avian influenza viruses of the H7N9 subtype (designated A/Shanghai/1/2013, A/Shanghai/2/2013, and A/Anhui/1/2013) from the first three cases. The sequences of the coding regions of all eight viral genes were deposited in the influenza sequence database of the Global Initiative on Sharing All Influenza Data (GISAID) on 31 March (Table 1). On 5 April 2013, the Hangzhou Center for Disease Control and Prevention deposited the haemagglutinin (HA), neuraminidase (NA), and matrix (M) gene sequences of A/Hongzhou/1/2013 virus (Table 1), which was isolated in cell culture from samples obtained from the 38 year-old man.

All four human influenza A(H7N9) viruses are similar at the nucleotide and amino acid levels, suggesting a common ancestor. The HA gene of the novel viruses belongs to the Eurasian lineage of avian influenza viruses and shares ca. 95% identity with the HA genes of low pathogenic avian influenza A(H7N3) viruses isolated in 2011 in Zhejiang province (south of Shanghai) (Figure 1, Table 2). The NA gene of the novel viruses is ca. 96% identical to the low pathogenic avian influenza A(H11N9) viruses isolated in 2010 in the Czech Republic (Figure 1, Table 2).

Table 1

Origin of influenza A(H7N9) isolates included in the phylogenetic analysis, China, February–April 2013 (n=7)

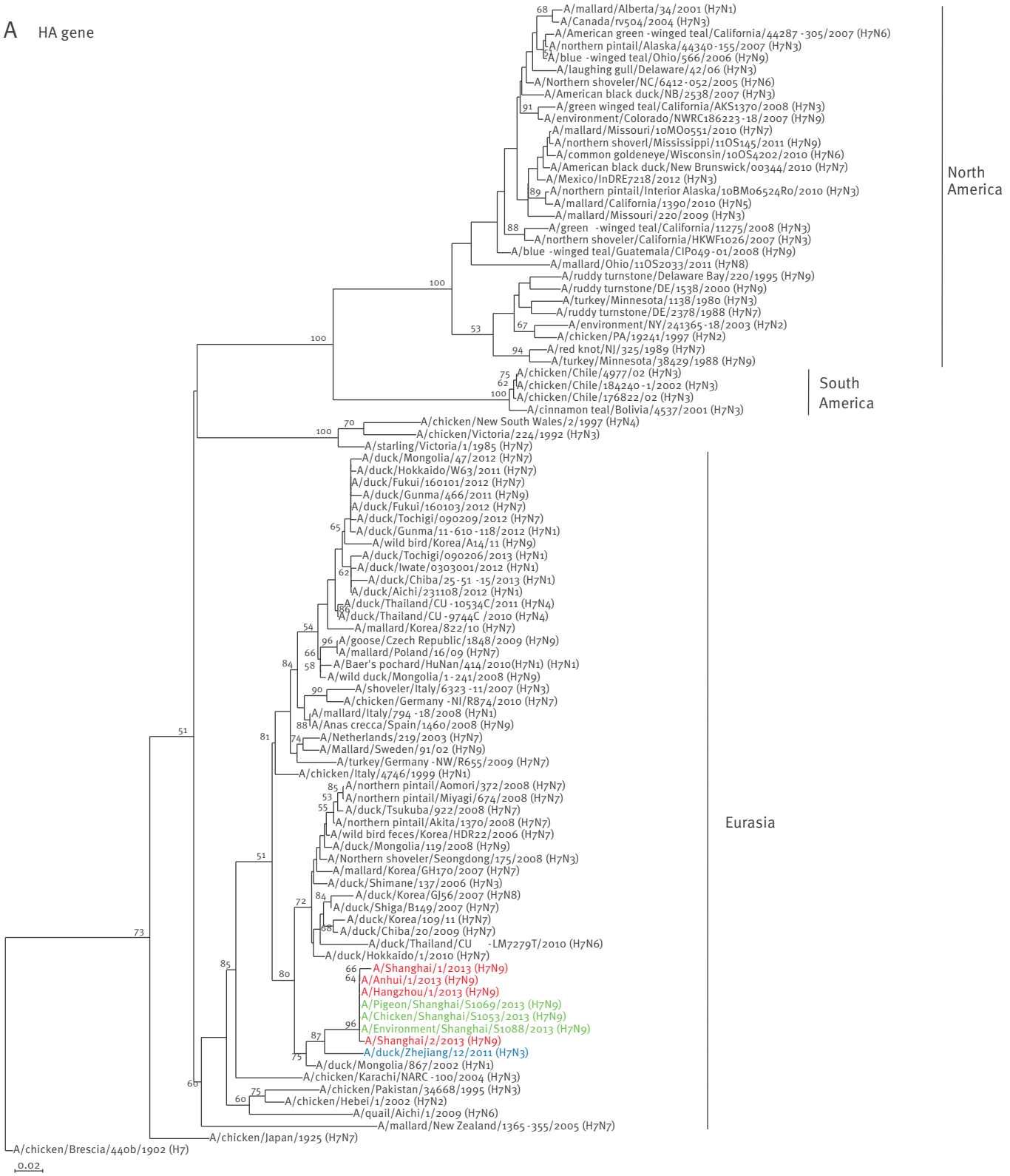
Segment ID	Segment	Isolate name	Collection date	Originating Laboratory	Submitting Laboratory	Submitter/ Authors
EPI439488	PB2	A/Shanghai/1/2013	2013	-	WHO Chinese National Influenza Center	Lei Yang
EPI439489	PB1					
EPI439490	PA					
EPI439486	HA					
EPI439491	NP					
EPI439487	NA					
EPI439493	M					
EPI439494	NS					
EPI439495	PB2	A/Shanghai/2/2013	2013	-		
EPI439501	PB1					
EPI439498	PA					
EPI439502	HA					
EPI439496	NP					
EPI439500	NA					
EPI439497	M					
EPI439499	NS					
EPI439504	PB2	A/Anhui/1/2013	2013	-		
EPI439508	PB1					
EPI439503	PA					
EPI439507	HA					
EPI439505	NP					
EPI439509	NA					
EPI439506	M					
EPI439510	NS					
EPI440095	HA	A/Hangzhou/1/2013	2013-03-24	Hangzhou Center for Disease Control and Prevention	Hangzhou Center for Disease Control and Prevention	Li,J; Pan,JC; Pu,XY; Yu,XF; Kou,Y; Zhou,YY
EPI440096	NA					
EPI440097	M					
EPI440682	PB2	A/Chicken/Shanghai /S1053/2013	2013-04-03	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Huihui Kong
EPI440683	PB1					
EPI440681	PA					
EPI440685	HA					
EPI440678	NP					
EPI440684	NA					
EPI440680	M					
EPI440679	NS					
EPI440690	PB2	A/Environment/ Shanghai /S1088/2013	2013-04-03			
EPI440691	PB1					
EPI440689	PA					
EPI440693	HA					
EPI440686	NP					
EPI440692	NA					
EPI440688	M					
EPI440687	NS					
EPI440698	PB2	A/Pigeon/Shanghai /S1069/2013	2013-04-02			
EPI440699	PB1					
EPI440697	PA					
EPI440701	HA					
EPI440694	NP					
EPI440700	NA					
EPI440696	M					
EPI440695	NS					

We gratefully acknowledge the authors and laboratories for originating and submitting these sequences to the EpiFlu database of the Global Initiative on Sharing All Influenza Data (GISAID); these sequences were the basis for the research presented here.

All submitters of data may be contacted directly via the GISAID website www.gisaid.org

Figure 1

Phylogenetic analysis of the haemagglutinin (A) and neuraminidase (B) genes of the novel influenza A(H7N9) viruses, China, February–April 2013 (n=7)



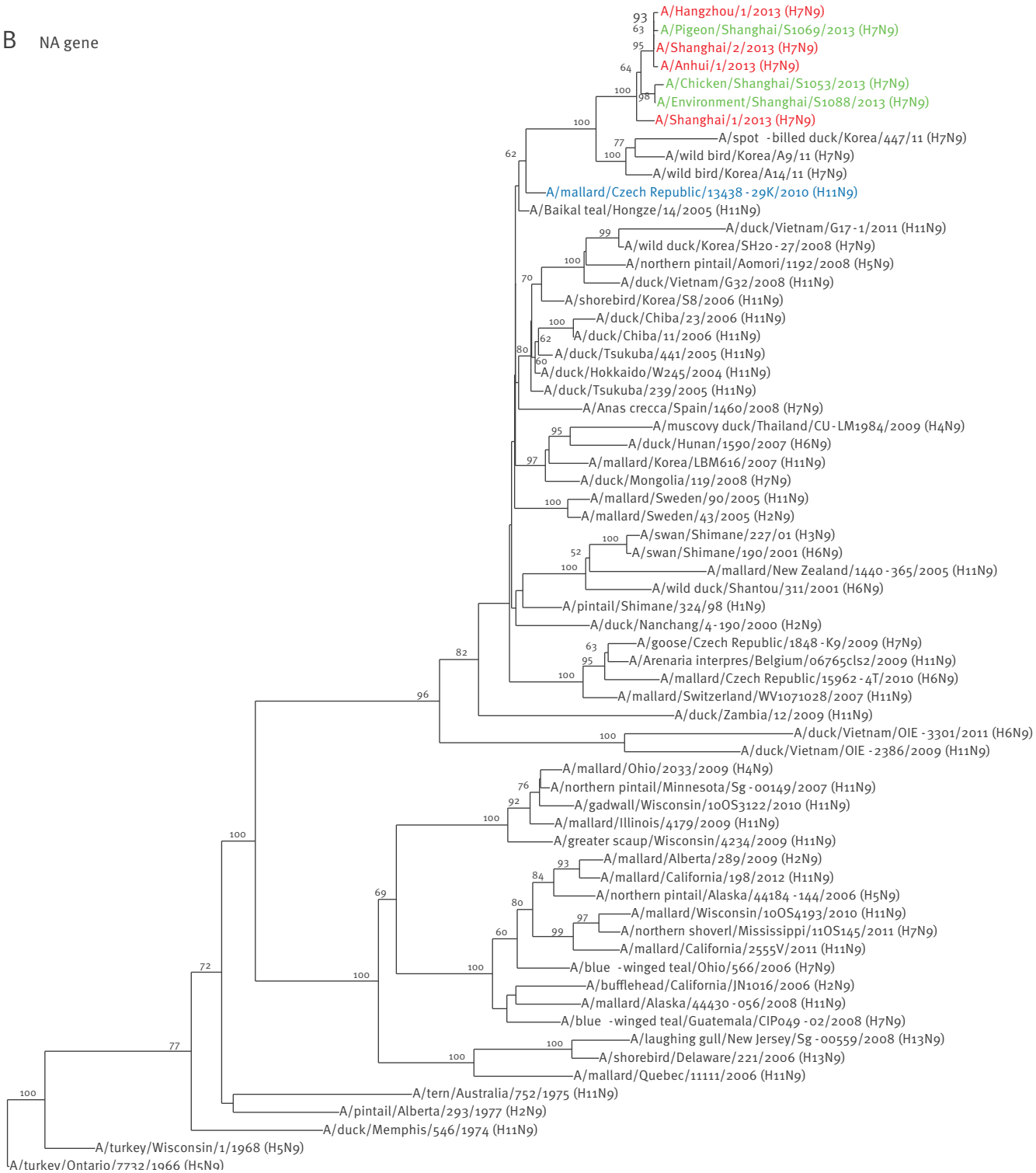
HA: haemagglutinin; NA: neuraminidase.

Multiple alignments were constructed by using the CLUSTAL W algorithm. Genetic distances were calculated by using the Kimura's 2-parameter method [26], and phylogenetic trees were constructed by using the neighbour-joining method with bootstrap analyses of 1,000 replicates in CLUSTAL W. Numbers next to nodes indicate bootstrap value percentages (>50%).

Novel human H7N9 viruses are shown in red; novel H7N9 viruses from birds and the environment are shown in green; viruses with the highest similarities to the novel viruses are shown in blue. The HA clade names, North America, South America, and Eurasia, are based on epidemiological studies of H7 viruses [27,28].

Figure 1

Phylogenetic analysis of the haemagglutinin (A) and neuraminidase (B) genes of the novel influenza A(H7N9) viruses, China, February–April 2013 (n=7)



HA: haemagglutinin; NA: neuraminidase.

Multiple alignments were constructed by using the CLUSTAL W algorithm. Genetic distances were calculated by using the Kimura's 2-parameter method [26], and phylogenetic trees were constructed by using the neighbour-joining method with bootstrap analyses of 1,000 replicates in CLUSTAL W. Numbers next to nodes indicate bootstrap value percentages (>50%).

Novel human H7N9 viruses are shown in red; novel H7N9 viruses from birds and the environment are shown in green; viruses with the highest similarities to the novel viruses are shown in blue. The HA clade names, North America, South America, and Eurasia, are based on epidemiological studies of H7 viruses [27,28].

Table 2

Nucleotide identity of novel influenza A(H7N9) virus genes and their closest relative, China, February–April 2013

Viral gene	Closest influenza virus relative	Nucleotide identity (%)
PB2	A/brambling/Beijing/16/2012(H9N2)	99
PB1	A/chicken/Jiangsu/Q3/2010(H9N2)	98
PA	A/brambling/Beijing/16/2012(H9N2)	99
HA	A/duck/Zhejiang/12/2011(H7N3)	95
NP	A/chicken/Zhejiang/611/2011(H9N2)	98
NA	A/mallard/Czech Republic/13438-29K/2010(H11N9)	96
M	A/chicken/Zhejiang/607/2011(H9N2)	98
NS	A/chicken/Dawang/1/2011(H9N2)	99

HA: haemagglutinin; M: matrix gene; NA: neuraminidase; NP: nucleoprotein; NS: non-structural gene; PA: RNA polymerase acidic subunit; PB1: RNA polymerase basic subunit 1; PB2: RNA polymerase basic subunit 2.

The sequences of the remaining viral genes are closely related (>97% identity) to avian influenza A(H9N2) viruses, which recently circulated in poultry in Shanghai, Zhejiang, Jiangsu, and neighbouring provinces of Shanghai (Table 2, Figure 2). These findings strongly suggest that the novel influenza A(H7N9) viruses are reassortants that acquired their H7 HA and N9 NA genes from avian influenza viruses, and their remaining genes from recent influenza A(H9N2) poultry viruses (Figure 1, Figure 3, Table 2).

At the nucleotide level, A/Shanghai/2/2013, A/Anhui/1/2013, and A/Hangzhou/1/2013 share more than 99% identity and differ by no more than three nucleotides per gene, even though they were isolated in different cities several hundred kilometres apart. On 7 April 2013, the Harbin Veterinary Research Institute deposited the full genome sequences of isolates from a pigeon (A/pigeon/Shanghai/S1069/2013), a chicken (A/chicken/Shanghai/S1053/2013), and an environmental sample (A/environment/Shanghai/S1088/2013) that were collected on 2 and 3 April from a Shanghai market (Table 1). All eight genes of these three isolates are similar to those of A/Shanghai/2/2013 and A/Anhui/1/2013 at the nucleotide level, except for the PB1 gene of A/pigeon/Shanghai/S1069/2013, which belongs to a different lineage than the PB1 of the other H7N9 isolates (Figures 1 and 2).

Interestingly, A/Shanghai/1/2013 and A/Shanghai/2/2013 differ by 52 nucleotides (for example, there are 13 nucleotide and nine amino acid differences in their HA sequences) even though these two cases were identified in the same city and at around the same time. These findings suggest that A/Shanghai/2/2013, A/Anhui/1/2013, A/Hangzhou/1/2013, as well as the viruses from the chicken and the environment, share a closely related source of infection, whereas A/Shanghai/1/2013 and A/pigeon/Shanghai/S1069/2013 are likely to have originated from other sources.

Highly pathogenic avian influenza viruses are characterised by a series of basic amino acids at the HA cleavage site that enable systemic virus spread. The HA cleavage sequence of the novel influenza A(H7N9) viruses possesses a single basic amino acid (EIPKGR*GL; *indicates the cleavage site), suggesting that these viruses are of low pathogenicity in avian species.

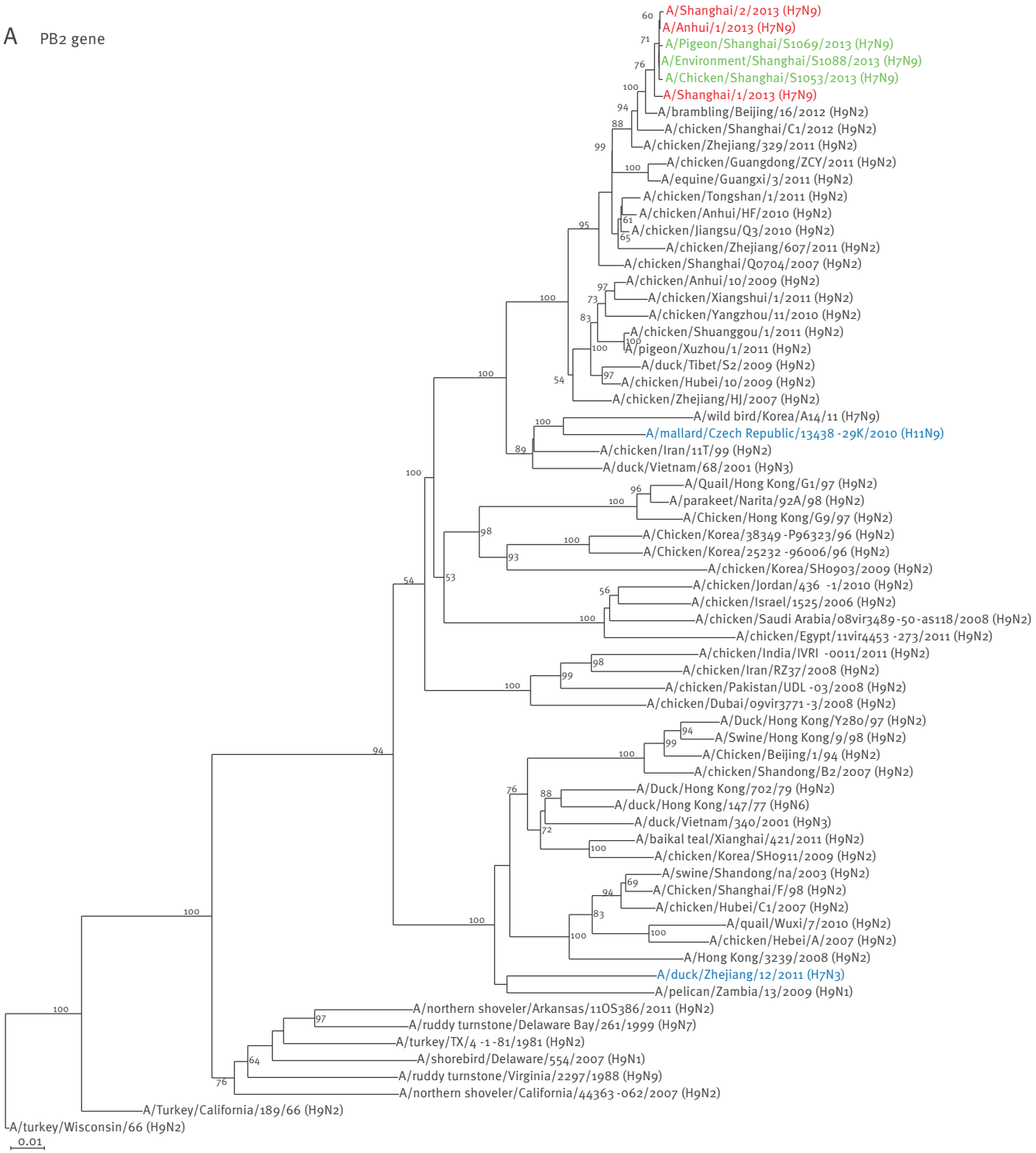
The amino acid sequence of the receptor-binding site (RBS) of HA determines preference for human- or avian-type receptors. At this site, A/Shanghai1/2013 encodes an A138S* mutation (H3 numbering; Figure 4, Table 3), whereas A/Shanghai/2/2013, A/Anhui/1/2013, the two avian isolates, and the virus from the environmental sample encode G186V and Q226L mutations; any of these three mutations could increase the binding of avian H5 and H7 viruses to human-type receptors [12-14]. The finding of mammalian-adapting mutations in the RBS of these novel viruses is cause for concern. The A/Hangzhou/1/2013 isolate encodes isoleucine at position 226, which is found in seasonal influenza A(H3N2) viruses.

In addition, all seven influenza A(H7N9) viruses possess a T160A substitution (H3 numbering; Table 3) in HA, which is found in recently circulating H7 viruses; this mutation leads to the loss of an *N*-glycosylation site at position 158 (H3 numbering; position 149 in H7 numbering), which results in increased virus binding to human-type receptors [15].

