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Influenza – the need to stay ahead of the virus

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As illustrated in a series of publications in the current and previous issue of *Eurosurveillance* [1-3], the ongoing influenza epidemics in Europe and North America are dominated by influenza A(H3N2) viruses. The majority of these appear to vary antigenically from the current northern hemisphere vaccine strain A/Texas/50/2012(H3N2) and more closely related to the vaccine strain A/Switzerland/9715293/2013(H3N2) recommended for the 2014/15 season of the southern hemisphere. In line with the observed antigenic mismatch between circulating and vaccine A(H3N2) viruses, preliminary estimates of influenza vaccine effectiveness (IVE) from Canada in the general population [3] and in hospitalised patients [4] and from the general population in the United Kingdom (UK) [5] complement previous data for the United States (US) [6]. All point to an overall substantially reduced vaccine effectiveness with point estimates of –8%, –16.8%, 3.4%, and 22%, respectively, as compared to seasons with a good match between circulating viruses and vaccine strains. This situation highlights the difficulties to accurately and timely anticipate antigenic changes of influenza viruses for inclusion of the proper antigenic (drift) variants in the vaccine.

Recommendations for the influenza vaccine composition are issued twice a year by the World Health Organization (WHO), in February and September, for the northern and southern hemisphere influenza seasons, respectively [7]. Recommendations are based on surveillance data and analysis of the virus characteristics provided by the National Influenza Centres from the WHO Global Influenza Surveillance and Response System (GISRS). For the four categories of seasonal influenza viruses, i.e. two influenza A virus subtypes A(H3N2) and A(H1N1)pdm09 and two influenza B lineage, B-Yamagata and B-Victoria viruses, data taken into account comprise epidemiological data as well as virological data in order to evaluate the genetic evolution of the viruses, their antigenic characteristics and susceptibility to antivirals, as well as their geographical distribution and impact. These are complemented by serological data aimed at evaluating the ability of post-vaccination sera from the previous season to neutralise the most recently circulating viruses with particular focus on potential drift variants [8]. The serological study in Finland in vaccinated healthcare workers by Haveri et al. in this issue points to a reduced cross-protection towards currently circulating drifted influenza A(H3N2) viruses [2].

Despite expansion of the GISRS network especially following the 2009 influenza A(H1N1) pandemic and continuous improved surveillance worldwide [9], predicting six months ahead of time which influenza variants will be predominating the next season remains a challenge. To achieve this, a better understanding of the link between genetic and antigenic evolution of the virus is required. Recent studies have provided information on key residues of the haemagglutinin that contribute to major antigenic changes for the influenza A(H3N2) and A(H1N1)pdm09 viruses [10,11]. Substitutions for at least one of these key residues (aa 159) were observed for the drifted A(H3N2) viruses from the current influenza season. However, in order to stay ahead of the virus, new means to better predict which genetic group of viruses will most likely become predominant are needed. This might be achieved through analysis of the evolutionary trajectories of the virus sequences taking into account minority variants that can be detected through Next Generation Sequencing. The feasibility on a large scale and benefits for the definition of the vaccine composition of an approach combining improved prediction of genetic variants likely to emerge and their impact on virus antigenicity, will require more research.

In spite of the challenges to define the vaccine composition, when excepting the 2009 pandemic, mismatches for viruses circulating in Europe occurred only once for A(H1N1) viruses and three times for A(H3N2) viruses in the past 12 years (Table).

In addition, for type B viruses, a mismatch occurred three times, in two instances related to the inclusion of the wrong influenza B lineage in the composition of the trivalent vaccine. Making global predictions for

Table

Antigenic match with vaccine strains of influenza viruses circulating in Europe from 2003/04 to 2014/15

Season (northern hemisphere)	Vaccine composition (northern hemisphere)			Circulating viruses[a]		
	A(H1N1)	A(H3N2)	Type B (lineage)	A(H1N1)	A(H3N2)	Type B lineage
2003/04	A/New Caledonia/20/99	A/Moscow/10/995	B/Hong Kong/330/2001 (VIC)		H3N2	
2004/05	A/New Caledonia/20/99	A/Fujian/411/02	B/Shanghai/361/02 (VIC)		H3N2	
2005/06	A/New Caledonia/20/99	A/California/7/2004	B/Shanghai/361/02 (VIC)	H1N1		VIC
2006/07	A/New Caledonia/20/99	A/Wisconsin/67/2005	B/Malaysia/2506/2004 (VIC)		H3N2	
2007/08	A/Solomon Islands/3/2006	A/Wisconsin/67/2005	B/Malaysia/2506/2004 (VIC)	H1N1		YAM
2008/09	A/Brisbane/59/2007	A/Brisbane/10/2007	B/Florida/4/2006 (YAM)		H3N2	VIC
2009/10	A/Brisbane/59/2007	A/Brisbane/10/2007	B/Brisbane/60/2008 (VIC)	H1N1pdm09		
2010/11	A/California/7/2009 (pdm)	A/Perth/16/2009	B/Brisbane/60/2008 (VIC)	H1N1pdm09		VIC
2011/12	A/California/7/2009 (pdm)	A/Perth/16/2009	B/Brisbane/60/2008 (VIC)		H3N2	
2012/13	A/California/7/2009 (pdm)	A/Victoria/361/2011	B/Wisconsin/1/2010 (YAM)	H1N1pdm09	H3N2[b]	YAM
2013/14	A/California/7/2009 (pdm)	A/Texas/50/2012	B/Massachusets/2/2012 (YAM)	H1N1pdm09	H3N2	
2014/15	A/California/7/2009 (pdm)	A/Texas/50/2012	B/Massachusets/2/2012 (YAM)		H3N2	

pdm: pandemic; VIC: Victoria; YAM: Yamagata.

[a] Only viruses accounting for more than 10% of the circulating viruses are mentioned ; mismatches are highlighted in grey.

[b] Mismatch related to antigenic changes of the vaccine strain upon growth in eggs.

influenza B viruses has proven particularly challenging as different influenza B lineages may predominate or co-circulate in different regions. Availability of tetravalent vaccines containing influenza B strains from both the B-Yamagata and B-Victoria lineage in addition to the two A(H3N2) and A(H1N1)pdm09 strains provides a solution but will not prevent a mismatch due to the emergence of a drift variant. Mismatch may also be related to antigenic changes of the vaccine strain upon growth in eggs as seen for the A(H3N2) strain during the 2012/13 season [12].

Mismatches concerning the A(H3N2) component of the vaccine impacted most on public health as A(H3N2) viruses are known to confer more severe illness with potential for complications especially in the elderly, a population that is also one of the main targets for vaccination. The extent to which a mismatch results in reduced IVE, however, is variable [13]. Vaccine effectiveness depends on the immunogenicity of the vaccine itself. This may vary with the type of vaccine (e.g. inactivated, presence of adjuvant, live attenuated), and for each vaccine strain. It also depends on the quality of the elicited immune response that is known to vary between individuals especially with age.

The role of pre-existing immunity that results from previous infection or vaccination also needs to be considered. In this respect, more serological data to inform, before the beginning of the season, about the antibody levels in the population against the various influenza viruses, including potential drift variants would be desirable. Finally, IVE depends on the extent of the mismatch between the vaccine strain and the circulating virus and the predominance of the drift variants among circulating viruses needs to be taken into account. This highlights the importance of quality surveillance that integrates virological and epidemiological data. Predicting the actual impact of a given mismatch on IVE is thus very challenging. It requires integration of virological, serological and epidemiological data that are not always available and knowledge for the establishment of correlations is lacking. For instance, the impact of repeat vaccination that has sometimes shown to have a negative effect on IVE as reported from Canada by Skowronski et al. [3] remains a complex and unresolved issue that requires further investigation [14].

In the absence of methodologies to predict the impact of a mismatch on IVE, real time epidemiological evaluation of IVE is the preferred option in order to guide appropriate responses to suboptimal vaccine effectiveness. Recent years have seen marked improvements in the capacity of generating early in-season epidemiological measures of IVE, despite the many pitfalls attached to such studies [15,16]. The first issue relates to the case definition. Indeed, a clinical outcome such as influenza-like illness (ILI) lacks specificity and may lead to underestimation of IVE. Therefore, laboratory confirmation of ILI, as done in the Canadian and the UK studies published last week and in the current *Eurosurveillance* issue, is increasingly considered as a standard. The second issue is bias. As all observational studies, IVE studies are prone to bias. Both negative and positive confounding can alter the quality of IVE, requiring the documentation of a minimum set of variables to be included as covariates in models.