Lysine at position 627 of the polymerase PB2 protein is essential for the efficient replication of avian influenza viruses in mammals [16] and has been detected in highly pathogenic avian influenza A(H5N1) viruses and in the influenza A(H7N7) virus isolated from the fatal case in the Netherlands in 2003 [17]. PB2-627K is rare among avian H9N2 PB2 proteins (i.e. it has been found in only five of 827 isolates). In keeping with this finding, the avian and environmental influenza A(H7N9)

Figure 2

Phylogenetic analysis of the six remaining genes of the novel influenza A(H7N9) viruses, China, February–April 2013 (n=7)



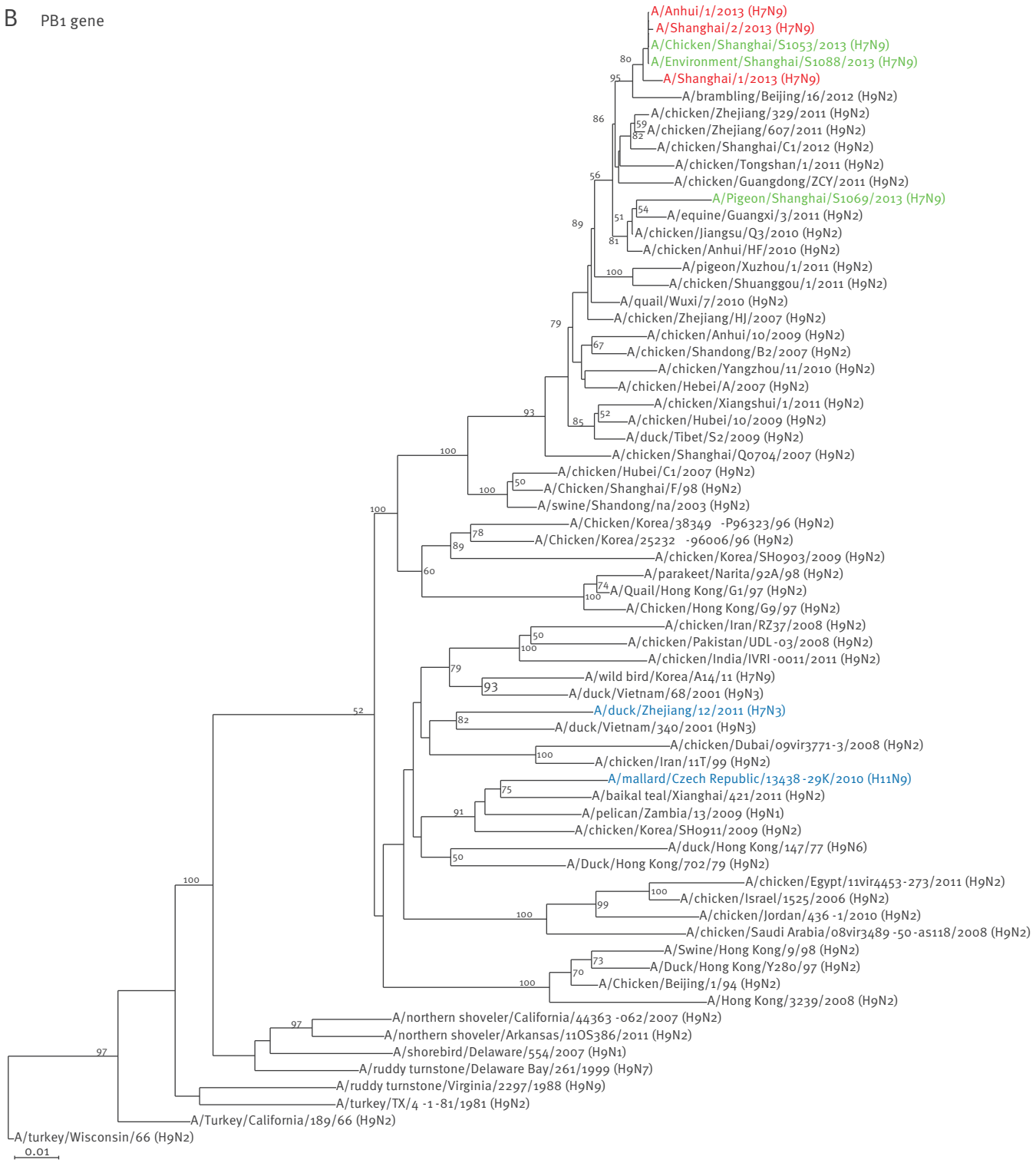
PB2: RNA polymerase basic subunit 2.

Multiple alignments were constructed by using the CLUSTAL W algorithm. Genetic distances were calculated by using the Kimura's 2-parameter method [26], and phylogenetic trees were constructed by using the neighbour-joining method with bootstrap analyses of 1,000 replicates in CLUSTAL W. Numbers next to nodes indicate bootstrap value percentages (>50%).

The novel human H7N9 viruses are shown in red; novel H7N9 viruses from birds and the environment are shown in green. Influenza viruses whose HA and NA genes are most closely related to the novel human H7N9 viruses are shown in blue.

Figure 2

Phylogenetic analysis of the six remaining genes of the novel influenza A(H7N9) viruses, China, February–April 2013 (n=7)



PB1: RNA polymerase basic subunit 1.

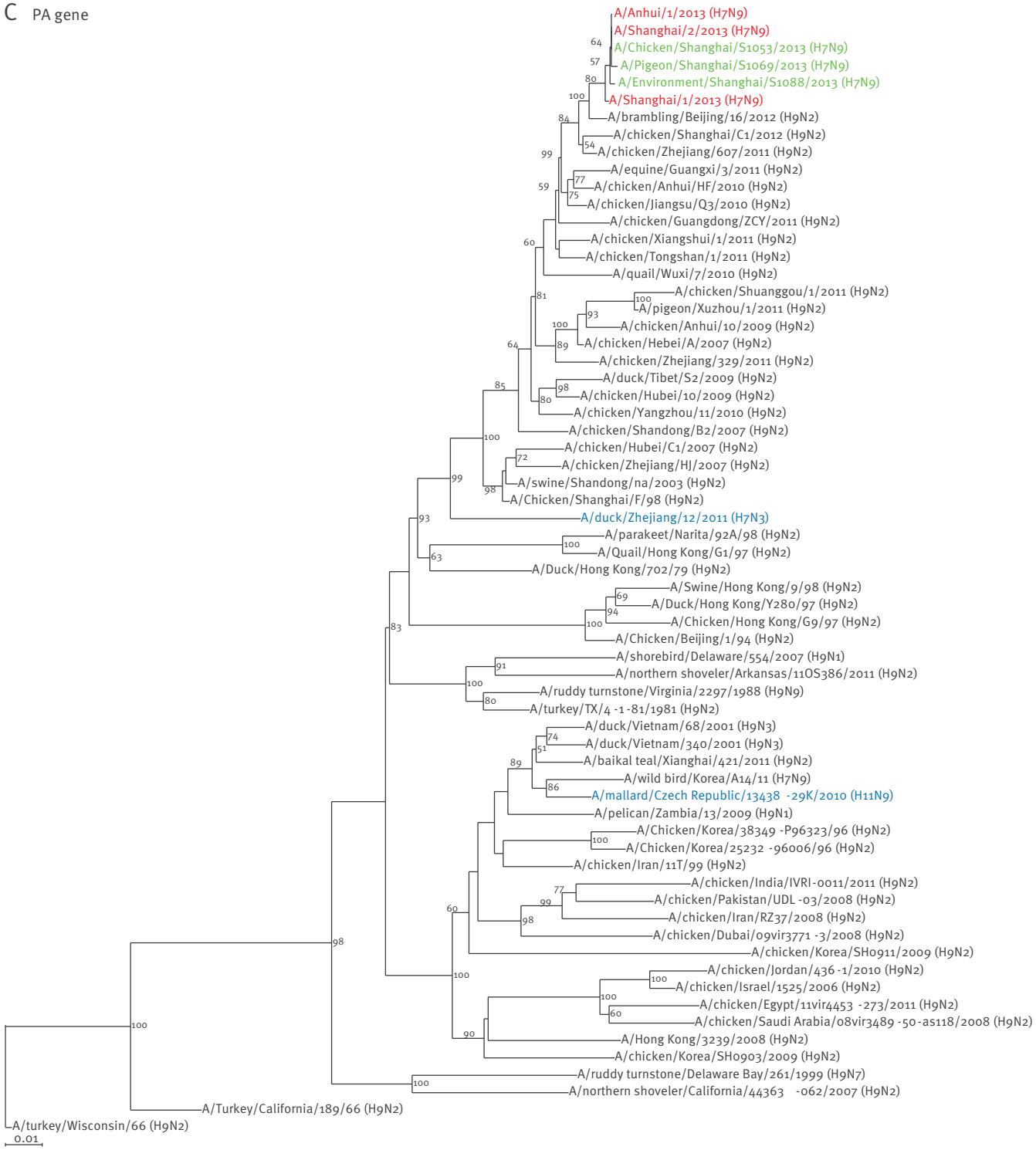
Multiple alignments were constructed by using the CLUSTAL W algorithm. Genetic distances were calculated by using the Kimura's 2-parameter method [26], and phylogenetic trees were constructed by using the neighbour-joining method with bootstrap analyses of 1,000 replicates in CLUSTAL W. Numbers next to nodes indicate bootstrap value percentages (>50%).

The novel human H7N9 viruses are shown in red; novel H7N9 viruses from birds and the environment are shown in green. Influenza viruses whose HA and NA genes are most closely related to the novel human H7N9 viruses are shown in blue.

Figure 2

Phylogenetic analysis of the six remaining genes of the novel influenza A(H7N9) viruses, China, February–April 2013 (n=7)

C PA gene



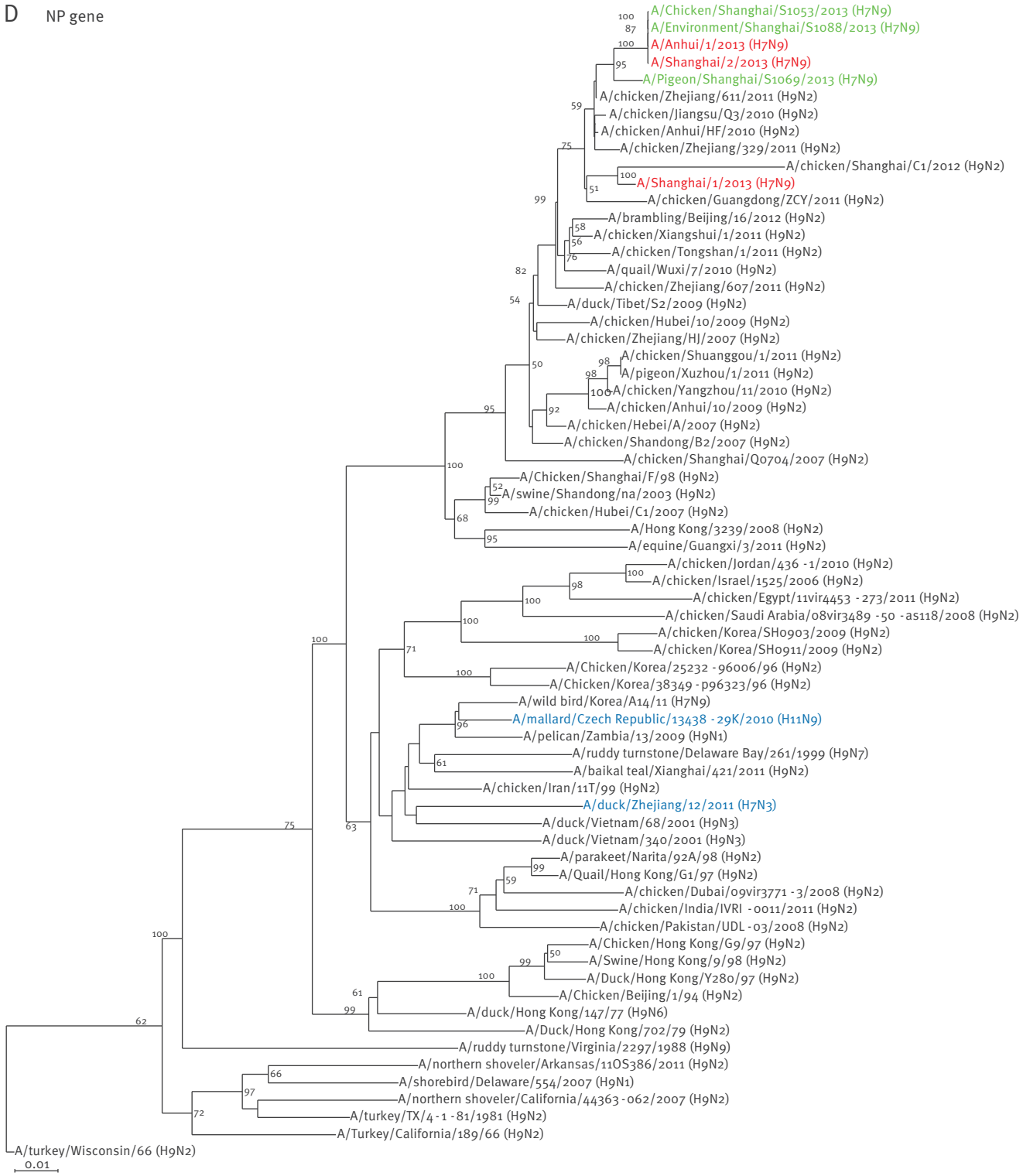
PA: RNA polymerase acidic subunit.

Multiple alignments were constructed by using the CLUSTAL W algorithm. Genetic distances were calculated by using the Kimura's 2-parameter method [26], and phylogenetic trees were constructed by using the neighbour-joining method with bootstrap analyses of 1,000 replicates in CLUSTAL W. Numbers next to nodes indicate bootstrap value percentages (>50%).

The novel human H7N9 viruses are shown in red; novel H7N9 viruses from birds and the environment are shown in green. Influenza viruses whose HA and NA genes are most closely related to the novel human H7N9 viruses are shown in blue.

Figure 2

Phylogenetic analysis of the six remaining genes of the novel influenza A(H7N9) viruses, China, February–April 2013 (n=7)



NP: nucleoprotein.

Multiple alignments were constructed by using the CLUSTAL W algorithm. Genetic distances were calculated by using the Kimura's 2-parameter method [26], and phylogenetic trees were constructed by using the neighbour-joining method with bootstrap analyses of 1,000 replicates in CLUSTAL W. Numbers next to nodes indicate bootstrap value percentages (>50%).

The novel human H7N9 viruses are shown in red; novel H7N9 viruses from birds and the environment are shown in green. Influenza viruses whose HA and NA genes are most closely related to the novel human H7N9 viruses are shown in blue.

Figure 2

Phylogenetic analysis of the six remaining genes of the novel influenza A(H7N9) viruses, China, February–April 2013 (n=7)

M: matrix gene.

Multiple alignments were constructed by using the CLUSTAL W algorithm. Genetic distances were calculated by using the Kimura's 2-parameter method [26], and phylogenetic trees were constructed by using the neighbour-joining method with bootstrap analyses of 1,000 replicates in CLUSTAL W. Numbers next to nodes indicate bootstrap value percentages (>50%).

The novel human H7N9 viruses are shown in red; novel H7N9 viruses from birds and the environment are shown in green. Influenza viruses whose HA and NA genes are most closely related to the novel human H7N9 viruses are shown in blue.

Figure 2

Phylogenetic analysis of the six remaining genes of the novel influenza A(H7N9) viruses, China, February–April 2013 (n=7)

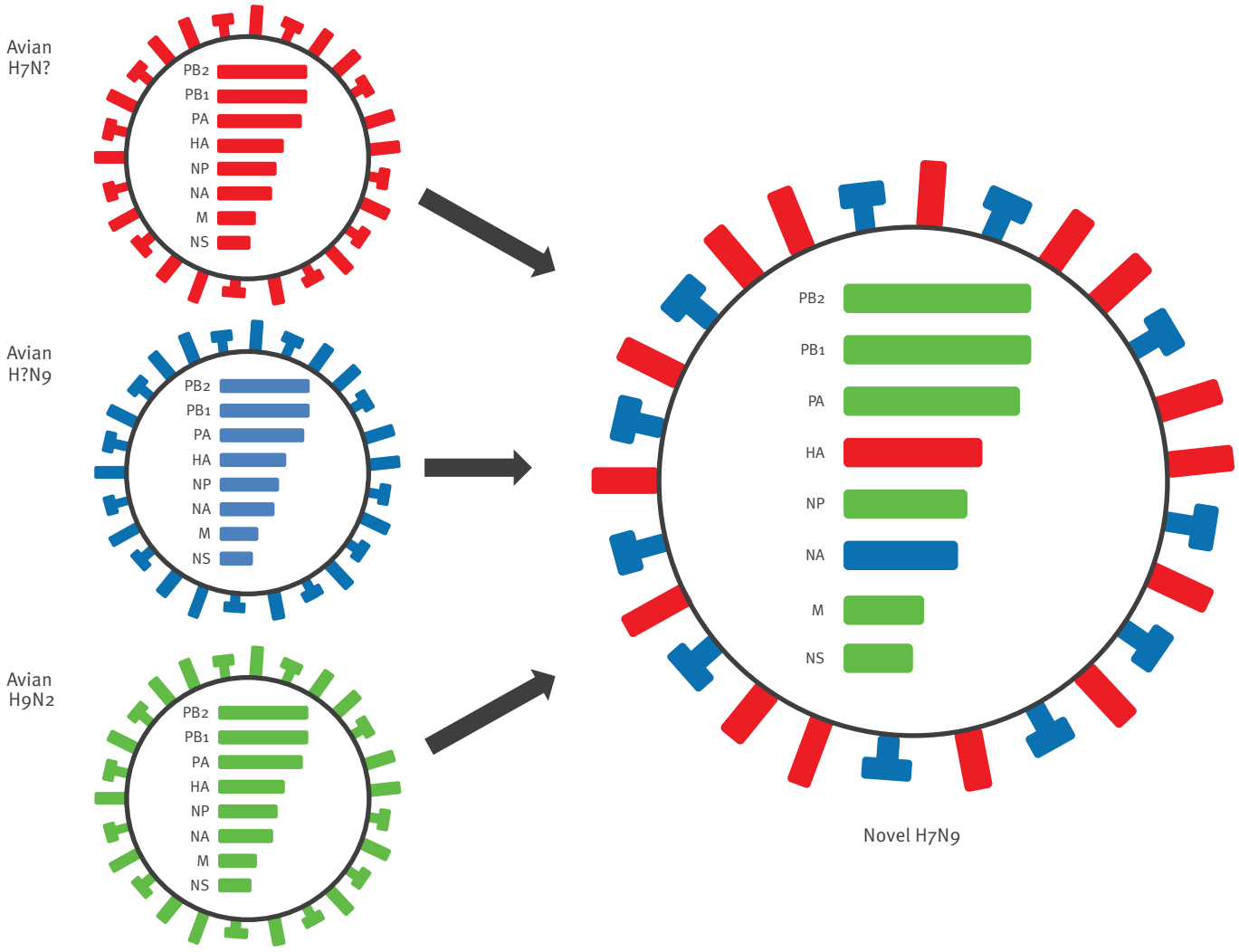
NS: non-structural gene.

Multiple alignments were constructed by using the CLUSTAL W algorithm. Genetic distances were calculated by using the Kimura's 2-parameter method [26], and phylogenetic trees were constructed by using the neighbour-joining method with bootstrap analyses of 1,000 replicates in CLUSTAL W. Numbers next to nodes indicate bootstrap value percentages (>50%).

The novel human H7N9 viruses are shown in red; novel H7N9 viruses from birds and the environment are shown in green. Influenza viruses whose HA and NA genes are most closely related to the novel human H7N9 viruses are shown in blue.

Figure 3

Schematic diagram of novel influenza A(H7N9) virus generation



HA: haemagglutinin; NA: neuraminidase.

The novel influenza A(H7N9) viruses are likely to have acquired their HA gene from an avian H7 virus of unknown NA subtype, their NA gene from an avian N9 virus of unknown HA subtype, and their remaining six viral segments from avian H9N2 viruses circulating in poultry.

viruses analysed here encode PB2-627E. By contrast, all four human H7N9 viruses analysed here encode PB2-627K (Table 3).

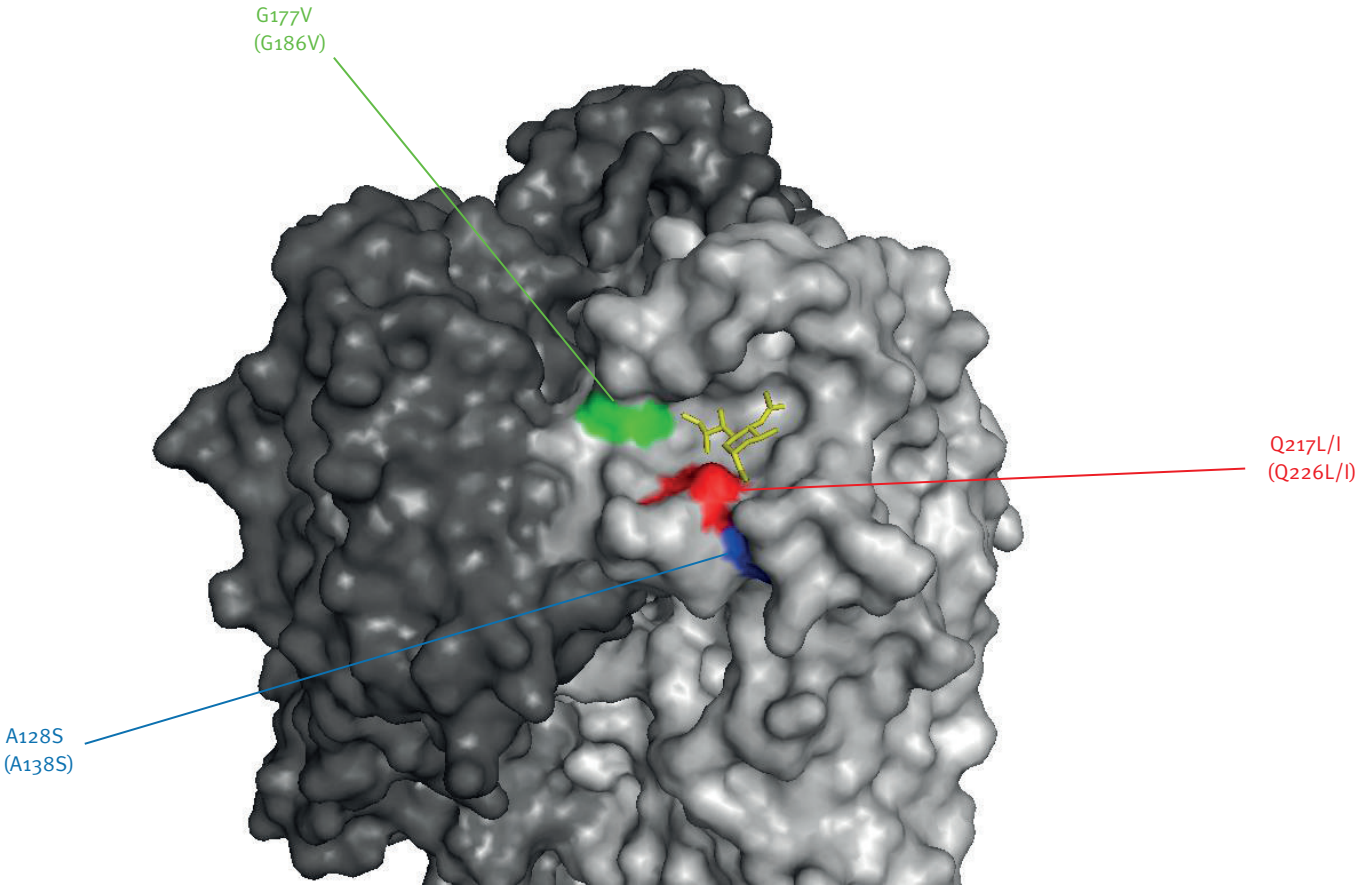
Antiviral compounds are the first line of defense against novel influenza viruses until vaccines become available. All seven novel influenza A(H7N9) viruses sequenced to date encode the S31N substitution in the viral ion channel M2 (encoded by the M segment) (Table 3), which confers resistance to ion channel inhibitors [18,19]. Based on the sequences of their NA proteins, all H7N9 viruses analysed here, with the exception of A/Shanghai/1/2013, should be sensitive to neuraminidase inhibitors (Table 3). However, the R294K mutation in the NA protein of A/Shanghai/1/2013 is known to confer resistance to NA inhibitors in N2 and N9 subtype viruses [20], and is therefore of great concern.

All H7N9 viruses encode a deletion at positions 69–73 of the NA stalk region (Table 3), which is reported to occur upon virus adaptation to terrestrial birds. This finding suggests that the novel H7N9 viruses (or their ancestor) may have circulated in terrestrial birds before infecting humans. Moreover, this deletion is associated with increased virulence in mammals [21].

The influenza A virus PB1-F2 protein (encoded by the PB1 segment) is also associated with virulence. The available sequences indicate that the H7N9 PB1 genes of all of the human viruses encode a full-length PB1-F2 of 90 amino acids, but lack the N66S mutation that is

Figure 4

Amino acid changes in the three novel influenza A(H7N9) viruses that may affect their receptor-binding properties, China, February–April 2013 (n=7)



Shown is the three-dimensional structure of three monomers (light and dark gray) of the influenza A(H7N7) virus (A/Netherlands/219/2003) haemagglutinin (accession code 4DJ8). Also shown is the part of 6'-sialyl-N-acetyllactosamine (a sialyloligosaccharide) to which human viruses bind preferentially (yellow). Indicated are amino acid changes in the H7N9 virus haemagglutinin protein at positions known to increase binding to human-type receptors.

associated with the increased pathogenicity of the 1918 pandemic virus and the highly pathogenic avian influenza A(H5N1) viruses [22]. Interestingly, the pigeon isolate encodes a truncated PB1-F2 of only 25 amino acids; the significance of this truncation is unknown.

The NS1 protein (encoded by the NS segment) is an interferon antagonist with several functions in the viral life cycle. All available H7N9 NS1 sequences lack the C-terminal PDZ domain-binding motif; the lack of the PDZ domain-binding motif may attenuate these viruses in mammals [23].

Other amino acids in the NS1 and matrix (M1; encoded by the M segment) proteins of the novel viruses are also associated with increased virulence (Table 3) [24.25]. However, these amino acids are found in many avian influenza viruses, and therefore, their significance for the biological properties of the novel influenza A(H7N9) viruses is currently unclear.

In conclusion, we here present a biological evaluation of the sequences of the avian influenza A(H7N9) viruses that caused fatal human infections in China. These viruses possess several characteristic features of mammalian influenza viruses, which are likely to contribute to their ability to infect humans and raise concerns regarding their pandemic potential.

*Authors' correction:

The mutation A138S was erroneously written as S138A in the original publication. This mistake was corrected on 13 April 2013

Table 3

Selected characteristic amino acids of the three novel influenza A(H7N9) viruses, China, February–April 2013 (n=7)

Viral protein	Amino acid position	Shanghai/1/2013	Shanghai/2/2013	Anhui/1/2013	Hangzhou/1/2013	Chicken/Shanghai/S1053/2013	Environment/Shanghai/S1088/2013	Pigeon/Shanghai/S1069/2013	Human influenza viruses	Avian influenza viruses	Comments	Reference(s)
PB2	627	**K**	**K**	**K**	Nd	E	E	E	K	E	E627K: Mammalian host adaptation	16
HA	128/138[a]	**S**	A	A	A	A	A	A	A	A[b]	S138A: Increased virus binding to human-type receptors	13
	151/160[a]	**A**	**A**	**A**	**A**	**A**	**A**	**A**	K	**A**[b]	T160A: Loss of N-glycosylation and increased virus binding to human-type receptors	15
	177/186[a]	G	**V**	**V**	**V**	**V**	**V**	**V**	G	G[b]	G186V: Increased virus binding to human-type receptors	14
	217/226[a]	Q	**L**	**L**	**I**	**L**	**L**	**L**	I	Q[b]	Q226L: Increased virus binding to human-type receptors	12
NA	69–73[c]	**Deletion**	**Deletion**	**Deletion**	**Deletion**	**Deletion**	**Deletion**	**Deletion**	No deletion	No deletion	Deletion of amino acids 69–73: Increased virulence in mice	21
	289/294/292[d]	**K**	R	R	R	R	R	R	R	R	R294K: Reduced susceptibility to oseltamivir and zanamivir	20
M1	30	D	D	D	D	D	D	D	D/(S)	D	N30D: Increased virulence in mice (most influenza A viruses encode 30D)	24
	215	A	A	A	A	A	A	A	A	A	T215A: Increased virulence in mice (most avian influenza A viruses encode 215A)	24
M2	31	**N**	**N**	**N**	**N**	**N**	**N**	**N**	S/N	S/(N)	S31N: Reduced susceptibility to amantadine and rimantadine	18,19
NS1	42	S	S	S	Nd	S	S	S	S	S/A	P42S: Increased virulence in mice (most avian influenza A viruses encode 42S)	25
	218–230	**Deletion**	**Deletion**	**Deletion**	Nd	**Deletion**	**Deletion**	**Deletion**	No deletion[e]	No deletion/Deletion	Lack of PDZ domain binding motif: Decreased virulence in mice	23

Substitutions of particular concern are shown in bold.

Nd: not determined.

[a] H7/H3 numbering.
[b] H7 virus.
[c] N9 numbering.
[d] H7N9/avian N9/N2 numbering.
[e] Influenza A(H1N1)pdm09 viruses from the 2009 influenza pandemic have the deletion.

Acknowledgements

We are grateful to Dr. Shu Yuelong, Chinese National Influenza Center, Chinese Center for Disease Control and Prevention, Beijing, China, for his rapid publication of the entire gene sequence data of A(H7N9) viruses isolated from human cases in China, and also for his information sharing and advice to this study. We also thank Susan Watson for scientific editing. This work was supported by Grants-in-Aid for Pandemic Influenza Research (TK, SF, HX, and MT) and Grant-in-Aid for Specially Promoted Research (MT) from the Ministry of Health, Labour and Welfare, Japan, by the NIAID-funded Center for Research on Influenza Pathogenesis (CRIP, HHSN266200700010C)(YK), by a Grant-in-Aid for Specially Promoted Research, by the Japan Initiative for Global Research Network on Infectious Diseases from the Ministry of Education, Culture, Sports, Science, and Technology, Japan (YK), and by ERATO, Japan (YK).

Authors contributions

Designed the analyses: TK, SF, ET, SY, GN, YK, MT. Analysed and interpreted data: TK, SF, ET, HX, SY, YU, GN, YK, MT. Drafted the article: TK, SF. Revised the article: ET, GN, TS, YK, MT.

Conflict of interest

None declared.

This manuscript was accepted on 11 April 2013 and published online on 12 April 2013

References

1. World Health Organization (WHO)/Global Influenza Programme. Cumulative number of confirmed human cases for avian influenza A(H5N1) reported to WHO, 2003-2013. Geneva: WHO: 12 Mar 2013. Available from: http://www.who.int/influenza/human_animal_interface/EN_GIP_20130312CumulativeNumberH5N1cases.pdf
2. Campbell CH, Webster RG, Breese SS Jr. Fowl plague virus from man. J Infect Dis. 1970;122(6):513-6. http://dx.doi.org/10.1093/infdis/122.6.513. PMid:5489075.
3. Centers for Disease Control and Prevention (CDC). Update: influenza activity-United States and worldwide, 2003-04 season, and composition of the 2004-05 influenza vaccine. MMWR Morb Mortal Wkly Rep. 2004;53(25):547-52. PMid:15229411.
4. Editorial team. Avian influenza A/(H7N2) outbreak in the United Kingdom. Euro Surveill. 2007;12(22): :pii=3206. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3206
5. Fouchier RA, Schneeberger PM, Rozendaal FW, Broekman JM, Kemink SA, Munster V, et al. Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. Proc Natl Acad Sci U S A. 2004;101(5):1356-61. http://dx.doi.org/10.1073/pnas.0308352100. PMid:14745020 PMCid:337057.
6. Hirst M, Astell CR, Griffith M, Coughlin SM, Moksa M, Zeng T, et al. Novel avian influenza H7N3 strain outbreak, British Columbia. Emerg Infect Dis. 2004;10(12):2192-5. http://dx.doi.org/10.3201/eid1012.040743. PMid:15663859. PMCid:3323367.
7. Koopmans M, Wilbrink B, Conyn M, Natrop G, van der Nat H, Vennema H, et al. Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. Lancet. 2004;363(9409):587-93. http://dx.doi.org/10.1016/S0140-6736(04)15589-X.
8. Kurtz J, Manvell RJ, Banks J. Avian influenza virus isolated from a woman with conjunctivitis. Lancet. 1996;348(9031):901-2. http://dx.doi.org/10.1016/S0140-6736(05)64783-6.
9. Nguyen-Van-Tam JS, Nair P, Acheson P, Baker A, Barker M, Bracebridge S, et al. Outbreak of low pathogenicity H7N3 avian influenza in UK, including associated case of human conjunctivitis. Euro Surveill. 2006;11(18): pii=2952. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2952. PMid:16816456.
10. Taylor HR, Turner AJ. A case report of fowl plague keratoconjunctivitis. Br J Ophthalmol. 1977;61(2):86-8. http://dx.doi.org/10.1136/bjo.61.2.86.
11. Tweed SA, Skowronski DM, David ST, Larder A, Petric M, Lees W, et al. Human Illness from Avian Influenza H7N3, British Columbia. Emerg Infect Dis. 2004;10(12):2196-9. http://dx.doi.org/10.3201/eid1012.040961. PMid:15663860 PMCid:3323407.
12. Srinivasan K, Raman R, Jayaraman A, Viswanathan K, Sasisekharan R. Quantitative description of glycan-receptor binding of influenza A virus H7 hemagglutinin. PLoS One. 2013;8(2):e49597. http://dx.doi.org/10.1371/journal.pone.0049597. PMid:23437033 PMCid:3577880.
13. Nidom CA, Takano R, Yamada S, Sakai-Tagawa Y, Daulay S, Aswadi D, et al. Influenza A(H5N1) viruses from pigs, Indonesia. Emerg Infect Dis. 2010;16(10):1515-23. http://dx.doi.org/10.3201/eid1610.100508. PMid:20875275. PMCid:3294999.
14. Yang H, Chen LM, Carney PJ, Donis RO, Stevens J. Structures of receptor complexes of a North American H7N2 influenza hemagglutinin with a loop deletion in the receptor binding site. PLoS Pathog. 2010;6(9):e1001081. http://dx.doi.org/10.1371/journal.ppat.1001081 PMid:20824086 PMCid:2932715.
15. Wang W, Lu B, Zhou H, Suguitan AL Jr, Cheng X, Subbarao K, et al. Glycosylation at 158N of the hemagglutinin protein and receptor binding specificity synergistically affect the antigenicity and immunogenicity of a live attenuated H5N1 A/Vietnam/1203/2004 vaccine virus in ferrets. J Virol. 2010;84(13):6570-7. http://dx.doi.org/10.1128/JVI.00221-10. PMid:20427525 PMCid:2903256.
16. Hatta M, Gao P, Halfmann P, Kawaoka Y. Molecular basis for high virulence of Hong Kong H5N1 influenza A viruses. Science. 2001;293(5536):1840-2. http://dx.doi.org/10.1126/science.1062882. PMid:11546875.
17. Munster VJ, de Wit E, van Riel D, Beyer WE, Rimmelzwaan GF, Osterhaus AD, et al. The molecular basis of the pathogenicity of the Dutch highly pathogenic human influenza A H7N7 viruses. J Infect Dis. 2007;196(2):258-65. http://dx.doi.org/10.1086/518792. PMid:17570113.
18. Hay AJ, Wolstenholme AJ, Skehel JJ, Smith MH. The molecular basis of the specific anti-influenza action of amantadine. EMBO J. 1985;4(11):3021-4. PMid:4065098 PMCid:554613.
19. Pinto LH, Holsinger LJ, Lamb RA. Influenza virus M2 protein has ion channel activity. Cell. 1992;69(3):517-28. http://dx.doi.org/10.1016/0092-8674(92)90452-I.
20. McKimm-Breschkin JL, Sahasrabudhe A, Blick TJ, McDonald M, Colman PM, Hart GJ, et al. Mutations in a conserved residue in the influenza virus neuraminidase active site decreases sensitivity to Neu5Ac2en-derived inhibitors. J Virol. 1998;72(3):2456-62. PMid:9499107. PMCid:109546.
21. Matsuoka Y, Swayne DE, Thomas C, Rameix-Welti MA, Naffakh N, Warnes C, et al. Neuraminidase stalk length and additional glycosylation of the hemagglutinin influence the virulence of influenza H5N1 viruses for mice. J Virol. 2009;83(9):4704-8. http://dx.doi.org/10.1128/JVI.01987-08. PMid:19225004 PMCid:2668507.
22. Conenello GM, Zamarin D, Perrone LA, Tumpey T, Palese P. A single mutation in the PB1-F2 of H5N1 (HK/97) and 1918 influenza A viruses contributes to increased virulence. PLoS Pathog. 2007;3(10):1414-21. http://dx.doi.org/10.1371/journal.ppat.0030141. PMid:17922571. PMCid:2000966.
23. Jackson D, Hossain MJ, Hickman D, Perez DR, Lamb RA. A new influenza virus virulence determinant: the NS1 protein four C-terminal residues modulate pathogenicity. Proc Natl Acad Sci U S A. 2008;105(11):4381-6. http://dx.doi.org/10.1073/pnas.0800482105. PMid:18334632 PMCid:2393797.
24. Fan S, Deng G, Song J, Tian G, Suo Y, Jiang Y, et al. Two amino acid residues in the matrix protein M1 contribute to the virulence difference of H5N1 avian influenza viruses in mice. Virology. 2009;384(1):28-32. http://dx.doi.org/10.1016/j.virol.2008.11.044. PMid:19117585.
25. Jiao P, Tian G, Li Y, Deng G, Jiang Y, Liu C, et al. A single-amino-acid substitution in the NS1 protein changes the pathogenicity of H5N1 avian influenza viruses in mice. J Virol. 2008;82(3):1146–54. http://dx.doi.org/10.1128/JVI.01698-07. PMid:18032512 PMCid:2224464
26. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 1980;16(2):111-20. http://dx.doi.org/10.1007/BF01731581. PMid:7463489.
27. González-Reiche AS, Morales-Betoulle ME, Alvarez D, Betoulle JL, Müller ML, Sosa SM, et al. Influenza A viruses from wild birds in Guatemala belong to the North American lineage. PLoS One. 2012;7(3):e32873. http://dx.doi.org/10.1371/journal.pone.0032873. PMid:22427902 PMCid:3302778.
28. Kim HR, Park CK, Lee YJ, Oem JK, Kang HM, Choi JG, et al. Low pathogenic H7 subtype avian influenza viruses isolated from domestic ducks in South Korea and the close association with isolates of wild birds. J Gen Virol. 2012;93(Pt 6):1278-87. http://dx.doi.org/10.1099/vir.0.041269-0. PMid:22422062.

First case of *Echinococcus vogeli* infection imported to the Netherlands, January 2013

C Stijnis (c.stijnis@amc.uva.nl)[1], A Bart[2], L Brosens[3], T Van Gool[2], M Grobusch[1], T van Gulik[4], J Roelfsema[5], P van Thiel[1]
1. Center for Tropical and Travel Medicine, Academic Medical Center, Amsterdam, the Netherlands
2. Department of Medical Microbiology, Section Parasitology, Academic Medical Center, Amsterdam, the Netherlands
3. Department of Pathology, Academic Medical Center, Amsterdam, the Netherlands
4. Department of Surgery, Academic Medical Center, Amsterdam, the Netherlands
5. National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands

Citation style for this article:
Stijnis C, Bart A, Brosens L, Van Gool T, Grobusch M, van Gulik T, Roelfsema J, van Thiel P. First case of Echinococcus vogeli infection imported to the Netherlands, January 2013. Euro Surveill. 2013;18(15):pii=20448. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20448

Article submitted on 28 March 2013 / published on 11 April 2013

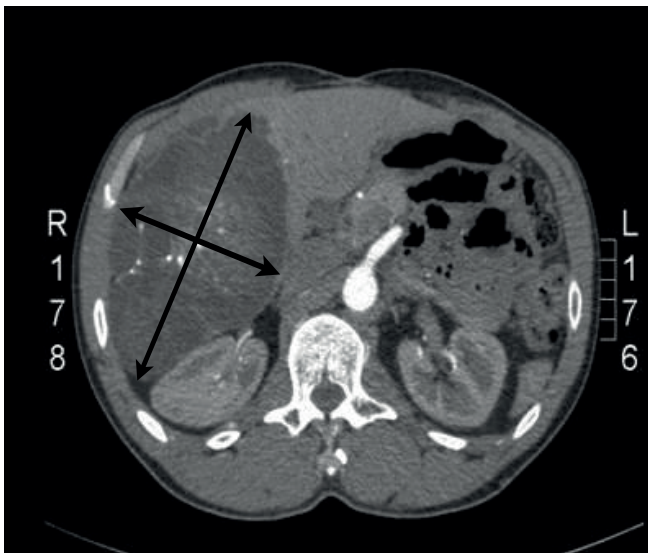
In January 2013 in the Netherlands, a man in his 50s from Suriname underwent hemihepatectomy because of a cystic liver mass, assumed to be a cystadenoma. Pathology revealed an echinococcal infection. PCR analysis of cyst material identified Echinococcus vogeli, causing polycystic hydatid disease. This echinococcus species is rarely diagnosed outside South America. The patient received adequate treatment, but this case emphasises the importance of awareness of this infection when treating patients with cystic tumours from endemic areas.

Case description

In October 2012, a man in his 50s of Surinamese descent noticed a painless mass in his right upper abdomen and was referred to the surgical department of the Academic Medical Center in Amsterdam, the Netherlands. Until 1984, he had been living in Suriname, where his work in hydrography entailed exploring all rivers in this country, frequently under primitive conditions. His medical history was otherwise unremarkable. Physical examination confirmed a palpable mass in the right abdomen. Routine haematology laboratory testing was normal, except for a slightly elevated percentage of eosinophils in the white blood cell differential (0.65%, no absolute count was performed). Liver biochemistry tests and alpha1-fetoprotein were not elevated. Imaging (abdominal computed tomography scan, Figure 1) showed a solitary, large multilocular cyst in the right side of the liver with diffuse calcifications.

Figure 1

Computed tomography scan of the abdomen of a patient with *Echinococcus vogeli* infection, the Netherlands, January 2013



The arrowed lines indicate the extent of the cyst.