The increasing use of the test–negative case–control design, whereby controls are individuals consulting for ILI and testing negative for influenza, allows reducing the potential bias linked to differential healthcare seeking behaviours according to vaccination status. The third issue relates to the power of the studies. Even in countries with a well-established General Practice (GP)-based sentinel surveillance system, it is difficult to conduct large scale studies allowing precise early estimates, especially for subgroup analysis. This is especially true for measurement of IVE in elderly patients as such patients, although the main target of seasonal influenza vaccination, are difficult to recruit in sufficient numbers at GP offices.

The European Centre for Disease Prevention and Control (ECDC)-funded Influenza Monitoring Vaccine Effectiveness (I-MOVE) network set up in 2007, including 22 partners from 17 European Union/European Economic Area (EU/EEA) countries with Epiconcept as the coordinating hub, has proven its ability to generate early reliable IVE estimates, taking into account the issues above [17]. To do so, I-Move partners have agreed on high quality standardised protocols allowing the pooling of the data at European level. Such an initiative, together with similar ones from other parts of the world e.g. in North America , South America, Australia [15] paves the way for providing IVE data to complement virological data, as basis for the decision-making process for the next season vaccine composition, at the WHO annual February meeting [18].

The new requirement from the European Medicines Agency (EMA) asking influenza vaccines market authorisation holders to provide annual brand-specific effectiveness data should bring more resources into the IVE studies [19]. This should result in more powered studies but requires, as a prerequisite, the set up of new mechanisms for public-private partnership in the sensitive area of monitoring and evaluation of immunisation programmes and related vaccines, that are acceptable to both sides. Several initiatives, including the Innovative Medicines Initiative (IMI) Advance project, are currently working on this issue [20]. More powered IVE studies conducted specifically in elderly should also be undertaken in the near future through the I-MOVE+ project currently under preparation.

However, despite those recent or soon to be expected improvements, unsolved challenges persist, in case of a mismatch. IVE estimates cannot always be obtained before the start of the epidemic in countries hit first and breakdown by virus (sub-)type or lineage is not always possible in case of mixed circulation of influenza viruses. Furthermore, it should be emphasised that extrapolation of IVE determined in a given context to other regions or settings is not always possible. Indeed, as mentioned above, differences in type of vaccine use, target populations for vaccination, pre-existing immunity resulting from previous circulation of influenza viruses, as well as the level of predominance of the drifted variants among circulating viruses will have an impact on IVE. However, the availability of interim assessments of IVE from other parts of the world and also from a European country, as presented in this issue for the UK, at a time where the influenza epidemic is still rising in most European countries, has proven useful in allowing national authorities, in line with the ECDC risk assessment, to issue recommendations for both health professionals and the lay public [5,21]. These mainly concern the strengthening of infection control measures and the early use of influenza antiviral medication for persons at higher risk for serious complications, either as post-exposure prophylaxis or treatment.

Although in the case of a mismatch reduced vaccine effectiveness can be anticipated towards the drifted variant, vaccination should still be recommended also for the ongoing season. Indeed, it will still provide protection towards the other viruses that match the vaccine strain. Despite the fact that in the older and more vulnerable population, IVE was very low as reported from Canada by McNeil et al. [4] in hospitalised adults presenting with acute respiratory illness, overall some cross-protection towards the drifted variant can be anticipated, in the sense that even if it does not prevent infection per se it could contribute to reduce disease severity leading to complications or even death [21,22].

Evidently, instead of a better measurement of low effectiveness a better vaccine is needed. This would mean, a more effective vaccine for all age groups, affording broad cross-protection within each sub-type or lineage of seasonal influenza viruses, thus allowing to avoid the need for annual vaccination and update of the vaccine composition. Of course, a universal vaccine covering all influenza A virus subtypes and protecting from potential pandemic strains would be ideal [23].

Conflicts of interest

Sylvie van der Werf: received financial support for scientific research (GSK and Roche), speaker's fees and attendance at international meeting (GSK).
Daniel Levy-Bruhl: none.

Authors' contributions

SW and DLB jointly drafted the manuscript and both approved the final version.

References

1. Broberg E, Snacken R, Adlhoch C, Beauté J, Galinska M, Pereyaslov D, et al., on behalf of the WHO European Region and the European Influenza Surveillance Network. Start of the 2014/15 influenza season in Europe: drifted influenza A(H3N2) viruses circulate as dominant subtype. Euro Surveill. 2015;20(4):21023.
2. Haveri A, Ikonen N, Julkunen I, Kantele A, Anttila VJ, Ruotsalainen E, et al. Reduced cross-protection against influenza A(H3N2) subgroup 3C.2a and 3C.3a viruses among

Finnish healthcare workers vaccinated with 2013/14 seasonal influenza vaccine. Euro Surveill. 2015;20(5):pii=21028.