Figure 2

Perioperative photograph of right hemihepatectomy of a patient with *Echinococcus vogeli* infection, the Netherlands, January 2013

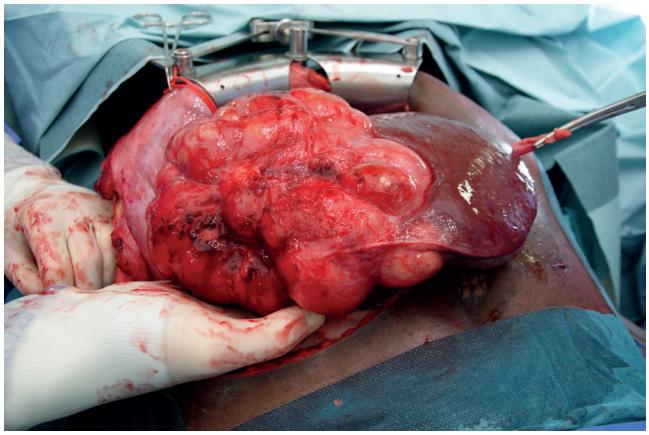
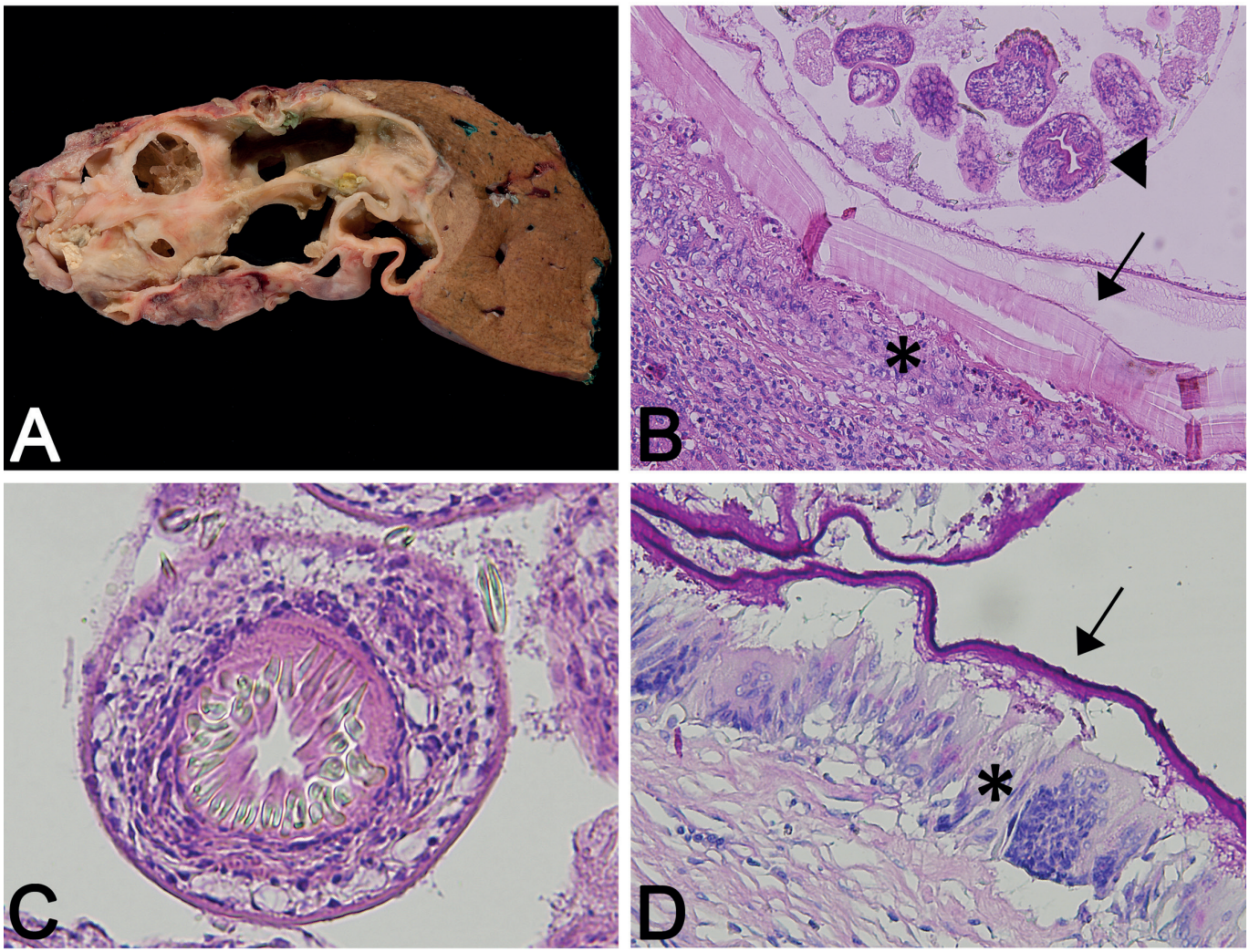


Figure 3

Histopathological examination of the liver of right hemihepatectomy of a patient with *Echinococcus vogeli* infection, the Netherlands, January 2013



A. Section of the liver showing a multilocular cystic tumour (diameter 18 cm).

B. Microscopic examination (hematoxylin and eosin stain, 100x) showing cystic spaces lined by laminated membranes (arrow) and surrounded by a rim of histiocytes (asterix). The cystic spaces were filled with necrotic debris and numerous protoscolices (arrowhead).

C. Detail of protoscolix with hooklets (hematoxylin and eosin stain, 400x).

D. Periodic acid-Schiff (PAS) stain (200x) showing laminated PAS-positive membranes (arrow) and rim of histiocytes and multinucleated giant cells facing the hydatid cysts (asterix).

Because a cystadenoma with possible malignant degeneration was suspected, he underwent right hemihepatectomy (Figure 2), in January 2013, which was successful. Much to the surprise of the surgeon, microscopic examination of the cyst fluid revealed protoscolices and hooklets, consistent with echinoccocal infection.

Histopathological examination of the liver showed a multilocular cystic tumour with a diameter of 12 cm. The cystic spaces were filled with necrotic debris, numerous protoscolices and hooklets and laminated periodic acid-Schiff-positive membranes. The lesion was surrounded by fibrosis with eosinophilic and lymphoplasmacytic inflammatory infiltrate and a rim of histiocytes and multinucleated giant cells directly facing the hydatid cysts (Figure 3).

Post-operative Echinococcus serology [1] was positive (indirect haemagglutin test 1: 320). Because of the atypical nature of the cyst, a specific polymerase chain reaction (PCR) was performed on cyst material., amplifying the mitochondrial 12S rRNA gene. The primers used were derived from the PCR developed by Trachsel et al. [2]. The reverse primer, Cest12Sr (GCGGTGTGTACITGAGITAAAC) was basically the same as Cest5 from Trachsel et al., but the forward primer, Ech12Sf (AAAIGGTTTGGCAGTGAGIGA) was designed by ourselves (unpublished data). The PCR results in a product of 285 nucleotides. Sequence analysis of the PCR product showed important similarity to *Echinococcus vogeli* (Figure 4).

Background

Most patients with echinococcis in the Netherlands are migrants from eastern Europe, northern Africa, Turkey and the Middle East and are diagnosed with cystic echinococcosis. The so-called hydatid cysts are caused by *Echinococcus granulosus* sensu lato, a tapeworm of dogs, the definite hosts. Livestock (cattle, pigs, goats, sheep, camels) become intermediate hosts* after ingestion of eggs, with cyst formation in visceral organs. Infected humans can become intermediate (but dead-end) hosts themselves, with cysts in several organs as well. The liver is most affected, but sometimes also the spleen, lungs, muscles, bones and even the central nervous system are involved. The cysts may cause pain or discomfort by their size, but are often asymptomatic and coincidentally diagnosed. Cyst rupture into the abdominal cavity is an uncommon but life-threatening complication, resulting in anaphylaxis and seeding of the infection. This may also be caused by surgical perforation of cysts [3].

Discussion

In the Center for Tropical and Travel Medicine in the Academic Medical Center in Amsterdam, a total of 133 patients with echinococcal disease have been treated in the last decade, with approximately 20 new cases being referred each year [4]. Although other hospitals in the Netherlands also diagnose new cases, most patients are referred to the Academic Medical Center because of its specific expertise in ultrasound diagnosis and treatment with percutaneous techniques and adjuvant chemotherapy with albendazol [5].

Figure 4

Sequence comparison of the mitochondrial 12S rRNA gene sequences of *Echinococcus* species from GenBank and from a patient with *E. vogeli* infection**, the Netherlands, January 2013

	11 14 17 18 24 37 39 47 48 55	59 60 62 63 64 65 67 69 81 86	93 110 113 114 121 136 137 140 142 143	144 145 148 149 152 155 157 158 159 160	161 162 163 164 165 166 184 190 191 195	212 213 214 215 222 223 226	Accession number
Patient	tttagaaaat	g-gtgatatg	ttga-gaatg	tggtaggaac	at--ga-gag	gtgactc	KC894948
E. vogeli	....	.-........	....-.....	....	..--..-...	......g	DQ408426.1
E. equinus	.g........	.-a.a...ga	....-a..g.	..t.tt...t	..--t.-t..	.a....g	EF143835.1
*E. gran*_buffel	...g...g.c	t-a..g.g..	c.ag-a..g.	..a.tttg.t	..--t.-t.a	a.ag..g	GQ168813.1
E. ortleppi	...g...g.c	t-a..g.g..	c.ag-a..g.	..a.tttg.t	..--t.-t.a	a.ag..g	FJ608743.1
E. can-G6	..c....g.c	t-a..g.g..	c..g-a..g.	....tttg.t	..--t.-t.t	a.....g	AB235846.1
E. can-G7	..c....g.c	t-a..g.g..	c..g-a..g.	....tttg.t	..--t.-t.t	a.....g	EU541210.1
E. can-G8	..c......c	t-a..g.g..	c..g-a..g.	....tttg.t	..--t.-t.t	a.a..cg	AB235847.1
E. multi	.a..t.g...	t-..a.g...	..a.-..gaa	.a..tttggt	..--tg-tgt	t.....g	AB235848.1
*E. gran*_sheep	.g..t.gg..	.-a.a.....	..tg-a..g.	.aa.t.tg.t	g.--..-t..	.a....g	EU043371.1
E. oligarthrus	a.......g.	.-.a......	.g..-.t..a	a..a...tgg	tg-t.t-t..	ta....g	AJ237779.1
E. felidis	.g..ag....	.sa.......	...gsa..g.	....t.tggt	..--..-t.t	ta..g.g	FJ426641.1

Alignment of polymorphic sites in the alignment of 230 nucleotides of the 12S rRNA gene sequence of various *Echinococcus* species retrieved from the GenBank nucleotide sequence database (accession numbers DQ408426.1, EF143835.1, GQ168813.1, FJ608743.1, AB235846.1, EU541210.1, AB235847.1, AB235848.1, EU043371.1, AJ237779.1, and FJ426641.1). The position of the polymorphic sites is indicated relative to position 94 in *E. vogeli* 12S rRNA gene with GenBank accession number DQ408426.1. The number of polymorphisms clearly shows similarity of the patient's sequence to the *E. vogeli* sequence in GenBank, and dissimilarity to other *Echinococcus* species. Identical positions are indicated with a dot, alignment gaps are indicated with a dash.

**The GenBank accession number was added on 17 April 2013.

Alveolar echinococcosis is caused by *Echinococcus multilocularis*, a tapeworm favouring the fox as a definite host. This is a more aggressive type of infection, often necessitating surgery and lifelong chemotherapy. Recently, a first case of probable locally acquired *E. multilocularis* in the Netherlands has been described [6]; in Denmark, the parasite has been detected in fox populations [7].

E. vogeli, as described in our patient, occurs in South America [8-13], including Suriname [8,13], as a tapeworm of the bush dog, with rodents as intermediate hosts. In humans, it may cause polycystic hydatid disease and is described in a small, but growing number of patients from this area [8-13]. To the best of our knowledge, this is the first report of *E. vogeli* in the Netherlands and probably also in Europe. As shown in our patient, the diagnosis is easily missed and may be mistaken for malignancy. Fortunately, the correct therapy was administered: radical surgery with no spill of cystic content, to be followed by at least six months of albendazol and lifelong surveillance. However, if this diagnosis had been considered before the operation, therapy with albendazol would probably have been started pre-operatively and antihistamnics administered peri-operatively.

Conclusion

In patients originating from endemic areas who have otherwise unexplained cystic masses, especially in the liver, the differential diagnosis must include echinococcosis. Most such patients in the Netherlands are diagnosed with *E. granulosus*. The case described here indicates that other pathogenic species such as E. vogeli, causing a different type of cystic disease, should also be considered. This, and earlier reports [8-13] from South America, show that *E. vogeli* is probably endemic to some parts of this continent.

Acknowledgements

Joke van der Giessen and Titia Kortbeek (National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands) for their advice, based on extensive expertise of echinococcosis.

Conflict of interest

None declared.

Authors' contributions

Cornelis Stijnis wrote the article. Aldert Bart, Lodewijk Brosens, Martin Grobusch, Thomas van Gulik, Jeroen Roelfsema and Pieter van Thiel revised drafts of the manuscript. Lodewijk Brosens analysed pathology results and produced the pathology figure. Tom van Gool analysed serology results. Thomas van Gulik performed surgery and postoperative photography. Jeroen Roelfsema analysed PCR results and produced the PCR figure.

* Authors' correction:

The authors are grateful to a reader for spotting an error in the background information. Rodents are intermediate hosts for *Echinococcus multilocularis*, not for *Echinococcus granulosus*. This was corrected on 16 April 2013.

** Addendum

The GenBank accession number was added to Figure 4 on 17 April 2013.

References

1. van Doorn HR, Hofwegen H, Koelewijn R, Gilis H, Wentink-Bonnema E, Pinelli E, et al. Reliable serodiagnosis of imported cystic echinococcosis with a commercial indirect hemagglutination assay. Diagn Microbiol Infect Dis. 2007;57(4):409-12. http://dx.doi.org/10.1016/j.diagmicrobio.2006.10.002. PMid:17189677.
2. Trachsel D, Deplazes P, Mathis A. Identification of taeniid eggs in the faeces from carnivores based on multiplex PCR using targets in mitochondrial DNA. Parasitology. 2007, 134(Pt 6):911-20.
3. Kern P. Echinococcus granulosus infection: clinical presentation, medical treatment and outcome. Langenbecks Arch Surg. 2003;388(6):413-20. http://dx.doi.org/10.1007/s00423-003-0418-y. PMid:14605887.
4. Herremans T, Verweij JJ, Schipper HG, Casparie M, van Lieshout L, Pinelli E, et al. [Decline of echinococcosis in the Netherlands; 1997-2008]. Ned Tijdschr Geneeskd. 2010. 154(18):A2297. Dutch.
5. Schipper HG, Laméris JS, van Delden OM, Rauws EA, Kager PA. Percutaneous evacuation (PEVAC) of multivesicular echinococcal cysts with or without cystobiliary fistulas which contain non-drainable material: first results of a modified PAIR method. Gut. 2002;50(5):718-23. http://dx.doi.org/10.1136/gut.50.5.718. PMid:11950823. PMCid:1773202.
6. van Dommelen L, Stoot JH, Cappendijk VC, Abdul Hamid MA, Stelma FF, Kortbeek LM, et al. The first locally acquired human infection of Echinococcus multilocularis in the Netherlands. J Clin Microbiol. 2012;50(5):1818-20. http://dx.doi.org/10.1128/JCM.06355-11. PMid:22357506. PMCid:3347149.
7. van der Giessen JW, Rombout YB, Franchimont JH, Limper LP, Homan WL. Detection of Echinococcus multilocularis in foxes in the Netherlands. Vet Parasitol. 1999;82(1):49-57. http://dx.doi.org/10.1016/S0304-4017(98)00263-5
8. Basset D, Girou C, Nozais IP, D'Hermies F, Hoang C, Gordon R, et al. Neotropical echinococcosis in Suriname: Echinococcus oligarthrus in the orbit and Echinococcus vogeli in the abdomen. Am J Trop Med Hyg. 1998;59(5):787-90.
9. D'Alessandro A, Rausch RL. New aspects of neotropical polycystic (Echinococcus vogeli) and unicystic (Echinococcus oligarthrus) echinococcosis. Clin Microbiol Rev. 2008;21(2):380-401. http://dx.doi.org/10.1128/CMR.00050-07. PMid:18400802. PMCid:2292577.
10. Knapp J, Chirica M, Simonnet C, Grenouillet F, Bart JM, Sako Y, et al. Echinococcus vogeli infection in a hunter, French Guiana. Emerg Infect Dis. 2009;15(12):2029-31. http://dx.doi.org/10.3201/eid1512.090940. PMid:19961693. PMCid:3044547.
11. Somocurcio JR, Sánchez EL, Náquira C, Schilder J, Rojas F, Chacón P, et al. First report of a human case of polycystic echinococcosis due to Echinococcus vogeli from neotropical area of Peru, South America. Rev Inst Med Trop Sao Paulo. 2004;46(1):41-2. http://dx.doi.org/10.1590/S0036-46652004000100008. PMid:15057334.
12. Tappe D, Stich A, Frosch M. Emergence of polycystic neotropical echinococcosis. Emerg Infect Dis. 2008;14(2):292-7. http://dx.doi.org/10.3201/eid1402.070742. PMid:18258123 PMCid:2600197.
13. Oostburg BF, Vrede MA, Bergen AE. The occurrence of polycystic echinococcosis in Suriname. Ann Trop Med Parasitol. 2000;94(3):247-52. http://dx.doi.org/10.1080/00034980050006429. PMid:10884869.

Automated mortality monitoring in Scotland from 2009

A P Wagner (apw40@medschl.cam.ac.uk)[1,2], E McKenzie[3], C Robertson[3,4,5], J McMenamin[5], A Reynolds[5], H Murdoch[5]
1. Cambridge Intellectual and Developmental Disabilities Research Group, Department of Psychiatry, University of Cambridge, Cambridge, United Kingdom
2. National Institute for Health Research (NIHR) Collaborations for Leadership in Applied Health Research and Care (CLAHRC) for Cambridgeshire and Peterborough, United Kingdom
3. Department of Mathematics and Statistics, University of Strathclyde, United Kingdom
4. International Prevention Research Institute, Lyon, France
5. Health Protection Scotland, Glasgow, United Kingdom

Citation style for this article:
Wagner AP, McKenzie E, Robertson C, McMenamin J, Reynolds A, Murdoch H. Automated mortality monitoring in Scotland from 2009. Euro Surveill. 2013;18(15):pii=20451. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20451

Article submitted on 23 April 2012 / published on 11 April 2013

Mortality monitoring systems are important for gauging the effect of influenza and other wide ranging health threats. We present the daily all-cause mortality monitoring system routinely used in Scotland, which differs from others by using two different statistical models for calculating expected mortality. The first model is an extended Serfling model, which captures annual seasonality in mortality using sine and cosine terms, and is frequently seen in other systems. Serfling models fit to summer seasonality well, but not to the winter peak. Thus, during the winter, there are frequent `excesses', higher than expected mortality, making it harder to directly judge if winter mortality is higher than in previous years. The second model, a Generalised Additive Model, resolves this by allowing a more flexible seasonal pattern that includes the winter peak. Thus, excesses under the second model directly indicate if winter mortality is higher than in previous years, useful, for example, in judging if a new strain of seasonal influenza is more likely to produce death than previous ones. As common in all-cause mortality monitoring systems, the Scottish system uses a reporting delay correction: we discuss the difficulties of interpretation when such a correction is used and possible avenues for future work that may address these difficulties.

Introduction

Mortality surveillance systems are used to monitor for unexpected increases in mortality and are important in monitoring public health. For example, they are used to gauge the effect of influenza [1-3] and to track heat waves and other wide-ranging public health threats [4-7]. Such systems are currently used in: Belgium [8]; England and Wales [9]; Portugal [4]; Sweden [5]; and the United States [1]. A project funded by the European Commission, called the European monitoring of excess mortality for public health action, EuroMOMO, worked to improve the real time monitoring of mortality in Europe [3,6,10]. Among its outcomes was the development of a common consensus system (A-MOMO) for use across Europe, to allow for comparable monitoring between Member States [3,6].

We present the Scottish daily all-cause mortality surveillance system used by Health Protection Scotland (HPS) [11]. This system uses mortality data collated by the National Records of Scotland (NRS) [12] to automatically carryout statistical analysis and produce supporting documentation. Our system differs from others by utilising two statistical models for calculating expected mortality, against which observed levels are compared to detect if mortality is unusually high.

The first model uses sine and cosine terms to model seasonal variation in mortality, extending an earlier model developed by Serfling [13], as is commonly used in other systems [2,4,7]. This model captures summer seasonality well, but does not follow the winter peak closely (Figure 1). Thus, in Serfling models, excess mortality essentially corresponds to mortality above that expected during the summer – during the winter months this is described as `excess winter mortality' [14]. While influenza may not be the primary driver of excess winter mortality, there is a strong association between them, making excess mortality a useful indicator of influenza [15,16].

The second model uses Generalised Additive Models (GAMs) [17]. GAMs allow for a less restrictive seasonal pattern and so can fit more closely to the winter peak (Figure 1; see [18] for a comparison of approaches to modelling seasonality). Thus, the increase of mortality during the winter is treated as part of the usual seasonal pattern (giving different excess mortality to that of the Serfling model). Consequently, the severity of any seasonal or pandemic influenza (for example, influenza A(H1N1) pdm09 [19]) can more readily be compared with what is usually expected during the winter.