3. Skowronski DM, Chambers C, Sabaiduc S, De Serres G, Dickinson JA, Winter AL, et al. Interim estimates of 2014/15 vaccine effectiveness against influenza A(H3N2) from Canada's Sentinel Physician Surveillance Network, January 2015. Euro Surveill. 2015;20(4):21022.
4. McNeil SA, Andrew MK, Ye L, Haguinet F, Hatchette TF, ElSherif M, et al. Interim estimates of 2014/15 influenza vaccine effectiveness in preventing laboratory-confirmed influenza-related hospitalisation from the Serious Outcomes Surveillance Network of the Canadian Immunization Research Network, January 2015. Euro Surveill. 2015;20(5):pii=21024
5. Pebody RG, Warburton F, Ellis J, Andrews N, Thompson C, von Wissmann B, et al. Low effectiveness of seasonal influenza vaccine in preventing laboratory-confirmed influenza in primary care in the United Kingdom: 2014/15 mid–season results. Euro Surveill. 2015;20(5):pii=21025
6. Flannery B, Clippard J, Zimmerman RK, Nowalk MP, Jackson ML, Jackson LA, et al.; Centers for Disease Prevention and Control. Early estimates of seasonal influenza vaccine effectiveness - United States, january 2015. MMWR Morb Mortal Wkly Rep. 2015;64(1):10-5. PMID:25590680
7. World Health Organization (WHO). WHO recommendations on the composition of influenza virus vaccines. Geneva: WHO. [Accessed 5 Feb 2015]. Available from: http://www.who.int/influenza/vaccines/virus/recommendations/en/
8. Barr IG, Russell C, Besselaar TG, Cox NJ, Daniels RS, Donis R, et al.; Writing Committee of the World Health Organization Consultation on Northern Hemisphere Influenza Vaccine Composition for 2013–2014. WHO recommendations for the viruses used in the 2013-2014 Northern Hemisphere influenza vaccine: Epidemiology, antigenic and genetic characteristics of influenza A(H1N1)pdm09, A(H3N2) and B influenza viruses collected from October 2012 to January 2013. Vaccine. 2014;32(37):4713-25. http://dx.doi.org/10.1016/j.vaccine.2014.02.014 PMID:24582632
9. Ampofo WK, Al Busaidy S, Cox NJ, Giovanni M, Hay A, Huang S, et al. Strengthening the influenza vaccine virus selection and development process: outcome of the 2nd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at the Centre International de Conférences (CICG) Geneva, Switzerland, 7 to 9 December 2011. Vaccine. 2013;31(32):3209-21. doi: 10.1016/j.vaccine.2013.05.049
10. Koel BF, Burke DF, Bestebroer TM, van der Vliet S, Zondag GC, Vervaet G, et al. Substitutions near the receptor binding site determine major antigenic change during influenza virus evolution. Science. 2013;342(6161):976-9. http://dx.doi.org/10.1126/science.1244730 PMID:24264991
11. Koel BF, Mögling R, Chutinimitkul S, Fraaij PL, Burke DF, van der Vliet S, et al. Identification of amino acid substitutions supporting antigenic change of A(H1N1)pdm09 viruses. J Virol. 2015 Jan 21. pii: JVI.02962-14. [Epub ahead of print]
12. Skowronski DM, Janjua NZ, De Serres G, Sabaiduc S, Eshaghi A, Dickinson JA, et al. Low 2012-13 influenza vaccine effectiveness associated with mutation in the egg-adapted H3N2 vaccine strain not antigenic drift in circulating viruses. PLoS One. 2014 Mar 25;9(3):e92153. doi: 10.1371/journal.pone.0092153.
13. Tricco AC, Chit A, Soobiah C, Hallett D, Meier G, Chen MH, Tashkandi M, Bauch CT, Loeb M. Comparing influenza vaccine efficacy against mismatched and matched strains: a systematic review and meta-analysis. BMC Med. 2013 Jun 25;11:153. doi: 10.1186/1741-7015-11-153. Review.PMID: 23800265.
14. Neuzil KM. How Can We Solve the Enigma of Influenza Vaccine-Induced Protection? J Infect Dis. 2014 Nov 21. pii: jiu651. [Epub ahead of print]. PMID: 2541681.
15. Lafond KE, Tam JS, Bresee JS, Widdowson MA. International meeting on influenza vaccine effectiveness, 3-4 December 2012, Geneva, Switzerland. Vaccine. 2014;32(49):6591-5. PMID:25446822
16. Valenciano M, Kissling E, Ciancio BC, Moren A. Study designs for timely estimation of influenza vaccine effectiveness using European sentinel practitioner networks. Vaccine. 2010;28(46):7381-8. http://dx.doi.org/10.1016/j.vaccine.2010.09.010 PMID:20851086
17. Valenciano M, Ciancio B; I-MOVE study team. I-MOVE: a European network to measure the effectiveness of influenza vaccines. Euro Surveill. 2012;17(39):20281. PMID:23041022
18. World Health Organization (WHO). Questions and Answers. Recommended composition of influenza virus vaccines for use in the northern hemisphere 2014-15 influenza seasonal and development of candidate vaccine viruses for pandemic preparedness. Geneva: WHO. [Accessed 4 Feb 2015]. Available from: http://www.who.int/influenza/vaccines/virus/recommendations/201402_qanda_recommendation.pdf?ua=1
19. European Medicines Agency (EMA). Explanatory note on the withdrawal of the Note for guidance on harmonisation of requirements for influenza Vaccines and of the core SmPC/PL for inactivated seasonal influenza vaccines. 23 Jan 2014. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2014/02/WC500161022.pdf)
20. Innovative Medicines Initiative (IMI). Key organisations unite to improve benefits and risk assessments of vaccines. Brussels: IMI. 26 Nov 2013. Available from: http://www.imi.europa.eu/content/press-release-advance
21. European Centre for Disease Prevention and Control (ECDC). Risk assessment. Seasonal influenza in the EU/EEA countries, 2014-2015. Stockholm: ECDC; Jan 2015. Available from: http://www.ecdc.europa.eu/en/publications/Publications/seasonal-influenza-risk-assessment.pdf
22. Beyer WE, McElhaney J, Smith DJ, Monto AS, Nguyen-Van-Tam JS, Osterhaus AD. Cochrane re-arranged: support for policies to vaccinate elderly people against influenza. Vaccine. 2013 Dec 5;31(50):6030-3. doi: 10.1016/j.vaccine.2013.09.063
23. Pica N, Palese P. Toward a universal influenza virus vaccine: prospects and challenges. Annu Rev Med. 2013;64:189-202. doi: 10.1146/annurev-med-120611-145115. Review. PMID:23327522.

Low effectiveness of seasonal influenza vaccine in preventing laboratory-confirmed influenza in primary care in the United Kingdom: 2014/15 mid–season results

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In 2014/15 the United Kingdom experienced circulation of influenza A(H3N2) with impact in the elderly. Mid-season vaccine effectiveness (VE) shows an adjusted VE of 3.4% (95% CI: −44.8 to 35.5) against primary care consultation with laboratory-confirmed influenza and −2.3% (95% CI: −56.2 to 33.0) for A(H3N2). The low VE reflects mismatch between circulating viruses and the 2014/15 northern hemisphere A(H3N2) vaccine strain. Early use of antivirals for prophylaxis and treatment of vulnerable populations remains important.

We present the 2014/15 mid-season estimates of influenza vaccine effectiveness (VE) for the United Kingdom of England, Wales, Scotland and Northern Ireland (UK). This season is dominated by early circulation of influenza A(H3N2) virus, and an overall VE in preventing medically attended laboratory-confirmed influenza in primary care of only 3.4% and against A(H3N2) of −2.3%. This report provides clear evidence of antigenic and genetic mismatch between circulating A(H3N2) viruses and the respective 2014/15 northern hemisphere vaccine strain.

Background

The UK has a long-standing selective influenza immunisation programme targeted at individuals at higher risk of severe disease, in particular all those 65 years and above and under 65-year olds in a clinical risk group, using inactivated trivalent influenza vaccine. The 2014/15 season is the second year where intranasally administered live attenuated influenza vaccine (LAIV) has been offered to pre-school age children in the UK with certain areas also vaccinating children of school-age [1]. This winter has been characterised by early influenza activity, with A(H3N2) virus the dominant circulating sub-type. In England, by week 4 2015 peak influenza activity levels had exceeded those seen in the past three seasons, but not approached the peak levels seen in 2010/11 and 2008/09 [2]. The current season has led to large numbers of care home outbreaks, often in highly vaccinated populations, hospitalisations and significant excess all-cause mortality in the over 65 year-old population.

The UK has well established methods to produce mid- and end-of-season estimates of VE in preventing primary care consultation due to laboratory-confirmed influenza infection [3,4]. The key aims of the present study were to provide early estimates of influenza VE in the UK to inform influenza prevention and control measures both for the remainder of this season and the forthcoming World Health Organization (WHO) convened meeting at the end of February, where vaccine composition is decided for the forthcoming northern hemisphere 2015/16 season.

Study population and period

Data were derived from five primary care influenza sentinel swabbing surveillance schemes from England (two schemes), Scotland, Wales and Northern Ireland. Details of the Royal College of General Practitioners (RCGP), Public Health England (PHE) Specialist Microbiology Network (SMN), Public Health Wales, Public Health Agency (PHA) of Northern Ireland and Health Protection Scotland (HPS) swabbing schemes have been published previously [4].

The study period ran from 1 October 2014 to 16 January 2015. Patients were swabbed as part of clinical care, with verbal consent. Cases were defined as persons presenting during the study period in a participating general practitioner (GP) practice with an acute influenza-like-illness (ILI) who were swabbed and then tested positive for influenza A or B viruses. An ILI case was defined as an individual presenting in primary care with an acute respiratory illness with physician-diagnosed fever or complaint of feverishness. Controls were individuals presenting with ILI in the same period that were swabbed and tested negative for influenza.

A standardised questionnaire was completed by the GP responsible for the patient during the consultation. Demographic, clinical and epidemiological information was collected from cases and controls, including date of birth, sex, pre-defined underlying clinical risk group, date of onset of respiratory illness, date of specimen collection, and influenza vaccination status for the 2014/15 season, with vaccination dates and route of administration (injection/intranasal) and whether resident in an area where a primary school vaccination programme was in operation.

Laboratory analysis

Laboratory confirmation was undertaken using comparable methods with real-time polymerase chain reaction (RT-PCR) assays capable of detecting circulating influenza A and influenza B viruses and other respiratory viruses [5,6]. Samples were sent to respective laboratories as previously described [4]. Further strain characterisation was also performed; influenza viruses were isolated in MDCK or MDCK-SIAT1 cells from RT-PCR positive samples from England as previously described [7,8]. Influenza A(H3N2) virus isolates with a haemagglutination titre ≥ 40 were characterised antigenically using post-infection ferret antisera in haemagglutination inhibition (HI) assays, with guinea pig red blood cells [9]. Nucleotide sequencing of the haemagglutinin (HA) gene of a subset of influenza A(H3N2) viruses selected to be representative of the range of patient's age, date of sample collection, geographical location, and antigenic characterisation of the influenza A(H3N2) virus isolate, if performed, was undertaken (primer sequences available on request), and phylogenetic trees were constructed with a neighbour-joining algorithm available in the Mega 6 software (http://www.megasoftware.net) [10]. HA sequences from reference strains used in the phylogenetic analysis were obtained from the EpiFlu database of the Global Initiative on Sharing Avian Influenza Data (GISAID) (Table 1).

Statistical analysis

Persons were defined as vaccinated if the date of vaccination with the 2014/15 seasonal influenza vaccine was 14 or more days before onset of illness. Those in whom the period between vaccination and onset of illness was less than 14 days were excluded, as were those where date of vaccination was missing. Those with a missing date of onset or an onset date more than seven days before the swab was taken were also excluded.

VE was estimated by the test negative case control (TNCC) design. In this design, VE is calculated as 1-(odds ratio) obtained using multivariable logistic regression models with influenza PCR results and seasonal vaccination status as the linear predictor. VE was also estimated for influenza A only and for A(H3N2); Influenza A(H1N1) and B numbers were too small to examine. In the analyses evaluating VE for a specific type or strain, those positive for other types were excluded. Age (coded into four standard age groups, < 18, 18–44, 45–64 and ≥ 65 years), sex, clinical risk group, surveillance scheme (RCGP, SMN, HPS, Wales, Northern Ireland), residence in an area where primary school age vaccination programme operated and date of onset (month) were investigated as potential confounding variables. All statistical analyses were carried out in Stata version 13 (StataCorp, College Station, Texas).

Results

A total of 2,278 individuals were swabbed in primary care during the study period and had a laboratory result available. The reasons for study inclusion and exclusion are outlined in Figure 1. Five persons were excluded because the influenza virus detected in them was a LAIV vaccine strain based either on sequence analysis or inferred based on influenza co-infection.

The details of the 1,341 individuals remaining in the study were stratified according to the swab result

Figure 1

Flowchart showing specimen inclusion and exclusion criteria, interim 2014/15 influenza vaccine effectiveness evaluation, United Kingdom, week 40 2014 to week 3 2015

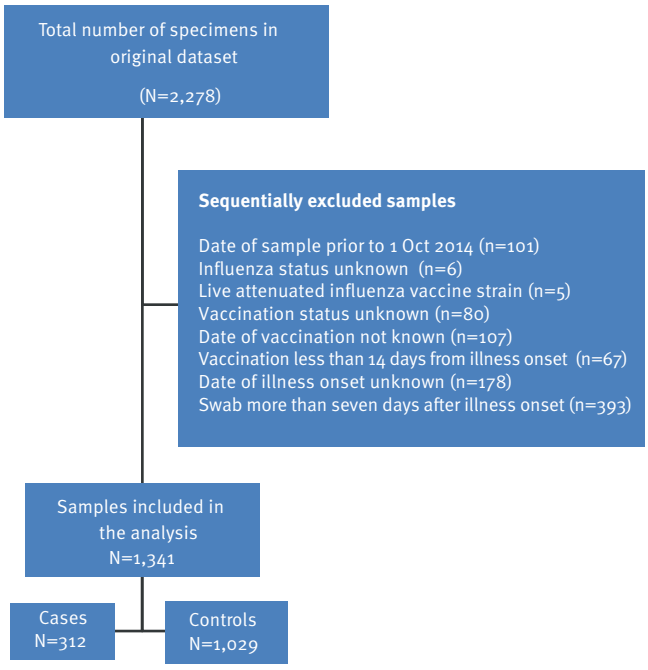


Table 1

Details for influenza A and B cases and controls, United Kingdom, week 40 2014 to week 3 2015 (1,029 controls and 312 cases)

	Controls	Influenza B cases	Influenza A[a] cases	Influenza A(H1N1) pdm09 cases	Influenza A(H3N2) cases	P-value[b]
	(n = 1,029)	(n = 16)	(n = 296)	(n = 14)	(n = 271)	
	n (%)	n (%)	n (%)	n (%)	n (%)	
Age group (years)						
<18	269(79.8)	3(0.9)	65(19.3)	2(0.6)	60(17.8)	0.40
18–44	412(76.7)	7(1.3)	118(22)	6(1.1)	106(19.7)	
45–64	244(74.8)	5(1.5)	77(23.6)	6(1.8)	69(21.2)	
65+	104(74.3)	1(0.7)	35(25)	0(0)	35(25)	
Missing information	0(0)	0(0)	1(100)	0(0)	1(100)	
Sex						
Female	592(77.2)	8(1)	167(21.8)	9(1.2)	153(19.9)	0.71
Male	432(76.3)	8(1.4)	126(22.3)	5(0.9)	115(20.3)	
Missing information	5(62.5)	0(0)	3(37.5)	0(0)	3(37.5)	
Scheme						
Northern Ireland	29(87.9)	1(3)	3(9.1)	0(0)	3(9.1)	<0.001
RCGP (England)	374(67)	6(1.1)	178(31.9)	10(1.8)	168(30.1)	
SMN (England)	138(77.1)	2(1.1)	39(21.8)	2(1.1)	32(17.9)	
Scotland	466(87.6)	7(1.3)	59(11.1)	2(0.4)	51(9.6)	
Wales	22(56.4)	0(0)	17(43.6)	0(0)	17(43.6)	
Risk group						
No	710(76.5)	15(1.6)	203(21.9)	10(1.1)	188(20.3)	0.93
Yes	215(76.2)	0(0)	67(23.8)	1(0.4)	63(22.3)	
Missing information	104(79.4)	1(0.8)	26(19.8)	3(2.3)	20(15.3)	
Interval onset to swab (days)						
0–1	140(69.7)	1(0.5)	60(29.9)	2(1)	56(27.9)	<0.001
2–4	498(74)	10(1.5)	165(24.5)	8(1.2)	149(22.1)	
5–7	391(83.7)	5(1.1)	71(15.2)	4(0.9)	66(14.1)	
Vaccination status and route						
Unvaccinated	852(77.5)	15(1.4)	232(21.1)	13(1.2)	210(19.1)	0.14[c]
Vaccinated	177(73.1)	1(0.4)	64(26.4)	11(4.5)	61(25.2)	
Injection	138(72.6)	1(0.5)	51(26.8)	0(0)	49(25.8)	
Intranasal	23(79.3)	0(0)	6(20.7)	1(3.4)	5(17.2)	
Missing route	16(69.6)	0(0)	7(30.4)	0(0)	7(30.4)	
Primary school age vaccination programme						
No	289(67.5)	4(0.9)	135(31.5)	10(2.3)	121(28.3)	<0.001
Yes	726(81.3)	12(1.3)	155(17.4)	4(0.4)	144(16.1)	
Missing information	14(70)	0(0)	6(30)	0(0)	6(30)	
Month of event						
Oct 2014	217(95.6)	3(1.3)	7(3.1)	1(0.4)	5(2.2)	<0.001
Nov 2014	343(94.2)	6(1.6)	15(4.1)	0(0)	15(4.1)	
Dec 2014	375(64.1)	4(0.7)	207(35.4)	6(1)	195(33.3)	
Jan 2015	94(57.3)	3(1.8)	67(40.9)	6(3.7)	56(34.1)	

RCGP: Royal College of General Practitioners' surveillance scheme; SMN: Specialist Microbiology Network.

[a] There were 11 cases of influenza A infection where the subtype was unknown.

[b] Controls vs any influenza case.

[c] Vaccinated vs unvaccinated.

(Table 1). Positivity rates differed significantly by month, scheme and primary school age vaccination programme area.

Influenza A(H3N2) strain characterisation

During the study period, a total of 127 A(H3N2) circulating viruses were isolated from all referred clinical samples and antigenically characterised by HI analysis. The majority of A(H3N2) viruses analysed (100/127; 79%) were antigenically similar to the A(H3N2) virus component of the 2014/15 northern hemisphere vaccine A/Texas/50/2012 (≤4-fold difference) with antiserum raised against A/Texas/50/2012 in antigenic characterisation by HI); however a proportion of A(H3N2) viruses (21%) showed reduced reactivity (>4-fold difference) (Table 2).