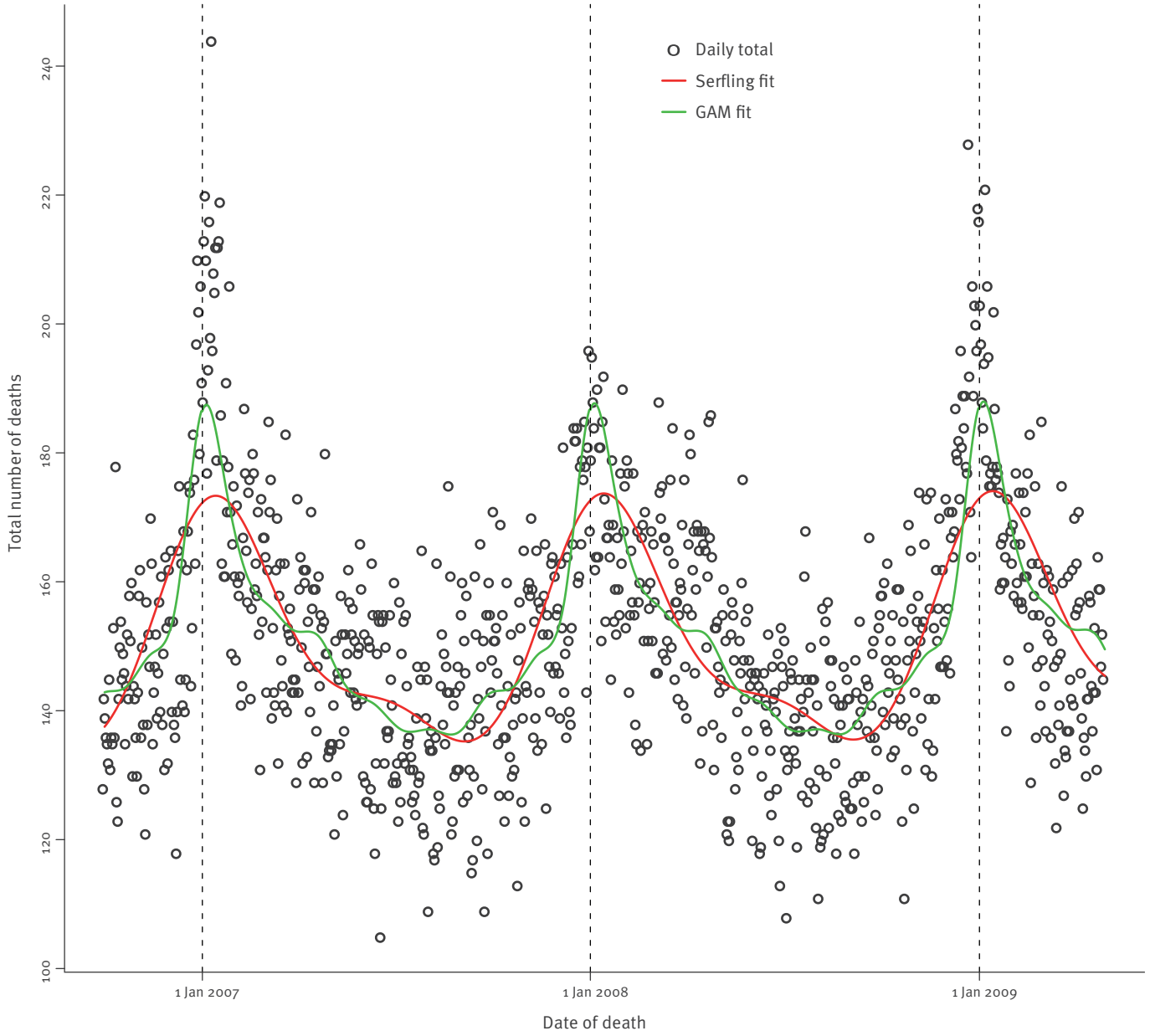
Methods

Collected data

Details of most deaths (≥95%) are transferred electronically to NRS from local Registrars' offices. Data is made available each weekday (Monday to Friday) to HPS on such deaths (currently, data is emailed but

Figure 1

The daily totals of deaths occurring in Scotland, 1 October 2006–29 April 2009



GAM: Generalised Additive Model.

The daily totals for all days of each year are shown. The dashed vertical black lines indicate 1 January for each year. The coloured lines give the fits of the two statistical models used to calculate expected numbers of deaths in the mortality surveillance system. The GAMs (green) follow the winter peaks, which occur at the beginning of each year, more closely, while the Serfling model (red) captures summer seasonality (corresponding with those times where the models are at their lowest values, the troughs between the winter peaks) more smoothly and consistently.

Table 1

Fit statistics of the Serfling models fitted to the daily totals of observed deaths occurring in Scotland, 1 October 2006–29 April 2009

Model description (unique elements, beyond those described in the caption)	Null deviance	Residual deviance	Explained deviance	% Deviance explained	Null degrees of freedom	Residual degrees of freedom	Used degrees of freedom	AIC
Interaction of age and first seasonal harmonic	105,993	12,277	93,716	88	11,303	11,277	26	54,059
Interaction of $Young_a$ and first seasonal harmonic	105,993	12,280	93,713	88	11,303	11,281	22	54,054
Second seasonal harmonic; second order interactions between both seasonal harmonics and each of age and $Young_a$	105,993	12,150	93,843	89	11,303	11,271	32	53,944
Model defined by Equation (1); as above, but no interaction between second seasonal harmonic and $Young_a$	105,993	12,152	93,841	89	11,303	11,277	26	53,934

AIC: Akaike Information Criterion.

All models include the first seasonal harmonic, factors for age group and sex, and second order interactions between: age group and sex; sex and the seasonal harmonics. Both by analyses of deviance and comparison of AICs (a lower AIC is favoured), the model defined by Equation (1) is the preferred model. It is not appropriate to use a goodness-of-fit test on this model, as it fits to very low counts in the youngest age groups, which violates the large sample assumptions of the test.

development is underway on automating the transfer). This arrangement is possible since all-cause mortality data has not been subject to the validity checking process whereby verification of the cause of death is established and ultimately published a number of months later as an official statistic. For each death, the sex, age at time of death, postcode of last known residence, date of death and date of registration are collected. Postcode sectors are used to link deaths with particular geographical regions in Scotland (typically, health boards, the 14 geographical subdivisions of the Scottish health service).

Data characteristics

On average, there are 152 deaths per day in Scotland, with little difference between days of the week [20]. Mortality reaches a peak around the turn of the year and is at its lowest during the summer. As would be expected in an industrialised country, there are relatively few deaths at young ages. We use the age groupings generally adopted by HPS: 0–14, 15–44, 45–64, 65–74, 75–84 and ≥85 years. There are different seasonalities in mortality among these groups and different levels between the sexes. The biggest differences are seen in the older groups (65–74, 75–84 and ≥85 year-olds), in both seasonal level and pattern.

Developing statistical models for the calculation of expected mortality

We fitted regression models to the daily totals of deaths aggregated by date of death (Figure 1). The following models were developed on data from 1 October 2006 to 14 May 2009, excluding the last two weeks to reduce the effect of unreported deaths.

Serfling model development

Serfling's model is extended to the following, where a Poisson Generalised Linear Model (GLM) is fitted to the daily mean of observed deaths, μ_{tsa} , for each age group a and sex s:

$$\begin{aligned}\log(\mu_{tas}) = {} & \beta_0 + Sex_s + Age.Gp_a + \beta_1 Trend_t \\ & + \beta_2 \sin(2\pi p_t) + \beta_3 \cos(2\pi p_t) \\ & + \beta_4 \sin(4\pi p_t) + \beta_5 \cos(4\pi p_t) \\ & + Sex_s : Age.Gp_a \\ & + Sex_s : \{\sin(2\pi p_t) \\ & + \cos(2\pi p_t) + \sin(4\pi p_t) + \cos(4\pi p_t)\} \\ & + Young_a : \{\sin(2\pi p_t) + \cos(2\pi p_t)\}\end{aligned} \quad (1)$$

where the β_k are coefficients; Sex_s is a factor with two levels; $Age.Gp_a$ is a factor with a level for each age group; $Trend_t$ is the number of day t as numbered from the first day ($Trend_t = t$); p_t gives the within-year time, which begins at zero on 1 January and increases

Table 2

Comparisons of Akaike Information Criterion between the Generalised Additive Models that adopt different approaches to modelling seasonality (separate models versus use of a factor)

Age in years	Separate GAM for each age and sex – Equation (2)			Sex factor – Equation (3)	Lowest AIC
	Females	Males	Sexes combined		
0–14	1,512.79	1,747.18	3,259.97	3,261.42	Equation (2)
15–44	3,263.70	4,140.83	7,404.53	7,411.06	Equation (2)
45–64	4,727.36	5,233.78	9,961.15	9,952.85	Equation (3)
65–74	5,084.46	5,426.35	10,510.81	10,500.31	Equation (3)
75–84	5,784.34	5,704.85	11,489.19	11,475.05	Equation (3)
≥85	5,944.66	5,211.47	11,156.13	11,158.30	Equation (2)

AIC: Akaike Information Criterion; GAM: Generalised Additive Model.

The GAM defined by Equation (2) has a separate spline for each age group and sex combination, while the GAM defined by Equation (3) uses the same spline for both sexes but utilises a factor to capture differences in seasonal levels. For the models defined by Equation (2), the AICs for each sex, within each age group, are summed ('Sexes combined') to allow for comparison with the models defined by Equation (3).

by 1/365 or 1/366 increments, depending on the numbers of days in that year; $Young_a$ is similar to $Age.Gp_a$, except that the three youngest groups (0–14, 15–44 and 45–64) share the same level; $A : B$ denotes the interaction of terms A and B. The inclusion of an interaction term allows two variables to affect the mean in a more complex way than simply additively; for example, $Sex_s : Age.Gp_a$ allows a different level for each sex and age combination. The development of this model is outlined in Table 1. The introduction of $Young_a$ allows for a parsimonious model while still allowing greater seasonal variation in the older groups.

Essentially, this model is very similar to those used in other mortality monitoring systems: there is a linear trend and seasonality is modelled with sine and cosine terms [3,4,7,9]. It differs from A-MOMO by being fitted to daily data, as in the approach by Cox et al. [7], and using data from the whole year, as described in the works of Cox et al. and Hardelid et al. [7,9]. In contrast with models from other systems, differences between age groups and sex are addressed in one model by using factors ($Age.Gp_a$, $Young_a$ and Sex_s), while other systems fit separate regression models to appropriate data subsets. A standard Poisson model suffices for the Scottish data [20].

Generalised Additive Models development

Using GAMs, we fitted models that follow the winter peak more closely. A GAM allows a `spline' to be fitted to data, a process by which the data range is split into separate sections, delineated by a series of `knot points', within which a simple curve is fitted to the data contained therein (see [21] for an introduction). The Mixed GAM Computation Vehicle with GCV/AIC/REML smoothness estimation (mgcv) package is used to fit the following GAMs [17].

We first determine if the daily means of deaths μ_t are best modelled with a separate spline for each age group a and sex s combination (n=12 models):

$$\log(\mu_t) = \beta_0 + \beta_1 Trend_t + f(p_t), \qquad (2)$$

or, if the seasonality for both sexes in each age group can be modelled with the same spline, with an additive sex factor to address differences in level (n=6 models):

$$\log(\mu_{ts}) = \beta_0 + \beta_1 Trend_t + Sex_s + f(p_t). \qquad (3)$$

In these Poisson GAMs, $f(p_t)$ is a cyclic cubic regression spline with 52 weekly knots, fitted to the within period time p_t. The use of a cyclic regression spline ensures a smooth seasonal pattern. We choose between these models by comparing their Akaike Information Criterions (AICs, [22]) as shown in Table 2. For each age group in the models defined by Equation (2), we sum the AICs of the models for each sex to allow for comparison with the models defined by Equation (3). The GAMs defined by Equation (3) are to be preferred in three age groups (45–64, 65–74 and 75–84 years), while in two groups there is little difference (0–14 and ≥85 years). Thus, we choose models defined by Equation (3).

Next, we investigate more parsimonious models resulting from using fewer knots. We choose from among sets of knots placed at regular intervals (weekly, fortnightly and monthly) and sets where knot locations vary from a higher density around the winter solstice, to a lower one around the summer solstice (Figure 2). The latter

Table 3

The Akaike Information Criterions of the Generalised Additive Models defined by Equation (3) with different sets of knot points

Age group in years	Sets of knots considered (n=number of knots)						
	Weekly (52)	Fortnightly (26)	Manually chosen–D (21)	Manually chosen–C (18)	Manually chosen–B (16)	Monthly (12)	Manually chosen–A (11)
0–14	3,261.42	3,261.72	3,263.55	3,263.21	3,258.56	3,258.56	3,258.56
15–44	7,411.06	7,411.07	7,411.08	7,411.13	7,411.14	7,411.12	7,411.22
45–64	9,952.85	9,953.32	9,952.22	9,954.31	9,951.37	9,961.44	9,962.50
65–74	10,500.31	10,503.66	10,503.53	10,501.76	10,506.11	10,506.23	10,506.44
75–84	11,475.05	11,475.76	11,480.02	11,482.72	11,485.77	11,485.98	11,485.85
≥85	11,158.30	11,158.43	11,157.82	11,158.72	11,158.65	11,164.53	11,162.58

Knots placed at regular intervals include those described as weekly, fortnightly and monthly. The position of the manually specified knot sets are shown in Figure 2.

sets of knots are motivated by noting an association between hours of daylight and levels of mortality during the winter [20].

The resulting AICs for fitting the models defined by Equation (3) to each age group and knot set are shown in Table 3. There is little change in AIC over this wide range of models, but the fit of the models in the older groups improves with the addition of more knots. The more clearly defined seasonal patterns in the older age groups, available from the greater number of deaths in these groups, can be more closely modelled by using a greater number of knots [20]. However, note that AICs cannot directly be used for choosing between knot sets. Instead, a pragmatic approach is adopted. For each age group, we choose the set of knots where the AIC levels off and is consistent with the fewest knots. However, to ease interpretation, we also want to use the same set of knots across all age groups. Given these constraints, we choose set B, containing 16 knots (Figure 2).

Reporting delay correction

There is a median reporting delay of one day. Thus, to increase the likelihood of detecting excesses more quickly, the empirical cumulative distributions of delays are used to inflate totals of reported deaths from recent days, which are likely to be underreported because of delayed reporting (similar methods are used by other authors [9,10]). As over 99% of deaths are reported within 14 days, only reporting totals from the last two weeks are corrected. Delays are measured in terms of working days, since negligible totals of deaths are reported at the weekend and on public holidays, when Registrars' offices are closed. Delay distributions are grouped into four day types: weekdays (Monday to Friday); Saturdays; Sundays; and public holidays. An example of these being used to correct national totals is given in Supplementary Table 1 (http://preview.tinyurl.com/c6ktyrr).

The following formula is used to calculate the delay corrected reporting total C_{isat} for age a, sex s, for day $t - i$ $(0 < i < 13)$ for use on day t:

$$C_{isat} = \frac{R_{sati}}{D_{d(t-i),g(a,s),w(i)}},$$

where R_{sati} is the total number of deaths occurring on day $t - i$ that have been reported by day t, and $D_{d(t-i),g(a,s),w(i)}$ is the proportion of deaths expected to be reported by $w(i)$ working days after day $t - i$, for the appropriate day type $d(t-i)$ (either weekday, Saturday, Sunday or public holiday), in group $g(a,s)$ (one of: 0–14, 45–64, 65–74, 75–84, ≥85 years age groups, but with separate groups for males and females aged 15–44 years). Currently, the $D_{d(t-i),g(a,s),w(i)}$ are calculated from the delays in the reporting of deaths occurring between 1 October 2006 and 29 April 2009. The groupings of $g(a,s)$ reflect that the delays for most age groups are similar across the sexes, except for the 15–44 year-old group, where reports for males tend to be delayed for longer, as they are frequently subject to autopsies following violent deaths.

Model for monitoring reporting levels

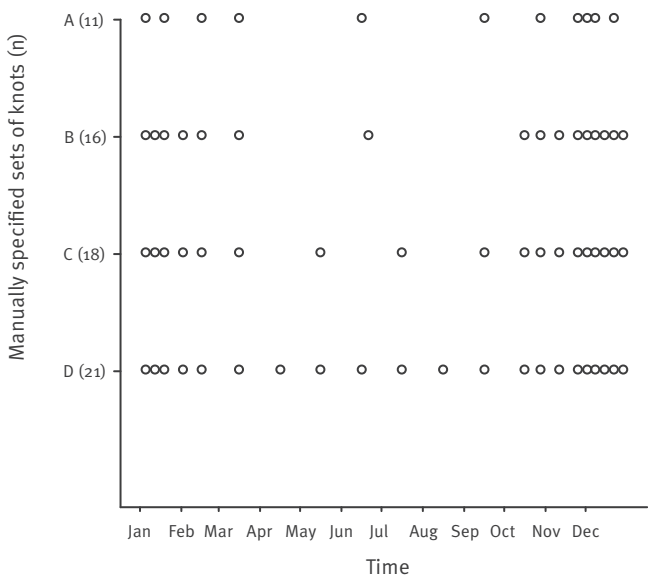
We fitted the following Poisson GAM to the mean daily total μ_{Rt} of reported deaths (that is, daily totals of reports aggregated by date of registration, rather than date of death as is used above):

$$\log(\mu_{Rt}) = \beta_0 + \beta_1 Trend_t + f(p_t) + Day_t + Holiday_t,$$

where: Day_t is a factor with a level for each week day (no deaths can be registered at the weekend); and $Holiday_t$ is a factor indicating if day t is a public holiday, modelling the lower levels of reporting on such days. Factor Day_t models the differences in reporting

Figure 2

The manually specified knot sets considered in the Generalised Additive Models



The letters A, B, C, D on the Y axis designate specific sets of knots. The number in parentheses next to each letter specifies the number of knots in the given set. Individual knots are depicted as small circles. In all sets, knots are placed more regularly around the winter solstice and then placed less frequently towards the summer solstice.

levels between days: for example, Mondays tend to have the highest level of reporting, since they are the first opportunity for weekend deaths to be reported. Linked with such deferred reporting, Dayt is set to Monday levels for any day following a public holiday. This model allows reporting levels to be monitored (Figure 3).

General system

The separate elements described thus far are brought together to produce a mortality surveillance system, as outlined in Figure 4. Details of the latest deaths are retrieved from NRS and used to update totals recorded within the system. The delay correction is used to inflate the reported totals for recent days to reduce the effect of delayed reporting. Expected mortality can be calculated from either the Serfling model or GAM, and then compared to the corrected totals. Plots and summaries of the expected and (delay corrected) observed values are made, and excesses determined. When an excess occurs, it can be investigated with the aid of the reporting level monitoring model, to determine whether an excess is genuine, or more likely to be an artificial product of the delay correction and an unusual reporting pattern.

In the HPS system, an excess occurs when the corrected reporting total for any sex, within any age group, on any day, exceeds the upper limit of the 99% prediction interval for the expected mortality for that group on that day. The size of an excess is defined as the difference between the expected and corrected reporting total. The total excess deaths for any day are found by summing the sizes of any excesses occurring across the twelve age/sex combinations. Obviously, excesses will vary with the model chosen for calculating the expected numbers of deaths, particularly during the winter.

System use and outputs

In preparation for the influenza season, the statistical models are annually re-estimated at the end of September, using data from 2001 to the present period. Predictions of expected levels are then made from October onwards for the coming year. Similarly, from 2012, the reporting delay distributions will also be annually updated (however, they are relatively stable). The system and its data are audited as part of the seasonal influenza review.

Plots produced by the system show daily totals of deaths, ranges of expected values and any excesses, allowing easy interpretation of the current state of mortality (for example, see Figure 3). Recent data, subject to the reporting delay correction, is highlighted to emphasise how totals for this period can fluctuate, as delayed reports continue to accumulate. Besides a graph of national figures (n=1 plot), other plots are also produced for each sex (n=2), age group (n=14) and health board (n=14).

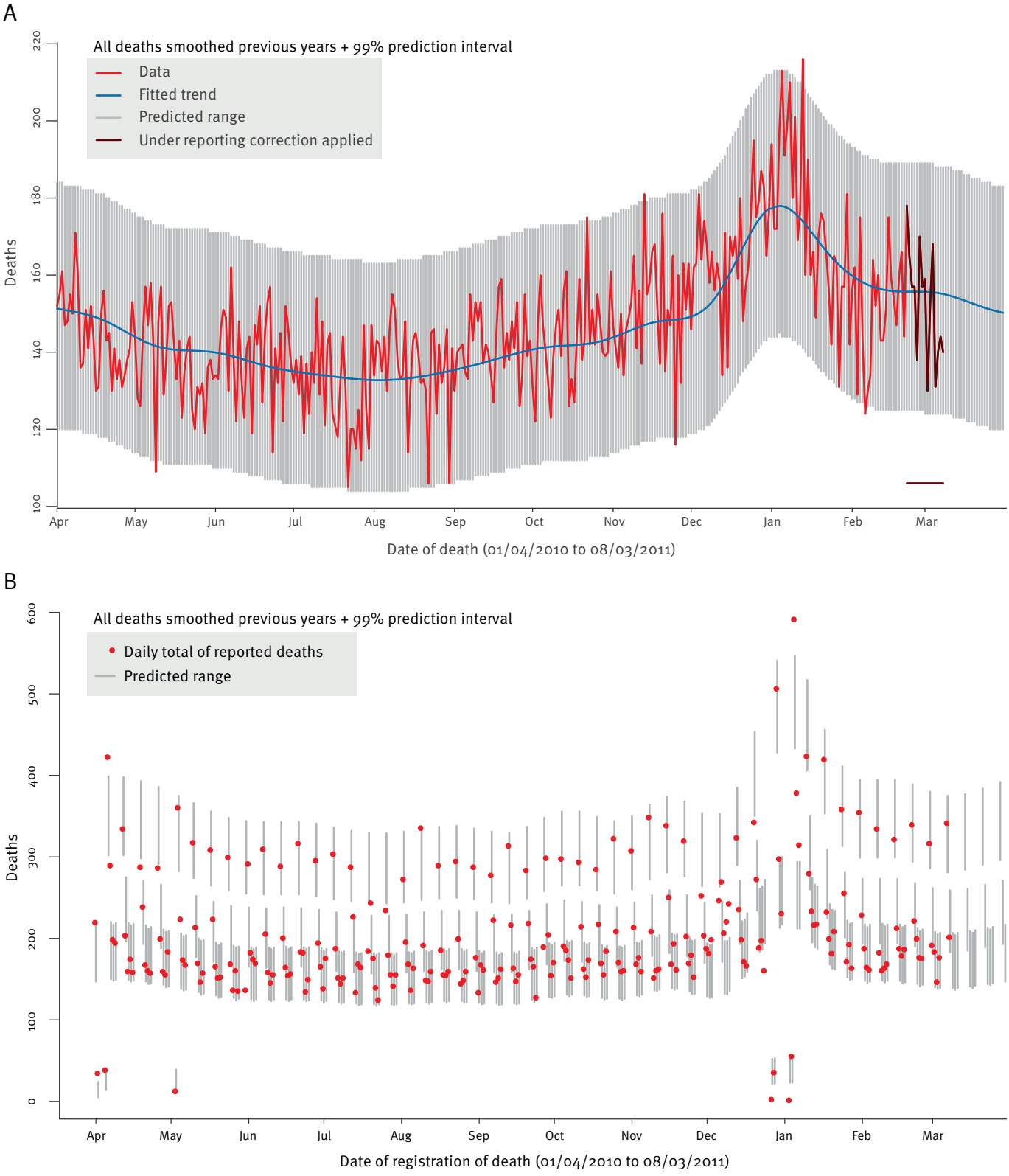
From 2011/12, the system is run daily at HPS (as it was during the influenza A(H1N1)pdm09 pandemic). Output from the system (primarily, the plots described above) is reviewed at least once a week by an epidemiologist on the Respiratory team (the team at HPS who take the lead on linking evidence of excess mortality to virus activity); during periods of intense monitoring, such as during the Olympics, output is reviewed daily (only Monday–Friday, as no new data is available at the weekend). Dependant on the review results, a protocol is followed to bring appropriate results to the attention of the consultant epidemiologist, who then decides if further investigation is warranted and if any excesses should be communicated to the NHS (for more detail see [20,23]).

Results

Output from running the system on a Tuesday is included in the weekly surveillance reports produced during the influenza season. For example, on 20 January 2011, HPS reported that an excess above the usual winter pattern had occurred during 3 to 9 January

Figure 3

Example output from the mortality surveillance system, Scotland, 01 April 2010–08 March 2011

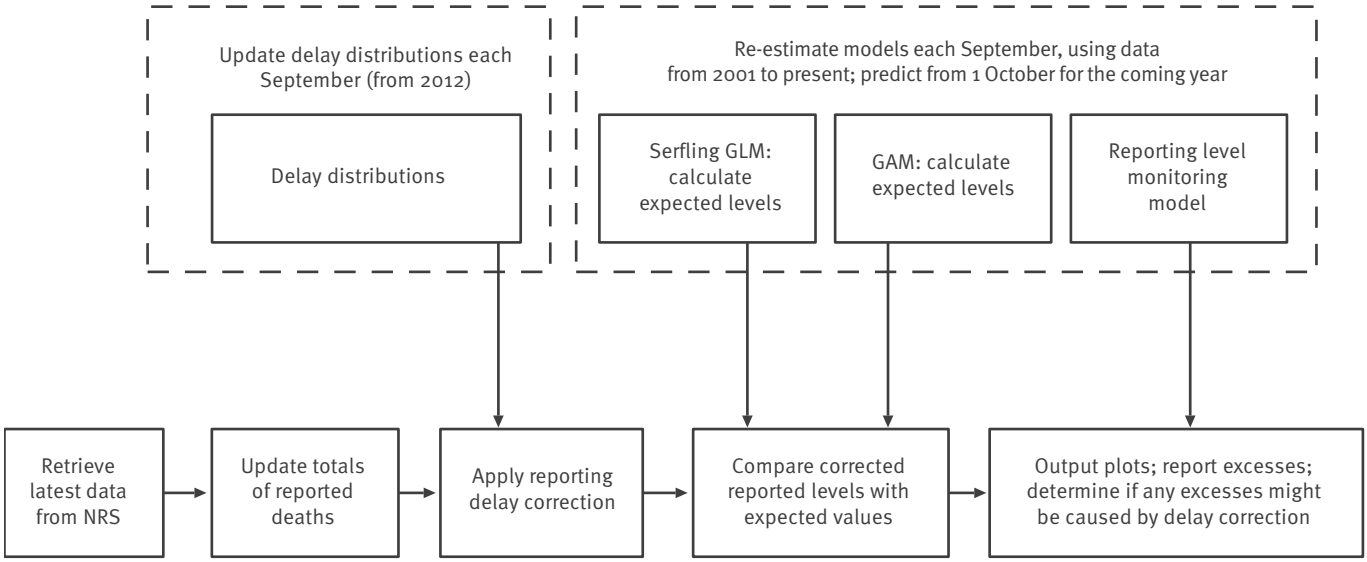


A. Daily national totals of deaths in Scotland, produced on 9 March 2011. The grey lines correspond to the 99% prediction intervals from the Generalised Additive Models; data points outside this, such as from the 3 to the 9 January, correspond to excesses of mortality. The inclusion of the horizontal line (in brown), indicating the period over which the delayed reporting correction is applied, helps to remind users that daily totals near to the present are subject to fluctuation, as delayed reports accumulate.

B. Output from the reporting level monitoring model. Red points are the daily totals of reported deaths and so there are only values for times corresponding to Monday through Friday, the only days on which deaths can be registered. The range of expected reporting levels are shown by the grey lines (99% prediction interval). The higher level of registration generally corresponds to report totals from Mondays.

Figure 4

A broad overview of the Scottish mortality surveillance system, from 2009



GAM: Generalised Additive Model; GLM: Generalised Linear Model; NRS: National Records of Scotland.

(shown in Figure 3, see [24] for details); no other excesses were reported in the winter of 2010/11.

Generally, the GAM and its associated output are preferred by the epidemiologists that use the system, due to its more direct and explicit interpretation. For example, the GAM based surveillance was a key element in demonstrating to the Scottish public that influenza A(H1N1)pdm09 had not significantly impacted upon general levels of mortality within Scotland (see, for example, [19]). However, later analysis has shown in other countries that influenza A(H1N1)pdm09 may have had an impact on mortality among the young [3,9]. Increases in the young (0–14 year-olds) are hard to detect, because even proportionately large increases result in only small increases in absolute totals.

The main challenge to using the system arises from the interpretation of the estimated daily deaths during the two week window in which the reporting delay correction is applied. Approximately 20% of deaths that occur on a working day are reported that same day, leading to a multiplication factor of five to convert the number of reported deaths to a `delay corrected' estimate of the number of deaths that have actually occurred. Thus, during the most recent few days, when the delay correction has its biggest effect, there are frequent temporary excesses that disappear with the accumulation of further data on subsequent days. The model monitoring levels of reporting can assist in determining genuine excesses, but this judgment generally requires a statistician.

As the system has only been running since winter 2009, it is too early to reliably comment on the sensitivity or specificity of the system (however, these are considered as part of the system's annual review). In any case, calculating sensitivity and specificity for all-cause mortality systems is difficult as the `relevant events are hard to define' and the `impact of known events is not certain, while other threats might not yet be known' [7]. However, to date, the system has not missed anything which has had an impact on deaths, though nothing untoward has occurred. It has detected days and periods with an excessive numbers of deaths and these have been investigated by epidemiologists. Examples from 2012 include 10 and 11 May (173 and 174 deaths respectively) and 22 May (188 deaths); these excesses may be associated with the atypical and very changeable temperatures during May.

Discussion and conclusions

Elsewhere, all-cause mortality surveillance systems that utilise Serfling models have been widely demonstrated to be useful in monitoring seasonal influenza [1,4,7,13,25]. Specifically, in Scotland, we have previously noted the strong association between levels of seasonal influenza and levels of `standard' excess mortality (deaths above the expected level as calculated

from a Serfling model) [14,16]. With the implementation of this system, Scotland now has an automated system for monitoring `standard' excesses in different age and sex combinations, at both the national and regional level.

The use of two statistical models for calculating expected totals of deaths increases the flexibility and utility of the system. By including Serfling excesses in the system, we have a measure of excess mortality that is more directly comparable to that produced by the systems of other countries. Further, as the Serfling part of the system is similar to EuroMOMO's A-MOMO, barring the differences noted earlier, the Serfling model supports Europe wide monitoring, particularly with those countries that have adopted EuroMOMO methodology [3,6,10].

The Serfling model gives an estimated number of excess deaths relative to a model which predicts a smooth change in deaths from summer to winter. Thus, virtually every year, there will be an excess of deaths from the Serfling model during the sharp winter peak. In contrast, by fitting to the winter peak, the GAM only produces excess deaths when mortality levels around the peak are worse than in previous years. This allows users of the system to more easily and directly detect if seasonal influenza and other factors are significantly increasing mortality above expected winter levels.

The GAMs have separate trends and cyclical components within each age group. It would have been possible to try and develop a Generalised Linear Mixed Model (GLMM) with random effects to control the deviation from the general trend and cyclical effects along the lines of Durban et al. [26]; however, the deviations are not random effects, but rather systematic ones associated with age group – the seasonal cycle becomes progressively more pronounced with increasing age group. Therefore, deviations from the population average are better represented by fixed differences with age, rather than random differences. Effects due to sex could be random but we did not find substantial evidence of differing seasonal components for men and women.

Interpreting output from the system is made more challenging due to the reporting delay correction. However, it is generally accepted that such corrections are needed if mortality surveillance systems are going to detect excesses in a more timely fashion [2,7,9,10]. While the reporting level monitoring model helps determine if an excess is likely to be an artefact of unusual reporting patterns, outputs from the system would be more easily interpretable if they were automatically `corrected' in some way to take account of the increased uncertainty arising from the use of the delay correction. We have considered some approaches for this, but further development is needed.

One approach would be to `inflate' the prediction interval that is used to give the predicted range of mortality (the grey lines in Figure 3, panel A), to reflect the increased variability arising from the use of the delay correction. We would expect the adjusted prediction interval to have a funnel shape, being widest in more recent days and then tapering to have the same width as the unadjusted prediction interval, to reflect the diminishing contribution of the correction further away from the most recent data. Ideally, the width of the adjusted prediction interval would be determined by a statistical model that takes into account differences in delays between age groups and day type. This approach may benefit from the delay corrections being modelled by probability distributions, rather than working with the raw empirical values as we have. For example, A-MOMO models delays with a binomial distribution [10]. We have investigated using standard geometric and negative binomial distributions, but fits have been poor [20] and further work is needed.

Little attention has been given to developing statistical methods for addressing reporting delay in mortality monitoring, or other types of prospective surveillance systems [2,27]. For example, the publications by Heisterkamp et al. and Kanieff et al. [28,29] are among the few papers that focus on the topic. Given that delay in mortality reporting is a common issue across Europe, further work in this area would be of wide benefit [29].

Currently, when the regression models are fitted to previous mortality data, every observation is given equal weight, including those corresponding to periods of excess mortality, which may increase expected mortality inappropriately. Ideally, the fit of these models, and consequently the expected levels, should be most heavily influenced by periods with no excesses. There are two main approaches to achieving this. The first of these, the one adopted by A-MOMO and others, is to fit the models to a subset of observations which are unlikely to include excesses of mortality [10]. The second approach refits the models a number of times and weights observations by the reciprocals of earlier fits [7,9]. Observations from periods of excesses should have larger residuals, resulting in smaller reciprocal weights, and so, should have a smaller influence on fit. We would need to adopt the latter approach, as otherwise the GAM might not reliably capture the annual seasonal cycle.

The ability of the system to detect smaller, but sustained, shifts away from expected mortality may be improved through the use of cumulative sums (CUSUMs), as used in other systems [2,9,30].

An enduring criticism of all-cause mortality data is that the cause of the excesses detected may be imputed but not known with certainty. Imputation is usually investigated by considering the temporal, demographic and geographic nature of the data in relation to other data sets. All-cause mortality data may also mask deaths

attributed to rare conditions. Therefore, HPS are considering the use of provisional cause-specific data. Pilot work is looking at accessing deaths where cause is provisionally recorded as influenza related and gauging how timely such a system might be.

This manuscript has presented the deaths surveillance system that has been running in Scotland since 2009. The strengths of the system are: the automatic daily processing of data from NRS; the use of a reporting delay correction; using both a Serfling model (for comparison with other European countries) and a GAM (for easier comparison with what is expected in Scotland based upon the pattern from previous years). The system provides timely information to epidemiologists and can be used on a weekly or daily basis as required.

Acknowledgements

This research was supported by a CASE PhD studentship, funded by the EPSRC and Health Protection Scotland. Results were written up in association with the NIHR CLAHRC for Cambridgeshire and Peterborough.

Funding to enable the creation of the data transfer mechanism between NRS and HPS was provided by Robert Kirkwood (of the Scottish Government Management Team) and the Scottish Government Resilience team.

We would like to thank Frank Dixon, Gordon Munro and Imran Hussain of NRS for their work on providing us with data during the development and early use of the system.

References

1. Simonsen L, Clarke MJ, Stroup DF, Williamson GD, Arden NH, Cox NJ. A method for timely assessment of influenza-associated mortality in the United States. Epidemiology. 1997;8(4):390-5. http://dx.doi.org/10.1097/00001648-199707000-00007 PMid:9209852
2. Unkel S, Farrington CP, Garthwaite PH, Robertson C, Andrews N. Statistical methods for the prospective detection of infectious disease outbreaks: a review. J R Stat Soc Ser A Stat Soc. 2012;175(1):49-82. http://dx.doi.org/10.1111/j.1467-985X.2011.00714.x
3. Mazick A, Gergonne B, Wuillaume F, Danis K, Vantarakis A, Uphoff H, et al. Higher all-cause mortality in children during autumn 2009 compared with the three previous years: pooled re-sults from eight European countries. Euro Surveill. 2010;15(5):pii=19480. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19480 PMid:20144446
4. Nogueira PJ, Machado A, Rodrigues E, Nunes B, Sousa L, Jacinto M, et al. The new automated daily mortality surveillance system in Portugal. Euro Surveill. 2010;15(13):pii=19529. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19529 PMid:20394709
5. Sartorius B, Jacobsen H, Törner A, Giesecke J. Description of a new all cause mortality surveillance system in Sweden as a warning system using threshold detection algorithms. Eur J Epidemiol. 2006;21(3):181-9. http://dx.doi.org/10.1007/s10654-005-5923-6 PMid:16547832
6. EuroMOMO – European monitoring of excess mortality for public health action. [Ac-cessed 22 Jul, 2011]. Available from: http://www.euromomo.eu/
7. Cox B, Wuillaume F, Van Oyen H, Maes S. Monitoring of all-cause mortality in Belgium (Be-MOMO): a new and automated system for the early detection and quantification of the mortality impact of public health events. Int J Pub Health. 2010;55(4):251-9. http://dx.doi.org/10.1007/s00038-010-0135-6 PMid:20376690
8. Cox B, Wuillaume F, Maes S, Van Oyen H. Belgian Mortality Monitoring (Be-MOMO). Public health and surveillance. Brussels: Scientific Institute of Public Health, Epidemiology Unit. [Ac-cessed 11 Apr 2013]. Available from: https://www.wiv-isp.be/epidemio/be-momo/
9. Hardelid P, Andrews N, Pebody R. Excess mortality monitoring in England and Wales during the influenza A(H1N1) 2009 pandemic. Epidemiol Infect. 2011;139(9):1431-9. http://dx.doi.org/10.1017/S0950268811000410 PMid:21439100
10. Mølbak K. European monitoring of excess mortality for public health action. [Accessed 20 Apr 2012]. Available from: http://ec.europa.eu/eahc/documents/news/technical_meetings/EuroMOMO.pdf
11. Health Protection Scotland (HPS). Health Protection Scotland – Protecting Scotland's Health. [Accessed 20 Apr 2012]. Glasgow: HPS. Available from: http://www.documents.hps.scot.nhs.uk/about-hps/hps-brochure-2009.pdf
12. General Register Office for Scotland. [Accessed 11 Apr 2013]. Available from: http://www.nrscotland.gov.uk/
13. Serfling RE. Methods for current statistical analysis of excess pneumonia-influenza deaths. Public Health Rep. 1963;78(6):494-506. http://dx.doi.org/10.2307/4591848 PMid:19316455 PMCid:1915276
14. General Register Office for Scotland. Increased Winter Mortality – Background. c2011. [Ac-cessed 20 Apr 2012]. Available from: http://www.gro-scotland.gov.uk/files2/stats/increased-winter-mortality/increased-winter-mortality-background-info.pdf
15. Healy JD. Excess winter mortality in Europe: a cross-country analysis identifying key risk fac-tors. J Epidemiol Community Health. 2003; 57(10):784-9. http://dx.doi.org/10.1136/jech.57.10.784 PMid:14573581 PMCid:1732295
16. Bowie N, Jackson G. The raised incidence of winter deaths. General Register Office for Scotland; 2002. [Accessed 11 Apr 2013]. Available from: http://www.gro-scotland.gov.uk/files2/stats/increased-winter-mortality/occ-paper-winter-deaths.pdf
17. Wood SN. Generalized Additive Models: An Introduction with R. London: Chapman & Hall/CRC; 2006.
18. Harvey A, Koopman SJ, Riani M. The Modeling and Seasonal Adjustment of Weekly Observations. J Bus Econ Stat. 1997;15(3):354-68.

19. Health Protection Scotland (HPS). Weekly Influenza Situation Report (Including H1N1v). Glasgow: HPS; 3 Feb 2010. Situation Report: 31. [Accessed 20 Apr 2012]. Available from: http://www.documents.hps.scot.nhs.uk/respiratory/swine-influenza/situation-reports/weekly-influenza-sitrep-2010-02-04.pdf
20. Wagner AP. Extending Scottish Exception Reporting Systems Spatially and Temporally [PhD thesis]. Glasgow: University of Strathclyde; 2010.
21. Zuur A, Ieno EN, Walker N, Saveliev AA, Smith GM. Mixed Effects Models and Extensions in Ecology with R. New York: Springer; 2009. http://dx.doi.org/10.1007/978-0-387-87458-6
22. Sakamoto Y, Ishiquro M, Kitagawa G. Akaike Information Criterion Statistics. 2nd ed. London: Springer; 1986.
23. Kavanagh K, Robertson C, Murdoch H, Crooks G, McMenamin J. Syndromic surveillance of influenza-like illness in Scotland during the influenza A H1N1v pandemic and beyond. J R Stat Soc Ser A Stat Soc. 2012;175(4):939-58. http://dx.doi.org/10.1111/j.1467-985X.2012.01025.x
24. Health Protection Scotland (HPS). Influenza Update Report (Week 2, 2010/11). Glasgow: HPS; 20 Jan 2011. [Accessed 20 Apr 2012]. Available from: http://www.documents.hps.scot.nhs.uk/respiratory/seasonal-influenza/flu-update/2011-01-20.pdf
25. World Health Organization (WHO). WHO statement: Comparing deaths from pandemic and seasonal influenza. Geneva: WHO. [Accessed 20 Apr 2012]. Available from: http://www.who.int/csr/disease/swineflu/notes/briefing_20091222/en/index.html
26. Durban M, Harezlak J, Wand MP, Carroll RJ. Simple fitting of subject-specific curves for longitudinal data. Stat Med. 2005;2498):1153-67.
27. Farrington CP, Andrews NJ, Beale AD, Catchpole MA. A statistical algorithm for the early detection of outbreaks of infectious diseases. J R Stat Soc Ser A Stat Soc. 1996;159(3):547-63. http://dx.doi.org/10.2307/2983331
28. Heisterkamp SH, Jager JC, Ruitenberg EJ, van Druten JA, Downs AM. Correcting reported AIDS incidence: a statistical approach. Stat Med. 1989;8(8):963-76. http://dx.doi.org/10.1002/sim.4780080807 PMid:2799125
29. Kanieff M, Rago G, Minelli G, Lamagni T, Sadicova O, Šelb J, et al. The potential for a concerted system for the rapid monitoring of excess mortality throughout Europe. Euro Surveill. 2010;15(43):pii=19697. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19697 PMid:21087579
30. Höhle M, Paul M. Count data regression charts for the monitoring of surveillance time series. Comput Stat Data Anal. 2008; 52(9):4357-68 http://dx.doi.org/10.1016/j.csda.2008.02.015

Prevailing effectiveness of the 2009 influenza A(H1N1) pdm09 vaccine during the 2010/11 season in Sweden

K Widgren (katarina.widgren@smi.se)[1], M Magnusson[1], P Hagstam[2], M Widerström[3], Å Örtqvist[4,5], I M Einemo[6], P Follin[7], A Lindblom[8], S Mäkitalo[9], O Wik[10], A Österlund[11], M Grünewald[1], I Uhnoo[12], A Linde[1]

1. Department of Analysis and Prevention, Swedish Institute for Communicable Disease Control, Solna, Sweden
2. Department of Communicable Disease Control and Prevention, Skåne County, Malmö, Sweden
3. Department of Communicable Disease Control and Prevention Jämtland County, Östersund, Sweden
4. Department of Communicable Disease Control and Prevention, Stockholm County, Stockholm, Sweden
5. Department of Medicine, Unit of Infectious Diseases, Karolinska Institute, Solna, Sweden
6. Department of Communicable Disease Control and Prevention, Jönköping Count, Jönköping, Sweden
7. Department of Communicable Disease Control and Prevention, Västra Götaland County, Gothenburg, Sweden
8. Department of Communicable Disease Control and Prevention, Dalarna County, Falun, Sverige
9. Department of Communicable Disease Control and Prevention, Gävleborg County, Gävle, Sweden
10. Department of Communicable Disease Control and Prevention, Värmland County, Karlstad, Sweden
11. Department of Communicable Disease Control and Prevention, Norrbotten County, Luleå, Sweden
12. Department of Diagnostics and Vaccinology, Swedish Institute for Communicable Disease Control, Solna, Sweden

Citation style for this article:
Widgren K, Magnusson M, Hagstam P, Widerström M, Örtqvist Å, Einemo IM, Follin P, Lindblom A, Mäkitalo S, Wik O, Österlund A, Grünewald M, Uhnoo I, Linde A. Prevailing effectiveness of the 2009 influenza A(H1N1)pdm09 vaccine during the 2010/11 season in Sweden. Euro Surveill. 2013;18(15):pii=20447. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20447

Article submitted on 23 April 2012 / published on 11 April 2013

Sixty per cent of the Swedish population received the monovalent AS03-adjuvanted pandemic influenza vaccine in the autumn of 2009. We assessed the age-specific effectiveness of this pandemic vaccine against hospitalisation with laboratory-confirmed influenza A(H1N1)pdm09 during the season 2010/11, in the age group from six months to 64 years in Sweden. The screening method was applied to available surveillance data. Our results suggest a prevailing effectiveness of 72% (95% confidence interval (CI): 63–80%) with the highest effectiveness among children, six months to nine years-old (92%, 95%CI: 80–97%). However, there were limitations in data quality and study design due to the lack of systematic recording of administered vaccinations, which underline the importance of preparing for an evaluation when planning for large public health actions. Despite these limitations, we believe the results reflect true, high prevailing vaccine effectiveness. Indeed, there were fewer deaths caused by influenza and the impact of influenza on intensive care units was less severe during the 2010/11 season in Sweden than in countries with lower pandemic vaccination coverage. The association between the pandemic vaccine and narcolepsy has increased the importance of assessing the risks and benefits of the vaccination; studies on the effectiveness and the duration of protection are needed for this.