These viruses were antigenically similar to A/Switzerland/9715293/2013, the recommended A(H3N2) component of the 2015 southern hemisphere vaccine. A>4-fold difference in HI assay titres with reference antiserum is considered to be significant antigenic drift. Of the 44 UK influenza A(H3N2) viruses analysed to date by sequencing, the HA genes of these viruses belonged in the genetic clade 3C, as does the 2014/15 A(H3N2) vaccine strain A/Texas/50/2012 and A/Switzerland/9715293/2013, one of the three recommended strains for the southern hemisphere 2015 vaccine composition. However, the majority (35/44; 79.5%) of the HA sequences of 2014/15 UK viruses analysed were further characterised within this clade to belong in subgroup 3C.2a of group 3C.2, with fewer (9/44 17.3%) in group 3C.3 (Figure 2). These groups are considered genetically distinct from the 2014/15 A/Texas/50/2012(H3N2)-like clade 3C.1 vaccine reference strain.

Model fitting for vaccine effectiveness estimation

When estimating vaccine effectiveness, age group, sex, month of onset, surveillance scheme and primary school age programme area were adjusted for in a multivariable logistic regression model. Only surveillance scheme, time period and primary school age programme area were significantly associated with having a positive swab, and all were confounders for vaccine effectiveness (changing the estimate by more than 5%). Information on risk group was missing for 131 samples (9.8%) and was therefore not included in the final model. If risk group was included, it was found not to be associated with being positive and the VE estimates remained similar.

Table 3 shows vaccine effectiveness estimates against all influenza, influenza A and influenza A(H3N2). There were inadequate samples to enable estimation of effectiveness against influenza A(H1N1)pdm09 or influenza B. The adjusted VE of influenza vaccine against all influenza was 3.4% and was very similar for A(H3N2), reflecting the fact that A(H3N2) is the dominant circulating virus strain this season.

Table 2

Influenza A(H3N2) viruses isolated from specimens with < 4-fold, 4-fold, or > 4-fold difference in haemagglutination inhibition (HI) assay titres compared with A/Texas/50/2012, the 2014/15 northern hemisphere influenza seasonal A(H3N2) influenza vaccine component, United Kingdom, week 40 2014 to week 3 2015 (n = 127)

Influenza virus	Change in reactivity with A/Texas/50/2012 antiserum							
	<4-fold		4-fold		>4-fold		Total	
	N	(%)	n	(%)	N	(%)	n	(%)
A(H3N2)	65	51.2	35	27.6	27	21.3	127	100

Discussion

This paper presents the mid-season estimates of influenza vaccine effectiveness for the UK. In a season dominated by early circulation of influenza A(H3N2) virus, we found the overall VE in preventing medically attended laboratory-confirmed influenza in primary care was only 3.4% and −2.3% specifically against A(H3N2). We also found clear evidence of antigenic and genetic mismatch between circulating A(H3N2) viruses and the 2014/15 northern hemisphere vaccine strain.

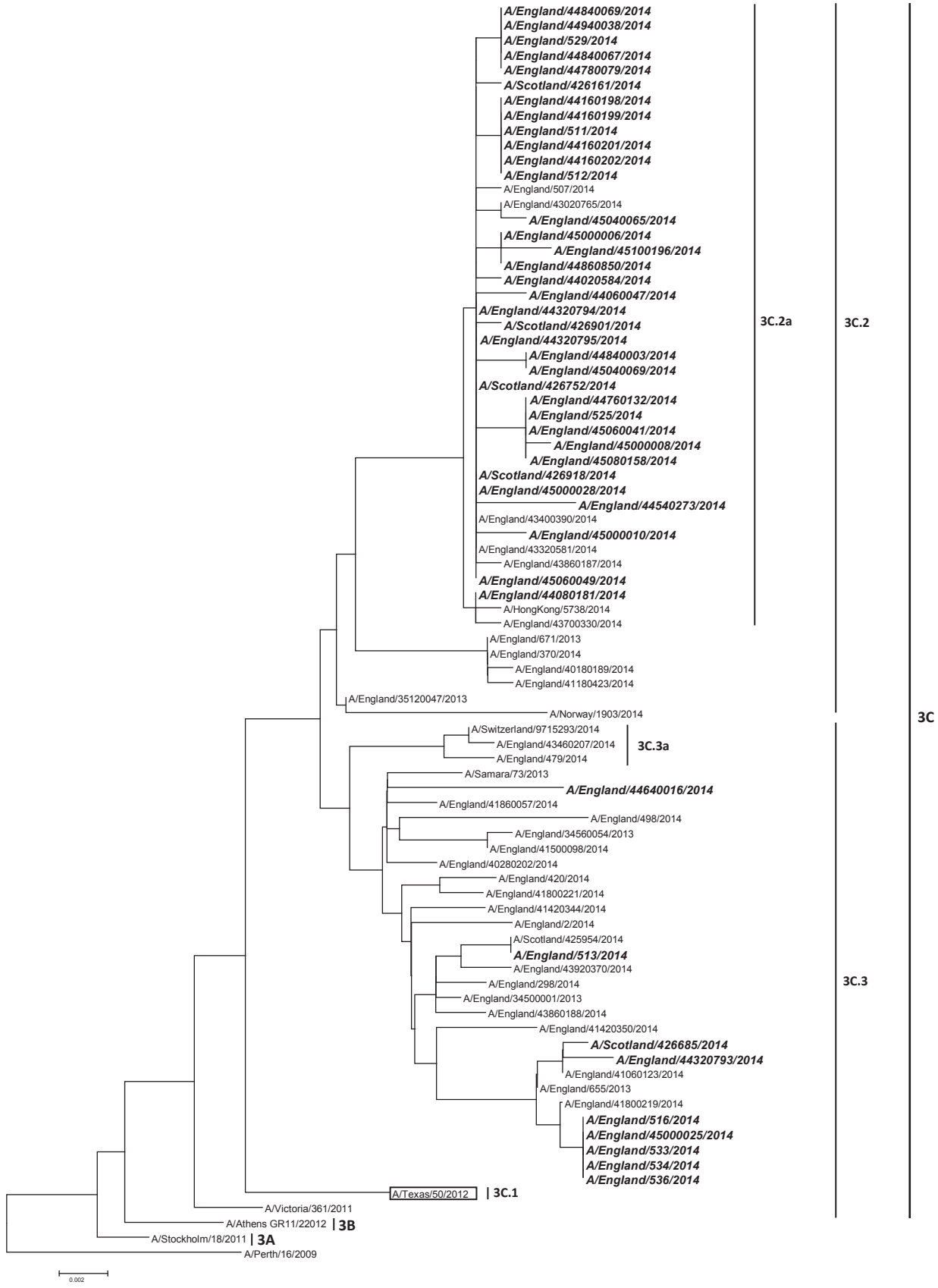
The UK, together with other European countries, the United States, Canada and Australia has well established systems to generate interim estimates of seasonal influenza VE. These early results are used to optimise in-season control and prevention measures, to inform other countries where the influenza season may have just started or is about to start, and to contribute to forthcoming WHO vaccine composition deliberations.

The UK, as observed in North America and some other European countries [11,12] has experienced a season dominated by early circulation of influenza A(H3N2) virus – with clear evidence of emergence of a drifted A(H3N2) strain, first seen in North America in spring 2014 [11], and then in Australia in mid-2014 [13]. This drifted strain has reduced antigenic reactivity with antiserum raised to the current A(H3N2) vaccine strain (A/Texas/50/2012), and is antigenically more closely related to A/Switzerland/9715293/2013, the A(H3N2) virus selected as one of the three recommended components for the 2015 southern hemisphere influenza vaccine [14].

Characterisation of circulating influenza viruses involves both genetic and antigenic characterisation. Genetic analysis focusses on detailed comparison of the HA genes of viruses, tracking changes over time and linking this to phylogenetic analysis of sequence clustering to determine emerging virus groups and changes in receptor binding and other important antigenic epitopes. Genetic variation does not always correlate with antigenic variation. The interpretation of both data sources is complex, as not all viruses

Figure 2

Phylogenetic analysis with sequences of the HA1 subunit of the haemagglutinin (HA) gene from reference viruses downloaded from the GISAID EpiFlu database and influenza A(H3N2) sequences derived from patients in the United Kingdom, week 40 2014 to week 3 2015



GISAID: Global Initiative on Sharing Avian Influenza Data.