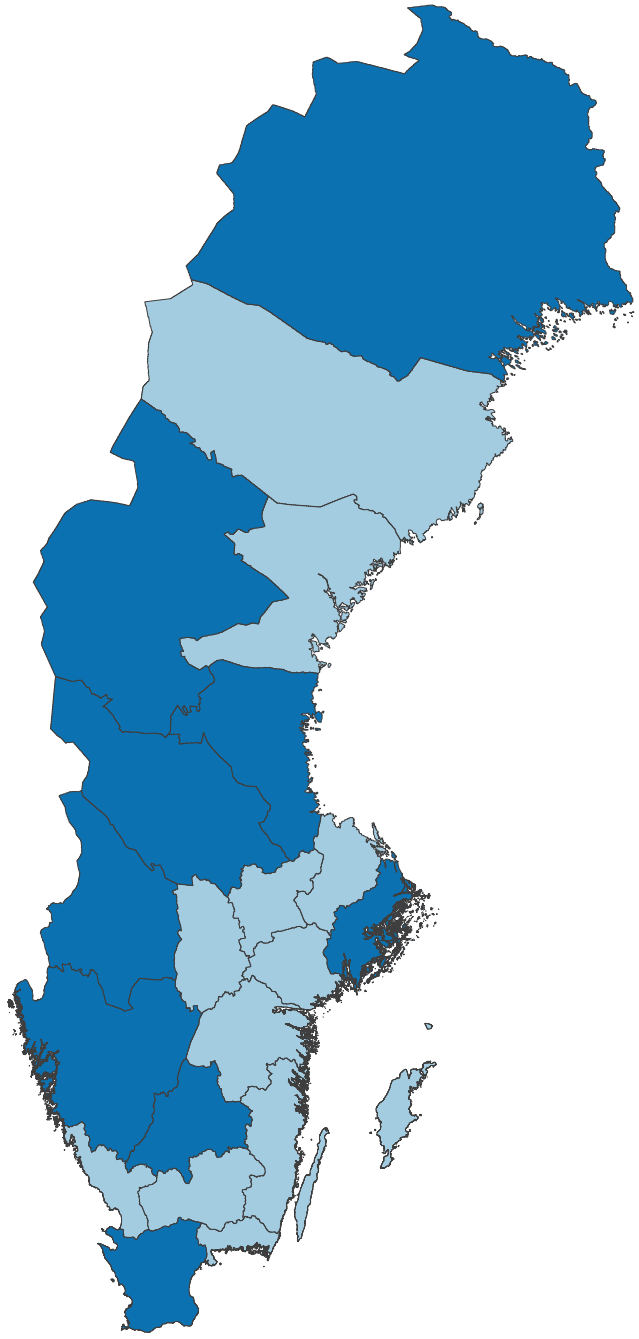
Background

In the pandemic situation of 2009, Sweden chose to conduct a mass vaccination campaign using the Pandemrix vaccine, a monovalent vaccine containing an influenza A/California/7/2009(H1N1)v-like strain, adjuvanted with AS03 (squalene, DL-alpha-tokoferol and polysorbate 80). This was the only pandemic vaccine available at the time in Sweden. The whole population was offered the vaccination free of charge. Within a period of 10 weeks from October to December 2009, 60% of the population received at least one dose of the pandemic influenza vaccine. Thus, Sweden had the highest national pandemic vaccination coverage in the European Union [1]. During seasonal influenza years, only non-adjuvanted trivalent inactivated influenza vaccines have been used in the country. Adjuvanted vaccines are considered to elicit a stronger, longer-lasting and broader immune response [2,3] and adjuvants make it possible to save time by producing larger quantities of vaccine with a smaller amount of antigen. Therefore, adjuvanted vaccines had been recommended by the World Health Organization in preparation for a pandemic of influenza A(H5N1) in 2005 [4] and were also supported for influenza A(H1N1)pdm09 vaccines in 2009 [5]. Accordingly, the Swedish government had already in 2007 closed a contract with a pharmaceutical company for the purchase of an adjuvanted vaccine for the entire population in the case of an influenza pandemic [6].

Several studies have been carried out worldwide to investigate the effectiveness of pandemic influenza vaccines during the first pandemic season 2009/10 [7-22]. Studies of AS03-adjuvanted, monovalent vaccines showed high effectiveness against influenza hospitalisation [17] and laboratory-confirmed influenza attended in primary care [18-21]. In Sweden, the weekly vaccine effectiveness against notified, laboratory-confirmed pandemic influenza was estimated at 87–95% in the population of Stockholm (ca. 2 million people) [22].

Figure 1

Counties providing age-specific coverage for the Pandemrix vaccine, Sweden, 2009



Dark blue: counties that provided coverage data.

In the subsequent influenza season, 2010/11, the pandemic virus strain was included in the trivalent seasonal vaccine, together with an influenza A/Perth/16/2009 (H3N2) strain and an influenza B/Brisbane/60/2008 strain. The seasonal vaccination was offered free of charge or at reduced cost to people belonging to risk groups and people aged 65 years and older. During that season the pandemic strain was in circulation, mainly around the New Year [23]. The close match between the 2009 pandemic vaccine strain and the 2010/11 influenza A(H1N1)pdm09 virus strain made it likely that the population could in the post-pandemic season still be protected by the pandemic vaccine administered more than one year earlier.

The pandemic mass vaccination in 2009 was a large and costly undertaking and therefore needed to be monitored and evaluated. However, at the time, a country-wide evaluation of the vaccine effectiveness was not planned, and data for it were not systematically collected in Sweden. Since evidence emerged on an association between the pandemic vaccination and the severe adverse event narcolepsy in children [24-29], an evaluation has become even more important. Studies on the effectiveness and the duration of protection induced by the pandemic vaccine are needed for an overall assessment of the vaccination and its risks and benefits. For this study, we used the best available data and methods to assess quickly the prevailing vaccine effectiveness against hospitalisation with laboratory-confirmed influenza A(H1N1)pdm09 one year after the pandemic vaccination campaign.

Methods

In Sweden, communicable disease control at the county level is coordinated by the 21 County Medical Officers (CMO). Currently, vaccination coverage data is also collected and administered by the CMOs. Different techniques for registering pandemic vaccination data were used in 2009, including: local vaccination registers, data extraction from medical charts, and a web-based vaccination register implemented in some counties (Svevac). Nine of 21 CMOs were able to provide the age-specific number of pandemic vaccinations (dose 1) administered during the autumn 2009. These nine counties comprise 68% of the Swedish population, they are scattered geographically (Figure 1) and cover the three major urban areas as well as the more scarcely populated areas in the north.

The organisation of the healthcare system does not differ much between the Swedish counties. The overall vaccination coverage in all counties versus those counties where age-specific vaccination coverage was available had overlapping ranges (54–70% versus 54–69%). We assumed that the vaccination coverage in counties with unknown age-specific coverage followed a normal distribution with the same, but unknown, mean as in the counties with known coverage. The total vaccination coverage in 2009, PPV, was estimated as follows:

$$\hat{PPV} = \sum_{i \in \mathcal{S}} w_i p_i + \hat{\mu} \sum_{i \notin \mathcal{S}} w_i$$

where $\mathbf{w}_i$ and $\mathbf{p}_i$ are the proportion of the Swedish population and vaccination coverage in county $\boldsymbol{I}$, respectively, $\boldsymbol{S}$ is the sample of counties with known vaccination coverage, and $\hat{\boldsymbol{\mu}}$ is the estimate of the mean vaccination coverage, based on the counties with known coverage in $\boldsymbol{S}$.

To calculate age-specific vaccination coverage we used population data as of December 2009 (Statistics Sweden).

Pandemic influenza was made notifiable with full patient identification in Sweden at the start of the 2009 pandemic and has since remained so. Laboratories are obliged by law to report all laboratory-confirmed cases to the CMO and the Swedish Institute for Communicable Disease Control (Smittskyddsinstitutet; SMI). The diagnostic method used is an in-house H1N1-specific real-time PCR. Sensitivity and specificity were assessed by quality control panels from SMI. Doctors who admit a patient to hospital for suspected pandemic influenza are also obliged by law to report the case. Clinical signs and symptoms are not specified in the national case definition, which is based on laboratory confirmation, as is the case for all notifiable diseases in Sweden. Swabbing of suspected cases is in principle mandatory, since pandemic influenza is a notifiable disease. Any delay between onset of symptoms and swabbing is accepted. Laboratory notifications of confirmed cases are matched in SmiNet (the national database for notifications) to notification forms of hospitalised cases. For hospitalised cases, the notification form contains voluntary questions on vaccination and risk group status. Risk groups are defined as people with chronic respiratory disease, cardiovascular disease, obesity of class III (body mass index >40kg/m²), neurological disorders with impaired breathing capacity, immunosuppression, chronic liver or kidney failure, severe diabetes, severe asthma and pregnancy as well as cerebral palsy or other neuromuscular disorders in children. All clinical notification forms were examined and updated by the CMOs at the end of the 2010/11 influenza season.

In this study, we included cases who had a laboratory-confirmed influenza A(H1N1)pdm09 infection and who

Table 1

Notified influenza A(H1N1)pdm09 cases[a] hospitalised in Sweden during the peak of the 2010/11 influenza A(H1N1)pdm09 season (n=252)

	Cases with known vaccination status (n=215)	Cases with unknown vaccination status (n=37)	p value
	Median	Median	
Age (years)	35	44	0.062[b]
	n (%)	n (%)	
Age group			
6 months–9 years	26 (12)	3 (8)	0.617[c]
10–19 years	18 (8)	2 (5)	
20–39 years	82 (38)	12 (32)	
40–64 years	89 (41)	20 (54)	
Sex			
Female	111 (52)	15 (41)	0.213[d]
Male	104 (48)	22 (59)	
Risk group			
Yes	92 (43)	9 (24)	0.160d[e]
No	107 (50)	19 (51)	0.003d[f]
Unknown	16 (7)	9 (24)	
Pandemic vaccination			
Yes	63 (29)	Missing data	Not applicable
No	152 (71)	Missing data	Not applicable

[a] Restricted to cases aged six months to 64 years at the time of the 2009 pandemic influenza vaccination.
[b] Wilcoxon rank-sum test.
[c] Fischer's exact test.
[d] Chi-square test.
[e] Excluding cases with unknown risk group status.
[f] Including all cases.

were admitted to hospital according to a notification form from a hospital doctor or according to the CMO. We only included cases who were hospitalised during the peak of the influenza A(H1N1)pdm09 season 2010/11, defined as the time period with at least 50 notified cases per week nationally, i.e. between week 52/2010 and week 7/2011. Cases with unknown pandemic vaccination status were excluded from the main analysis. The excluded cases were compared to cases with known vaccination status with regard to potential confounding factors, such as age, sex and risk group status, using appropriate statistical tests (Table 1).

Vaccine effectiveness (VE) was assessed using the screening method, where data on vaccination coverage among cases (proportion of cases vaccinated, PCV) and in the population (proportion of population vaccinated, PPV) were inserted in the formula VE=((PPV-PCV)/(PPV(1-PCV))*100 [30]. We used the method described by Farrington to obtain confidence intervals (CI) [31] and used the point estimate of PPV for the calculations.

For both PCV and PPV estimates, age at the time of the 2009 pandemic vaccination was used, i.e. more than a year before the outcome. Overall age-adjusted vaccine effectiveness was estimated as well as age-specific vaccine effectiveness for the following age groups: six months to nine years, 10 to 19 years, 20 to 39 years and 40 to 64 years. Children younger than six months at the time of the vaccination campaign were not eligible for the vaccination. Those aged 65 years and older had high vaccination coverage of the 2010/11 seasonal vaccine, which could have interfered with the results. Thus, both groups were excluded from the analysis. There was not enough statistical power to stratify both by age group and county. We chose to stratify by age group as this is a biologically more plausible confounder. We also carried out an analysis restricted only to cases that did not belong to risk groups. Since we did not have data on vaccination coverage in risk groups in the population, the entire general population of the same age was used as control group for this analysis.

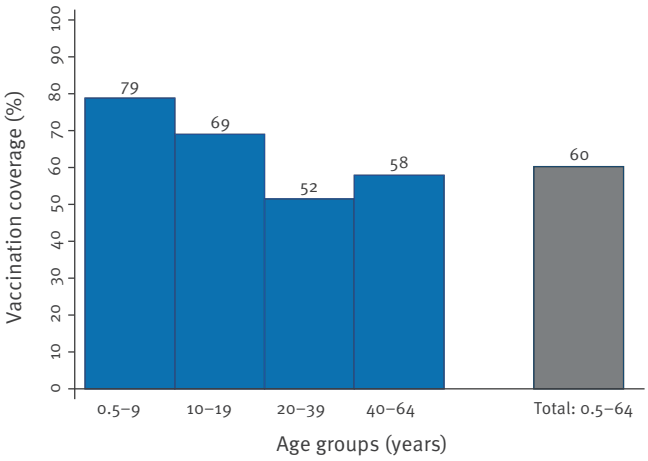
Results

Some 320 cases with influenza A(H1N1)pdm09 infection were hospitalised during the peak period (week 52/2010 to week 7/2011). Thirty-eight cases were younger than six months in the autumn of 2009 and 30 cases were 65 years or older, and therefore excluded. Vaccination status regarding the pandemic vaccine was known for 215 (85%) of the remaining 252 cases, and these were kept in the final analysis. A majority of cases were between 20 and 64 years-old (Table 1). There were no statistically significant differences with regard to age, sex or risk group status between the groups with and without known vaccination status (Table 1).

Vaccination coverage was estimated at 60% in the general population (aged six months to 64 years), with the highest coverage of 79% in children between the age of six months and nine years (Figure 2).

Figure 2

Age-specific coverage of pandemic influenza vaccination modelled on reports from nine[a] of 21 counties in Sweden, 2009



[a] The nine counties represent 68% of the population in Sweden.

The overall age-adjusted (six months to 64 years) vaccine effectiveness was 72% (95% CI: 63–80%), and highest in the youngest age group six months to nine years, with 92% (95% CI: 80–97%) (Table 2).

At least 43% of hospitalised influenza cases belonged to a risk group (Table 1). When restricting the analysis only to cases not belonging to a risk group, the vaccine effectiveness estimate increased to 82% (95%CI: 72–89%).

Table 2

Age-specific effectiveness for the pandemic influenza vaccine administered during the autumn of 2009 against hospitalisation with influenza A(H1N1)pdm09 infection during the peak of the influenza A(H1N1)pdm09 season in 2010/11 in Sweden

Age group	Vaccine effectiveness % (95% CI)
6 months–9 years	92% (80–97%)
10–19 years	78% (40–92%)
20–39 years	68% (47–80%)
40–64 years	63% (43–76%)
Total (6 months–64 years)	**72% (63–80%)**

CI: confidence interval.

Discussion

We applied the screening method to available surveillance data to assess the impact in the post-pandemic season 2010/11 of the adjuvanted pandemic influenza vaccine administered more than one year previously. Our results suggest a high prevailing vaccine effectiveness against hospitalisation with laboratory-confirmed influenza A(H1N1)pdm09 among the Swedish population aged between six months and 64 years, with the highest protection in the young children. If verified by other studies, this could have important public health implications. Trivalent inactivated vaccines against seasonal influenza have not successfully induced protective immunity in children naïve to the virus [32-34]. Adjuvanted vaccines induce higher levels of haemagglutination inhibition (HI) and neutralising antibodies against influenza virus in naïve children [35-37] and in adults [38] than the non-adjuvanted trivalent vaccines. High antibody persistence one year after vaccination was demonstrated in children who had received two doses of the AS03-adjuvanted vaccine in the United Kingdom, with a significant difference compared with children who had received a non-adjuvanted vaccine [39]. In a study of children in Canada, persistent antibody titres were found after one dose of an AS03-adjuvanted influenza vaccine [40]. In addition, Swedish serological data confirmed that more than 80% of 5–14 year-old Swedish children had sustained elevated HI-antibody titres of ≥40 in May 2011, 18 months after the pandemic vaccination campaign [41]. Although antibody titres are an unspecific marker for influenza immunity, these results are in line with our findings of persistent protection. Such lasting protection and the ability to induce priming in children are extremely beneficial in a pandemic situation.

However, the association between the pandemic influenza vaccine and narcolepsy seen in children in Finland, Ireland, Norway and Sweden [24-29] has raised serious concerns about the safety of Pandemrix. The European Medicines Agency recommends a restricted use of the vaccine in persons younger than 20 years [42]. An association with such a severe adverse event makes recommendations to use Pandemrix and similarly adjuvanted vaccines very difficult to adopt and implement, probably even in the context of a new pandemic. Thus, the association between the vaccine and narcolepsy needs to be thoroughly understood in order to make decisions on the future use of similar vaccines. Many studies on the link between narcolepsy and the pandemic vaccine are ongoing and will hopefully provide guidance on this issue.

Before the pandemic, very few studies had looked at the lasting protection of influenza vaccination. However, several studies have now investigated the prevailing vaccine effectiveness of pandemic influenza vaccines in the 2010/11 season [43-48]. Firstly, some of these studies have found that the vaccine effectiveness was higher for people who had received both the pandemic vaccine and the seasonal 2010/11 trivalent influenza vaccine than for those who had received only one [44,45]. Secondly, compared with our estimates, some have found lower prevailing vaccine effectiveness after receipt of only the pandemic vaccine [43-46]. However, the results from these studies cannot be easily compared with ours for one or several reasons. (i) They used another outcome: primary care-attended laboratory-confirmed influenza instead of severe influenza; (ii) they used other pandemic vaccines or a mix of vaccines; (iii) they included different age groups and/or restricted the analysis to risk groups or people with comorbidities. Moreover, these studies used more elaborate study designs such as the test-negative case–control design or cohort design. By using the screening method, our study is more prone to bias and confounding, and differences could be explained by positive confounding in our study.

Nonetheless, our findings of a high prevailing effectiveness of the pandemic vaccine, particularly in the young, are supported by results from Canada, where a similar vaccine was used. In a test-negative case–control study the prevailing effectiveness of an AS03-adjuvanted monovalent pandemic vaccine was 66% in all patients and 76% among young adults [47]. In addition, the high degree of protection from vaccination in Sweden may have reduced the virus burden in the population, resulting in lower infectious doses with a more limited possibility of overcoming the vaccine-induced protection.

Our aim was to achieve an assessment of prevailing vaccine effectiveness easily and quickly, but it proved to be a cumbersome process, due to the lack of vaccination registers. This underlines the importance of concurrent planning of the implementation and the evaluation of large public health actions. We used the best available data and method. However, both have inherent limitations and the results need to be interpreted with caution. We tried to address the potential limitations one by one. To increase the quality of the influenza surveillance data in the country, the CMOs were asked to cross-check and validate vaccination and hospitalisation status for each notified case at the end of the season. The completeness of the data was more difficult to improve or assess. All laboratory-confirmed cases are subject to mandatory reporting, which is mainly an automated process. Swabbing of suspected cases is in principle also mandatory as pandemic influenza is a notifiable disease, but we could not assess the extent to which this was done and thus how complete the reporting was. As expected, when working with surveillance data we faced issues with missing data. Because we aimed at a quick and easy estimate of prevailing vaccine effectiveness, we chose to exclude cases without data on vaccination status rather than using imputation techniques. There were no apparent differences between cases with and without data on vaccination status in the descriptive analysis (Table 1). Nonetheless, there could have been differences in these groups that we have failed to adjust for.

Vaccine effectiveness studies can be affected both by 'confounding by indication' and by confounding due to 'healthy vaccinee' effect, leading to an underestimation and an overestimation of results, respectively [49]. Also, the screening method may overestimate the vaccine effectiveness when the coverage is high [30]. Generally, the main confounder in vaccine effectiveness studies is age; to address this we stratified and adjusted our analysis by age group. Furthermore, since we used surveillance data, we were not able to adjust for receipt of the trivalent 2010/11 seasonal influenza vaccine, which was a major confounder in other studies [44,45]. In order to address this limitation, we restricted our analysis to an age group where seasonal vaccination is generally not recommended except for risk groups. Only 7% (10 of 141 cases with known seasonal vaccination status; data not shown) of hospitalised cases had had the seasonal vaccination, and according to vaccination coverage surveys carried out by SMI and others [50], this is in line with the seasonal vaccination coverage in this age group in previous years. Hence, this factor should not play a major role as a confounder in our analysis. On the other hand, the impact of natural immunity due to infection during the pandemic season is difficult to predict. Mass vaccination was carried out concurrently with the peak of the pandemic. Refusal, delay or even request of vaccination due to ongoing or previous influenza symptoms as well as boosting of vaccine response due to subclinical infection is plausible. Again, both an overestimation and an underestimation of the results are possible.

Yet another factor to take into account is risk group status. Nearly half of the hospitalised cases included in the study belonged to a risk group. There is no national data on exact risk group prevalence in the general population, although, according to surveys carried out by SMI between 4% and 14% of people in the relevant age groups consider themselves to belong to a risk group for influenza vaccination. It is fair to say that people belonging to risk groups are overrepresented among our cases. These groups were targeted and prioritised for pandemic vaccination and higher vaccination coverage among them is anticipated, making risk group status a likely confounder in our study. Since we could not adjust for this factor, we carried out a sensitivity analysis; vaccine effectiveness slightly increased when we restricted the analysis to cases that did not belong to risk groups, an indication that failing to adjust for risk factor status have at least not lead to an overestimation of results in our main analysis.

Sweden had 1.1 influenza-related deaths per 10^6 population during the winter 2010/11 [23], a rate dramatically lower than described in countries with lower pandemic vaccination coverage [51,52], and the impact on intensive care units was substantially lower in Sweden than elsewhere [51-53]. In fact, some countries have described a higher burden of severe disease in the post-pandemic season than during the pandemic season [51,54], which was not the case for Sweden [23]. Notwithstanding the limitations above, we believe our estimates reflect a high, prevailing influenza vaccine effectiveness of the adjuvanted vaccine administered in 2009, protecting a large part of the Swedish population against hospitalisation with influenza A(H1N1) pdm09 infection also during the 2010/11 season.

Acknowledgements

We would like to thank reporting doctors. We would also like to thank Marta Valenciano at EpiConcept, Baltazar Nunes at the Instituto Nacional de Saúde Dr Ricardo Jorge and the I-MOVE network as well as AnnaSara Carnahan at the Swedish Institute for Communicable Disease Control.

References

1. European Centre for Disease Prevention and Control (ECDC). Seasonal Influenza 2010-2011 in Europe (EU/EEA countries). Stockholm: ECDC; 2011. Available from: http://ecdc.europa.eu/en/publications/publications/110125_ra_seasonal_influenza_eu-eea_2010-2011.pdf
2. Walker WT, Faust SN. Monovalent inactivated split-virion AS03-adjuvanted pandemic influenza A (H1N1) vaccine. Expert Rev Vaccines. 2010;9(12):1385-98. http://dx.doi.org/10.1586/erv.10.141. PMid:21105775.
3. Nichol KL, Treanor JJ. Vaccines for seasonal and pandemic influenza. J Infect Dis. 2006;194 Suppl 2:S111-8. http://dx.doi.org/10.1086/507544. PMid:17163383.
4. World Health Organization (WHO). Influenza pandemic preparedness and response, Report by the secretariat. Geneva: WHO; 2005. Contract No.: EB115/44. Available from: http://www.wpro.who.int/emerging_diseases/documents/docs/B115_44en.pdf
5. Pandemic influenza A (H1N1) 2009 virus vaccine - conclusions and recommendations from the October 2009 meeting of the immunization Strategic Advisory Group of Experts. Wkly Epidemiol Rec. 2009;84(49):505-8. PMid:19960623.
6. Influensa A(H1N1) 2009 - Utvärdering och förberedelser för hantering av pandemin. [Influenza A(H1N1) 2009 - Evaluation and preparation for managament of the pandemic]. Stockholm: Socialstyrelsen, Karlstad: Myndigheten för samhällsskydd och beredskap; 2011. ISBN: 978-91-86585-84-6. Swedish. Available from: http://www.socialstyrelsen.se/Lists/Artikelkatalog/Attachments/18243/2011-3-3.pdf
7. Cheng AC, Kotsimbos T, Kelly HA, Irving LB, Bowler SD, Brown SG, et al. Effectiveness of H1N1/09 monovalent and trivalent influenza vaccines against hospitalization with laboratory-confirmed H1N1/09 influenza in Australia: A test-negative case control study. Vaccine. 2011;29(43):7320-5. http://dx.doi.org/10.1016/j.vaccine.2011.07.087. PMid:21810450.
8. Valenciano M, Kissling E, Cohen JM, Oroszi B, Barret AS, Rizzo C, et al. Estimates of pandemic influenza vaccine effectiveness in Europe, 2009-2010: results of Influenza Monitoring Vaccine Effectiveness in Europe (I-MOVE) multicentre case-control study. PLoS Med. 2011;8(1):e1000388. http://dx.doi.org/10.1371/journal.pmed.1000388. PMid:21379316. PMCid:3019108.
9. Hardelid P, Fleming DM, McMenamin J, Andrews N, Robertson C, SebastianPillai P, et al. Effectiveness of pandemic and seasonal influenza vaccine in preventing pandemic influenza A(H1N1)2009 infection in England and Scotland 2009-2010. Euro Surveill. 2011;16(2):pii=19763. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19763
10. Griffin MR, Monto AS, Belongia EA, Treanor JJ, Chen Q, Chen J, et al. Effectiveness of Non-Adjuvanted Pandemic Influenza A Vaccines for Preventing Pandemic Influenza Acute Respiratory Illness Visits in 4 U.S. Communities. PloS One. 2011;6(8):e23085. http://dx.doi.org/10.1371/journal.pone.0023085. PMid:21857999. PMCid:3155536.
11. Pelat C, Falchi A, Carrat F, Mosnier A, Bonmarin I, Turbelin C, et al. Field effectiveness of pandemic and 2009-2010 seasonal vaccines against 2009-2010 A(H1N1) influenza: estimations from surveillance data in France. PloS One. 2011;6(5):e19621. http://dx.doi.org/10.1371/journal.pone.0019621. PMid:21573005. PMCid:3091864.
12. Steens A, Wijnans EG, Dieleman JP, Sturkenboom MC, van der Sande MA, van der Hoek W. Effectiveness of a MF-59-adjuvanted pandemic influenza vaccine to prevent 2009 A/H1N1 influenza-related hospitalisation; a matched case-control study. BMC Infect Dis. 2011;11:196. http://dx.doi.org/10.1186/1471-2334-11-196. PMid:21767348. PMCid:3154871.
13. Steens A, van der Hoek W, Dijkstra F, van der Sande M. Influenza vaccine effectiveness, 2010/11. Euro Surveill. 2011;16(15):pii=19843. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19843. PMid:21507318.
14. Hardelid P, Fleming DM, Andrews N, Barley M, Durnall H, Mangtani P, et al. Effectiveness of trivalent and pandemic influenza vaccines in England and Wales 2008-2010: results from a cohort study in general practice. Vaccine. 2012;30(7):1371-8. http://dx.doi.org/10.1016/j.vaccine.2011.12.038. PMid:22178524.
15. Fielding JE, Grant KA, Garcia K, Kelly HA. Effectiveness of seasonal influenza vaccine against pandemic (H1N1) 2009 virus, Australia, 2010. Emerg Infect Dis. 2011;17(7):1181-7. http://dx.doi.org/10.3201/eid1707.101959. PMid:21762570. PMCid:3381383.
16. Puig-Barbera J, Arnedo-Pena A, Pardo-Serrano F, Tirado-Balaguer MD, Perez-Vilar S, Silvestre-Silvestre E, et al. Effectiveness of seasonal 2008-2009, 2009-2010 and pandemic vaccines, to prevent influenza hospitalizations during the autumn 2009 influenza pandemic wave in Castellon, Spain. A test-negative, hospital-based, case-control study. Vaccine. 2010;28(47):7460-7. http://dx.doi.org/10.1016/j.vaccine.2010.09.042. PMid:20875486.
17. Gilca R, Deceuninck G, De Serres G, Boulianne N, Sauvageau C, Quach C, et al. Effectiveness of Pandemic H1N1 Vaccine Against Influenza-Related Hospitalization in Children. Pediatrics. 2011;128(5):e1084-91. http://dx.doi.org/10.1542/peds.2010-3492. PMid:21987710.
18. Skowronski DM, Janjua NZ, De Serres G, Hottes TS, Dickinson JA, Crowcroft N, et al. Effectiveness of AS03 adjuvanted pandemic H1N1 vaccine: case-control evaluation based on sentinel surveillance system in Canada, autumn 2009. BMJ. 2011;342:c7297. http://dx.doi.org/10.1136/bmj.c7297. PMid:21292718. PMCid:3033439.
19. Wichmann O, Stocker P, Poggensee G, Altmann D, Walter D, Hellenbrand W, et al. Pandemic influenza A(H1N1) 2009 breakthrough infections and estimates of vaccine effectiveness in Germany 2009-2010. Euro Surveill. 2010;15(18):pii=19561. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19561. PMid:20460094.
20. Emborg HD, Krause TG, Hviid A, Simonsen J, Mølbak K. Effectiveness of vaccine against pandemic influenza A/H1N1 among people with underlying chronic diseases: cohort study, Denmark, 2009-10. BMJ. 2011;344:d7901. http://dx.doi.org/10.1136/bmj.d7901. PMid:22277542.
21. Andrews N, Waight P, Yung CF, Miller E. Age-specific effectiveness of an oil-in-water adjuvanted pandemic (H1N1) 2009 vaccine against confirmed infection in high risk groups in England. J Infect Dis. 2011;203(1):32-9. http://dx.doi.org/10.1093/infdis/jiq014. PMid:21148494. PMCid:3086445.
22. Örtqvist A, Berggren I, Insulander M, de Jong B, Svenungsson B. Effectiveness of an adjuvanted monovalent vaccine against the 2009 pandemic strain of influenza A(H1N1)v, in Stockholm County, Sweden. Clin Infect Dis. 2011;52(10):1203-11. http://dx.doi.org/10.1093/cid/cir182. PMid:21507917.
23. Smittskyddsinstitutet (SMI). Influensa i Sverige, säsongen 2010-2011. [Influenza in Sweden, the 2010-2011 season]. Stockholm: SMI; 2011. Swedish. Available from: http://www.smittskyddsinstitutet.se/upload/Publikationer/Influensa-i-Sverige-2010-11-artikelnr-2011-15-2.pdf
24. Läkemedelsverket. Occurrence of narcolepsy with cataplexy among children and adolescents in relation to the H1N1 pandemic and Pandemrix vaccinations - Results of a case inventory study by the MPA in Sweden during 2009-2010. Stockholm: Läkemedelsverket; 2011. Available from: http://www.lakemedelsverket.se/upload/nyheter/2011/Fallinventeringsrapport_pandermrix_110630.pdf
25. National Narcolepsy Task Force Interrim Report. Helsinki: National Institute for Health and Welfare; 2011. Available from: http://www.thl.fi/thl-client/pdfs/dce182fb-651e-48a1-b018-3f774d6d1875
26. Nohynek H, Jokinen J, Partinen M, Vaarala O, Kirjavainen T, Sundman J, et al. AS03 Adjuvanted AH1N1 Vaccine Associated with an Abrupt Increase in the Incidence of Childhood Narcolepsy in Finland. PloS One. 2012;7(3):e33536. http://dx.doi.org/10.1371/journal.pone.0033536. PMid:22470453. PMCid:3314666.
27. Partinen M, Saarenpaa-Heikkila O, Ilveskoski I, Hublin C, Linna M, Olsen P, et al. Increased Incidence and Clinical Picture of Childhood Narcolepsy following the 2009 H1N1 Pandemic Vaccination Campaign in Finland. PloS One. 2012;7(3):e33723. http://dx.doi.org/10.1371/journal.pone.0033723. PMid:22470463. PMCid:3314680.
28. Investigation of an increase in the incidence of narcolepsy in children and adolescents in 2009 and 2010 - Final Report of National Narcolepsy Study Steering Committee. Dublin: Irish Department of Health; 2012. Available from: http://healthupdate.gov.ie/wp-content/uploads/2012/04/Final_Report_of_National_Narcolepsy_Study_Steering_Committee-latest1.pdf
29. Folkehelseinstituttet (FHI). Rapport om utredning av narkolepsi etter Pandemrix [Report from the investigation of narcolepsy after Pandemrix]. Oslo: FHI; 2011. Norwegian. Available from: http://www.fhi.no/dav/842b470e7d.pdf
30. Orenstein WA, Bernier RH, Dondero TJ, Hinman AR, Marks JS, Bart KJ, et al. Field evaluation of vaccine efficacy. Bull World Health Organ. 1985;63(6):1055-68. PMid:3879673. PMCid:2536484.
31. Farrington CP. Estimation of vaccine effectiveness using the screening method. Int J Epidemiol. 1993;22(4):742-6. http://dx.doi.org/10.1093/ije/22.4.742. PMid:8225751.
32. Jefferson T, Rivetti A, Harnden A, Di Pietrantonj C, Demicheli V. Vaccines for preventing influenza in healthy children. Cochrane Database Syst Rev. 2008(2):CD004879. PMid:18425905.

33. Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2011. MMWR Morb Mortal Wkly Rep. 2011;60(33):1128-32. PMid:21866086.

34. Granström M, Voordouw AC. Registration of influenza vaccines for children in Europe. Vaccine. 2011;29(43):7572-5. http://dx.doi.org/10.1016/j.vaccine.2011.08.017. PMid:21820474.

35. Waddington CS, Walker WT, Oeser C, Reiner A, John T, Wilkins S, et al. Safety and immunogenicity of AS03B adjuvanted split virion versus non-adjuvanted whole virion H1N1 influenza vaccine in UK children aged 6 months-12 years: open label, randomised, parallel group, multicentre study. BMJ. 2010;340:c2649. http://dx.doi.org/10.1136/bmj.c2649. PMid:20508026. PMCid:2877808.

36. Vesikari T, Knuf M, Wutzler P, Karvonen A, Kieninger-Baum D, Schmitt HJ, et al. Oil-in-water emulsion adjuvant with influenza vaccine in young children. N Engl J Med. 2011;365(15):1406-16. http://dx.doi.org/10.1056/NEJMoa1010331. PMid:21995388.

37. Vesikari T, Pellegrini M, Karvonen A, Groth N, Borkowski A, O'Hagan DT, et al. Enhanced immunogenicity of seasonal influenza vaccines in young children using MF59 adjuvant. Pediatr Infect Dis J. 2009;28(7):563-71. http://dx.doi.org/10.1097/INF.0b013e31819d6394. PMid:19561422.

38. Ferguson M, Risi G, Davis M, Sheldon E, Baron M, Li P, et al. Safety and long-term humoral immune response in adults after vaccination with an H1N1 2009 pandemic influenza vaccine with or without AS03 adjuvant. J Infect Dis. 2012;205(5):733-44. http://dx.doi.org/10.1093/infdis/jir641. PMid:22315336.

39. Walker WT, de Whalley P, Andrews N, Oeser C, Casey M, Michaelis L, et al. H1N1 Antibody Persistence 1 Year After Immunization With an Adjuvanted or Whole-Virion Pandemic Vaccine and Immunogenicity and Reactogenicity of Subsequent Seasonal Influenza Vaccine: A Multicenter Follow-on Study. Clin Infect Dis. 2012;54(5):661-9. http://dx.doi.org/10.1093/cid/cir905. PMid:22267719. PMCid:3275760.

40. Gilca V, De Serres G, Hamelin ME, Boivin G, Ouakki M, Boulianne N, et al. Antibody persistence and response to 2010-2011 trivalent influenza vaccine one year after a single dose of 2009 AS03-adjuvanted pandemic H1N1 vaccine in children. Vaccine. 2011;30(1):35-41. http://dx.doi.org/10.1016/j.vaccine.2011.10.062. PMid:22063386.

41. Mörner A, Bråve A, Kling AM, Kühlmann-Berenzon S, Krook K, Hedenskog M, et al. Pandemic Influenza A(H1N1) pdm09 Seroprevalence in Sweden before and after the Pandemic and the Vaccination Campaign in 2009. PloS One. 2012;7(12):e53511. http://dx.doi.org/10.1371/journal.pone.0053511. PMid:23285299 PMCid:3532299.

42. European Medicines Agency (EMA). European Medicines Agency recommends restricting use of Pandemrix. Press release. London: EMA; 2011. [Accessed 19 Apr 2012]. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/public_health_alerts/2011/07/human_pha_detail_000034.jsp&mid=WC0b01ac058001d126&jsenabled=true#press-release

43. Kissling E, Valenciano M, Cohen JM, Oroszi B, Barret AS, Rizzo C, et al. I-MOVE multi-centre case control study 2010-11: overall and stratified estimates of influenza vaccine effectiveness in Europe. PloS One. 2011;6(11):e27622. http://dx.doi.org/10.1371/journal.pone.0027622. PMid:22110695 PMCid:3216983.

44. Pebody RG, Andrews N, Fleming DM, McMenamin J, Cottrell S, Smyth B, et al. Age-specific vaccine effectiveness of seasonal 2010/2011 and pandemic influenza A(H1N1) 2009 vaccines in preventing influenza in the United Kingdom. Epidemiol Infect. 2012;13:1-11.

45. Castilla J, Moran J, Martinez-Artola V, Reina G, Martinez-Baz I, Garcia Cenoz M, et al. Effectiveness of trivalent seasonal and monovalent influenza A(H1N1)2009 vaccines in population with major chronic conditions of Navarre, Spain: 2010/11 mid-season analysis. Euro Surveill. 2011;16(7):pii=19799. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19799. PMid:21345321.

46. Savulescu C, Jiménez-Jorge S, de Mateo S, Ledesma J, Pozo F, Casas I, et al. Effectiveness of the 2010/11 seasonal trivalent influenza vaccine in Spain: preliminary results of a case-control study. Euro Surveill. 2011;16(11):pii=19820. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19820. PMid:21435330.

47. Skowronski DM, Janjua NZ, De Serres G, Winter AL, Dickinson JA, Gardy JL, et al. A sentinel platform to evaluate influenza vaccine effectiveness and new variant circulation, Canada 2010-2011 season. Clin Infect Dis. 2012;55(3):332-42. http://dx.doi.org/10.1093/cid/cis431. PMid:22539661.

48. Jiménez-Jorge S, Savulescu C, Pozo F, de Mateo S, Casas I, Ledesma J, et al. Effectiveness of the 2010-11 seasonal trivalent influenza vaccine in Spain: cycEVA study. Vaccine. 2012;30(24):3595-602. http://dx.doi.org/10.1016/j.vaccine.2012.03.048. PMid:22472792.

49. Valenciano M, Ciancio B, Moren A, the influenza vaccine effectiveness working group. First steps in the design of a system to monitor vaccine effectiveness during seasonal and pandemic influenza in EU/EEA Member States. Euro Surveill. 2008;13(43):pii=19015. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19015. PMid:18947520.

50. Kroneman MW, van Essen GA. Variations in influenza vaccination coverage among the high-risk population in Sweden in 2003/4 and 2004/5: a population survey. BMC Public Health. 2007;7:113. http://dx.doi.org/10.1186/1471-2458-7-113. PMid:17570837. PMCid:1906854.

51. Athanasiou M, Baka A, Andreopoulou A, Spala G, Karageorgou K, Kostopoulos L, et al. Influenza surveillance during the post-pandemic influenza 2010/11 season in Greece, 04 October 2010 to 22 May 2011. Euro Surveill. 2011;16(44):pii=20004. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20004

52. Health Protection Agency (HPA). Surveillance of influenza and other respiratory viruses in the UK, 2010/11. London: HPA; 2011. Available from: http://www.hpa.org.uk/Publications/InfectiousDiseases/Influenza/1105influenzareport/

53. Glismann SO, Knudsen LK, Gubbels S, Mølbak K, Bragstad K, Nielsen LP. Influenza season 2010/2011. EpiNews. 2011;23. Available from: http://www.ssi.dk/English/News/EPI-NEWS/2011/No%2023%20-%202011.aspx

54. Bolotin S, Pebody R, White PJ, McMenamin J, Perera L, Nguyen-Van-Tam JS, et al. A New Sentinel Surveillance System for Severe Influenza in England Shows a Shift in Age Distribution of Hospitalised Cases in the Post-Pandemic Period. PloS One. 2012;7(1):e30279. http://dx.doi.org/10.1371/journal.pone.0030279. PMid:22291929. PMCid:3264602.

The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011 has been published

Eurosurveillance editorial team (eurosurveillance@ecdc.europa.eu)[1]
1. European Centre for Disease Prevention and Control, Stockholm, Sweden

Citation style for this article:
Eurosurveillance editorial team. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011 has been published. Euro Surveill. 2013;18(15):pii=20449. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20449

Article published on 11 April 2013

On 9 April 2013, the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) launched their joint annual report on zoonoses and food-borne outbreaks in 2011, the 'European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011' [1].

The report provides a comprehensive overview of zoonotic infections and disease outbreaks. It reports an increase in human infections from *Campylobacter* and *Escherischia coli* while the number of reported human cases of *Salmonella* infection fell in the reporting period. A total of 220,209 *Campylobacter* cases in humans were reported in 2011 which is an increase of 2.2 % from the previous year. The number of reported human cases of Shiga toxin/verocytotoxin-producing *E. coli* (STEC/VTEC) has increased since 2008 and the 2011 outbreak in Germany contributed further to the trend – 9,485 human cases were reported in 2011. Another zoonotic disease showing an increasing trend is alveolar echinococcosis, caused by the *Echinococcus multilocularis* parasite. A total of 781 cases of echinococcosis were reported in 2011, an increase of 3.3 % compared to 2010.

With 95,548 reported cases of salmonellosis in 2011, the trend continued downwards for this the second most reported zoonotic disease in humans. It reflects the continued efforts to eradicate *Salmonella* infections in poultry populations, especially in laving hens and hence also in eggs, as well as in chicken. The eradication is carried out via the European Union (EU) *Salmonella* control programmes, implemented by Member States and managed by the European Commission.

A total of 5,648 food-borne outbreaks were recorded in the EU in 2011. A food-borne outbreak is defined in the report as including two or more human cases having eaten the same, contaminated, food.

More information is available from the EFSA and ECDC websites.

References

1. European Food Safety Authority (EFSA), European Centre for Disease Prevention and Control (ECDC), 2013. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2011; EFSA Journal 2013,11(4):3129, 250 pp. doi:10.2903/j.efsa.2013.3129.