Table 3

Samples positive (cases) and negative (controls) for influenza according to vaccination status and vaccine effectiveness estimates, United Kingdom, week 40 2014 to week 3 2015

	Cases (vaccinated : unvaccinated)	Controls (vaccinated : unvaccinated)	Crude VE (95% CI)	Adjusted[a] VE (95% CI)
All influenza (A and B)	65 : 247	177 : 825	−26.7% (−74.0 to 7.8)	3.4% (−44.8 to 35.5)
All influenza A	64 : 232		−32.2% (−82.2 to 4.0)	−0.7% (−52.0 to 33.2)
Influenza A(H3N2) only	61 : 210		−39.8% (−94.1 to −0.7)	−2.3% (−56.2 to 33.0)

CI: confidence interval; VE: vaccine effectiveness.

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

can be cultivated in sufficient quantity for antigenic characterisation, so that viruses for which sequence information is available may not be antigenically characterised, leading to potential bias in interpretation. This is particularly relevant to the discussion of antigenic characterisation data for A(H3N2) viruses in the 2014/15 winter season. Some circulating A(H3N2) viruses are difficult to grow in tissue culture as a result of genetic drift affecting receptor binding properties [15], and viruses grown in eggs may have egg adaptive changes which make the analysis of antigenic drift complex. Most A(H3N2) strains seen since February 2014 fell into the HA genetic clade (3C) for which A/Switzerland/9715293/2013 was a prototype representative strain. Antigenic and genetic characterisation data indicate that A/Switzerland/9715293/2013-like strains have circulated in the UK during winter 2014/15. There is a clear antigenic mismatch between the northern hemisphere H3N2 vaccine strain and the circulating variant in winter of 2014/15. The full picture of virological variation requires further detailed analysis, not possible at this stage of the 2014/15 season.

Our observation of an absence of significant effectiveness in preventing medically-attended laboratory-confirmed influenza in primary care due to A(H3N2) are congruent with the findings recently reported from the US [16] who report low effectiveness of 22% (95% confidence interval (CI): 5–35) and from Canada who report a VE of −8% (95% CI: −50 to 23) against laboratory-confirmed, medically-attended influenza A(H3N2) virus infection in primary care [17]. The observation of low or non-significant effectiveness in 2014/15 in the UK and in North America correlates with the direct mismatch seen between the vaccine virus and A(H3N2) strains circulating this winter. Vaccine mismatch due to circulation of drifted strains does occasionally occur, although this is the lowest estimate of influenza VE reported by the UK over the past decade using the TNCC approach to measure VE [3,4]. It is also important to highlight the uncertainty of our estimate. The upper 95% CI of 35% shows we can be confident that VE is low at this point although we cannot be clear that influenza vaccine has no effectiveness this season. Indeed the significantly lower influenza positivity in areas where children of school age were vaccinated compared to non-pilot areas (Table 1) is suggestive of a possible impact of the childhood influenza vaccination programme. Furthermore, this mid-season analysis does not preclude the likelihood that the vaccine should offer protection from different types of influenza, such as influenza B that may still circulate later in the season. All these elements will form part of the end-of-season analysis including stratification by age-group and scheme.

The WHO has made their recommendations for the composition of the influenza vaccine for the 2015 southern hemisphere winter in September 2014, including a switch to a A/Switzerland/9715293/2013 (H3N2)-like virus [18]. The WHO influenza vaccine composition group will convene shortly, at the end of February 2015, to consider recommendations for the forthcoming northern hemisphere 2015/16 winter, and the findings in this paper will contribute to their deliberations. The observation of low vaccine effectiveness this season highlights the vital importance of implementing other prevention and control measures for the remainder of this season, in particular the early use of influenza antivirals for post-exposure prophylaxis and treatment of vulnerable populations, such as the elderly, together with appropriate infection control measures.

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Conflicts of interest

None declared.

Authors' contributions

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

References

1. Department of Health. Public Health England. The national flu immunisation programme 2014/15. London: Public Health England. 28 Apr 2014. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/316007/FluImmunisationLetter2014_accessible.pdf
2. Public Health England (PHE). PHE Weekly National Influenza Report – week 4 report. London: PHE. 22 Jan 2015. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/397454/Weekly_report_current_week_4x.pdf
3. Fleming DM, Andrews NJ, Ellis JS, Bermingham A, Sebastianpillai P, Elliot AJ, et al. Estimating influenza vaccine effectiveness using routinely collected laboratory data. J Epidemiol Community Health. 2010;64(12):1062-7. http://dx.doi.org/10.1136/jech.2009.093450 PMID:19910645
4. Andrews N, McMenamin J, Durnall H, Ellis J, Lackenby A, Robertson C, et al. Effectiveness of trivalent seasonal influenza vaccine in preventing laboratory-confirmed influenza in primary care in the United Kingdom: 2012/13 end of season results. Euro Surveill. 2014;19(27): 20851. http://dx.doi.org/10.2807/1560-7917.ES2014.19.27.20851 PMID:25033051
5. Ellis J, Iturriza M, Allen R, Bermingham A, Brown K, Gray J, et al. Evaluation of four real-time PCR assays for detection of influenza A(H1N1)v viruses. Euro Surveill. 2009;14(22):19230. PMID:19497254
6. Gunson R, Maclean A, Davies E, Bennett S, Miller R, Carman WF. Development of a multiplex real-time RT-PCR that allows universal detection of influenza A viruses and simultaneous typing of influenza A/H1N1/2009 virus. J Virol Methods. 2010;163(2):258-61. http://dx.doi.org/10.1016/j.jviromet.2009.10.006 PMID:19854220
7. Galiano M, Agapow P-M, Thompson C, Platt S, Underwood A, Ellis J, et al. Evolutionary pathways of the pandemic influenza A (H1N1) 2009 in the UK. PLoS ONE. 2011;6(8):e23779. http://dx.doi.org/10.1371/journal.pone.0023779 PMID:21887318
8. Matrosovich M, Matrosovich T, Carr J, Roberts NA, Klenk HD. Overexpression of the alpha-2,6-sialyltransferase in MDCK cells increases influenza virus sensitivity to neuraminidase inhibitors. J Virol. 2003;77(15):8418-25. http://dx.doi.org/10.1128/JVI.77.15.8418-8425.2003 PMID:12857911
9. Zambon M. Laboratory Diagnosis of Influenza. In: Nicholson K, Hay A, Webster RG, editors. Textbook of Influenza. Oxford: Blackwell Science; 1998. pp. 291-313].
10. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013;30(12):2725-9. http://dx.doi.org/10.1093/molbev/mst197 PMID:24132122
11. Centers for Disease Prevention and Control (CDC). CDC Health Advisory Regarding the Potential for Circulation of Drifted Influenza A (H3N2) Viruses. Atlanta: CDC. 3 Dec 2014. Available from: http://emergency.cdc.gov/HAN/han00374.asp
12. Broberg E, Snacken R, Adlhoch C, Beauté J, Galinska M, Pereyaslov D, et al. Start of the 2014/15 influenza season in Europe: drifted influenza A(H3N2) viruses circulate as dominant subtype. Euro Surveill. 2015;20(4):pii=21023. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21023
13. World Health Organization (WHO). Review of the 2014 influenza season in the southern hemisphere. Wkly Epidemiol Rec. 2014;89(48):529-41.
14. ECDC Circulation of drifted influenza A(H3N2) viruses in the EU/EEA http://ecdc.europa.eu/en/publications/Publications/RRA-InfluenzaA-H3N2-Dec-2014.pdf
15. Wilson IA, Cox NJ. Structural basis of immune recognition of influenza virus hemagglutinin. Annu Rev Immunol. 1990;8(1):737-87. http://dx.doi.org/10.1146/annurev.iy.08.040190.003513 PMID:2188678
16. Flannery B, Clippard J, Zimmerman RK, Nowalk MP, Jackson ML, Jackson LA, et al. Early estimates of seasonal influenza vaccine effectiveness - United States, january 2015. MMWR Morb Mortal Wkly Rep. 2015;64(1):10-5. PMID:25590680
17. Skowronski DM, Chambers C, Sabaiduc S, De Serres G, Dickinson JA, Winter AL, et al. Interim estimates of 2014/15 vaccine effectiveness against influenza A(H3N2) from Canada's Sentinel Physician Surveillance Network, January 2015. Euro Surveill. 2015;20(4):21022.
18. World Health Organization (WHO). Recommended composition of influenza virus vaccines for use in the 2015 southern hemisphere influenza season. Geneva: WHO; 22-24 Sept 2014. Available from: http://www.who.int/influenza/vaccines/virus/recommendations/consultation201409/en/

Reduced cross-protection against influenza A(H3N2) subgroup 3C.2a and 3C.3a viruses among Finnish healthcare workers vaccinated with 2013/14 seasonal influenza vaccine

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Virus strains in the seasonal influenza vaccine for the 2014/15 northern hemisphere season remained unchanged from those in 2013/14. During spring 2014, drifted influenza A(H3N2) viruses, subgroup 3C.3a, were detected in Finland; another subgroup, 3C.2a, emerged in the 2014/15 season and has predominated. We monitored antibody responses against vaccine and epidemic strains (2013/14 and 2014/15) among Finnish healthcare workers after influenza vaccination with the 2013/14 vaccine. The data suggest reduced cross-protection towards both subgroups of drifted A(H3N2) viruses.

Early in the 2014/15 influenza season, drifted influenza A(H3N2) viruses have predominated in Europe [1,2]. In Finland, the season started earlier in the year than the previous season did (December 2014 as opposed to January 2014). We characterised a subset of circulating 2013/14 and 2014/15 influenza A(H3N2) viruses genetically to monitor changes in the circulating strains. On the basis of the genetic changes identified, representatives of these strains were selected for serological study. The strains recommended by the World Health Organization (WHO) for inclusion in the 2014/15 trivalent influenza vaccine (TIV) for the northern hemisphere [3] are the same as those in 2013/14 [4]: A/California/07/2009, A/Texas/50/2012 and B/Massachusetts/02/2012. By exploring the antibody responses to the 2013/14 TIV in Finnish healthcare workers (HCWs), we evaluated the seroprotection level against viruses included in TIV and compared it with vaccine-induced cross-protection towards selected epidemic virus strains from the 2013/14 and 2014/15 seasons. Our findings suggest reduced cross-protection towards the two subgroups of drifted A(H3N2) viruses detected in Finland (genetic subgroups 3C.3a and 3C.2).

Genetic characterisation of influenza A(H3N2) viruses in Finland in 2013/14 and 2014/15

As part of virological surveillance of influenza in Finland, a subset of influenza A(H3N2)-positive samples from sites in a sentinel influenza surveillance network and non-sentinel sites are selected throughout the season for genetic characterisation on the basis of their geographical origin and temporal distribution.

The sentinel network consists of healthcare centres collecting specimens from patients with influenza-like illness or acute respiratory infection and most also report clinical data. Healthcare centres of garrisons, also included in the network, only collect specimens. While intensive-care units are also part of the network, collecting specimens only, they are not considered as sentinel sites, as their participation is not agreed in advance (unlike that of healthcare centres). Other non-sentinel sites include clinical microbiology laboratories, for example.

Phylogenetic analysis of the haemagglutinin gene was performed as described previously [5]. Reference influenza A(H3N2) virus sequences for the phylogenetic tree were obtained from the EpiFlu database of the Global Initiative on Sharing Avian Influenza Data (GISAID) (Table 1).

During the 2013/14 season, a total of 27 influenza A(H3N2) viruses were analysed: 25 belonged to group 3C.3 and two to group 3C.2 (Figure 1, Table 1). Of the

Table 1A

Origin of the haemagglutinin sequence information of influenza A(H3N2) viruses included in the phylogenetic analysis

Isolate name	Segment ID	Country	Collection date	Originating laboratory	Submitting laboratory	Authors
A/Alabama/05/2010	EPI278808	United States	2010-Jul-13	US Air Force School of Aerospace Medicine	Centers for Disease Control and Prevention	
A/Athens GR/112/2012	EPI358885	Greece	2012-Feb-01	Hellenic Pasteur Institute	National Institute for Medical Research	
A/Hong Kong/146/2013	EPI426061	Hong Kong (SAR)	2013-Jan-11	Government Virus Unit	National Institute for Medical Research	
A/Hong Kong/5738/2014	EPI539806	Hong Kong (SAR)	2014-Apr-30	Government Virus Unit	National Institute for Medical Research	
A/Iowa/19/2010	EPI335923	United States	2010-Dec-30	Iowa State Hygienic Laboratory	Centers for Disease Control and Prevention	
A/Ireland/M28426/2013	EPI467996	Ireland	2013-Apr-02	National Virus Reference Laboratory	National Institute for Medical Research	
A/Johannesburg/3495/2012	EPI405940	South Africa	2012-Jul-04	Sandringham, National Institute for Communicable D	National Institute for Medical Research	
A/Madagascar/0648/2011	EPI319276	Madagascar	2011-Feb-21	Institut Pasteur de Madagascar	National Institute for Medical Research	
A/Nebraska/4/2014	EPI539619	United States	2014-Mar-11	Centers for Disease Control and Prevention	National Institute for Medical Research	
A/Norway/1186/2011	EPI326137	Norway	2011-Mar-16	Norwegian Institute of Public Health	National Institute for Medical Research	
A/Norway/1330/2010	EPI302231	Norway	2010-Dec-03	WHO National Influenza Centre	National Institute for Medical Research	
A/Norway/1903/2014	EPI539623	Norway	2014-May–20	WHO National Influenza Centre	National Institute for Medical Research	
A/Perth/16/2009	EPI211334	Australia	2009	WHO Collaborating Centre for Reference and Research on Influenza	Centers for Disease Control and Prevention	
A/Samara/73/2013	EPI460558	Russian Federation	2013-Mar-12	WHO National Influenza Centre Russian Federation	National Institute for Medical Research	
A/Stockholm/18/2011	EPI326139	Sweden	2011-Mar-28	Swedish Institute for Infectious Disease Control	National Institute for Medical Research	
A/Switzerland/9715293/2013	EPI540526	Switzerland	2013-Dec-06	National Institute for Medical Research	Centers for Disease Control and Prevention	
A/Texas/50/2012	EPI391247	United States	2012-Apr-15	Texas Department of State Health Services-Laboratory Services	Centers for Disease Control and Prevention	
A/Victoria/361/2011	EPI349106	Australia	2011-Oct-24	Melbourne Pathology	WHO Collaborating Centre for Reference and Research on Influenza	Deng, Y-M; Caldwell, N; Iannello, P; Komadina, N.
A/Finland/385/2013	EPI502957	Finland	2013-Dec-11	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/404/2014	EPI556921	Finland	2014-Feb-06	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/410/2014	EPI556922	Finland	2014-Feb-26	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/428/2014	EPI556939	Finland	2014-Feb-17	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/433/2014	EPI557055	Finland	2014-Feb-07	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/437/2014	EPI557056	Finland	2014-Mar-24	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/438/2014	EPI557057	Finland	2014-Apr-03	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/439/2014	EPI557058	Finland	2014-Apr-23	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M

Table 1B

Origin of the haemagglutinin sequence information of influenza A(H3N2) viruses included in the phylogenetic analysis

Isolate name	Segment ID	Country	Collection date	Originating laboratory	Submitting laboratory	Authors
A/Finland/440/2014	EPI557059	Finland	2014-Apr-28	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/461/2014	EPI557060	Finland	2014-Oct-22	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/462/2014	EPI557061	Finland	2014-Oct-08	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/463/2014	EPI557062	Finland	2014-Nov-20	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/464/2014	EPI557063	Finland	2014-Nov-24	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/465/2014	EPI557064	Finland	2014-Dec-01	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/466/2014	EPI557065	Finland	2014-Nov-21	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/467/2014	EPI557066	Finland	2014-Nov-24	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/468/2014	EPI557067	Finland	2014-Nov-27	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/469/2014	EPI557068	Finland	2014-Nov-27	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M

All reference and Finnish haemagglutinin sequences are available from the Global Initiative on Sharing Avian Influenza Data (GISAID) EpiFlu database.

25 group 3C.3 viruses, six represented a new drifted A(H3N2) type, group 3C.3a viruses. In Finland, these viruses were first detected in February 2013.

At the beginning of the 2014/15 season, all nine influenza A(H3N2) viruses analysed belonged to another drifted group, 3C.2a.

Monitoring antibody response after influenza vaccination in a cohort of healthcare workers

A total of 79 clinically healthy HCWs (12 men, 67 women), median age 46 years (range: 22–66), were recruited on a voluntary basis during autumn 2013 from the personnel of the Department of Medicine at the Helsinki University Hospital and the Viral Infections Unit at the National Institute for Health and Welfare, Helsinki. The employer vaccinated each participant with the 2013/14 seasonal influenza vaccine, which was trivalent, non-adjuvanted, containing the three WHO-recommended influenza virus strains. One dose was administered intramuscularly. Serum samples were collected before vaccination (day 0) and three weeks and six months after vaccination.

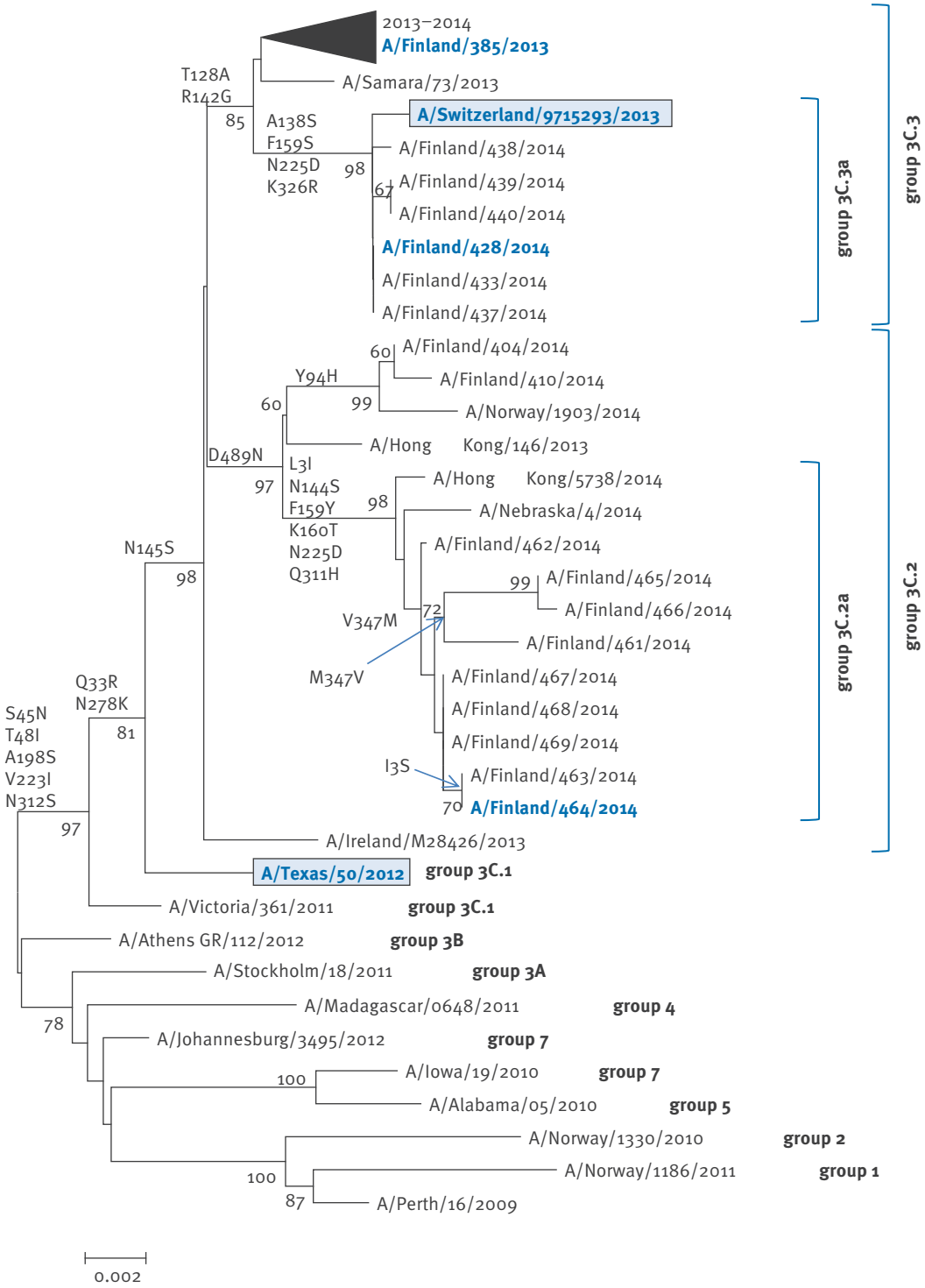
The study protocol was approved by the Ethic Committee of the Department of Medicine, Helsinki University Hospital (298/13/03/00/12) and the Finnish Medicines Agency (EudraCT 2012–003727–38). Written informed consent was provided by all participants.

The serum samples were tested by the haemagglutination inhibition (HI) test for the presence of antibodies against TIV vaccine viruses and Finnish influenza virus isolates from the 2013/14 and 2014/15 seasons. TIV strains for the northern hemisphere 2013/14 and 2014/15 seasons were A/California/07/2009 (group 1), A/Texas/50/2012 (group 3C.1) and B/Massachusetts/02/2012 (clade 2). For comparison, we also included B/Wisconsin/01/2010 (clade 3), the vaccine strain for the northern hemisphere 2012/13 season, and A/Switzerland/9715293/2013 (H3N2) (group 3C.3a), the vaccine strain for the southern hemisphere 2015 season. We also included A/Finland/420/2014 (group 6B), which represented the Finnish A(H1N1) pdm09 strain that circulated in 2013/14. In addition, A/Finland/385/2013 (2013/14), A/Finland/428/2014 (2013/14) and A/Finland/464/2014 (2014/15) were selected as representative of circulating Finnish A(H3N2) viruses for groups 3C.3, 3C.3a and 3C.2a, respectively.

The assay was performed as previously described [6] using erythrocytes from turkeys for A(H1N1)pdm09 viruses and guinea pigs for A(H3N2) and influenza B viruses. A(H3N2) viruses were assayed in the presence of 20 nM oseltamivir carboxylate (Roche). For statistical analyses, serum specimens with HI titres <10 were assigned a titre value of 5. We calculated the geometric mean titres (GMT) with 95% confidence intervals and presumable seroprotection rate (using the commonly accepted European Medicines Agency criteria [7]: HI titre $\geq$ 1:40) for each virus. Statistical significance of

Figure 1

Phylogenetic analysis of the haemagglutinin sequences of influenza A(H3N2) viruses from Finnish surveillance data during two epidemic seasons, 2013/14 and 2014/15



All sequences included in the phylogenetic tree were 1,650 nucleotides long. The tree was constructed using the neighbour-joining method with Mega software version 5.1 and with 1,000 bootstrap replicates. Bootstrapping values ≥ 60 are shown. The viruses used in serological tests are in bold blue. In addition, the vaccine viruses for northern and southern hemisphere are shown in boxes. Arrowed lines represent the location of amino acid substitutions.

Table 2

Geometric mean titres against influenza A(H1N1)pdm09, A(H3N2) and B viral strains measured by haemagglutination inhibition test before and after vaccination of 79 healthcare workers with 2013/14 trivalent influenza vaccine, Finland

Influenza virus strain	Group	Geometric mean titres (95% CIs)		
		Day 0 n=79	Day 21 n=77	Day 180 n=72
A(H1N1)pdm09				
A/California/07/2009[a]	1	31.5 (26.0–40.4)	63.9 (51.9–74.5)	38.8 (32.3–48.6)
A/Finland/420/2014	6B	34.8 (29.6–50.4)	85.3 (68.8–105.5)	45.9 (37.4–61.7)
A(H3N2)[b]				
A/Texas/50/2012[a]	3C.1	33.2 (26.5–41.0)	70.3 (57.8–86.0)	50.7 (39.7–60.4)
A/Finland/385/2013	3C.3	25.3 (20.7–31.0)	46.5 (38.1–56.0)	26.8 (21.6–32.4)
A/Switzerland/9715293/2013[c]	3C.3a	11.4 (8.9–14.3)	19.5 (15.1–26.4)	12.1 (9.6–16.0)
A/Finland/428/2014	3C.3a	8.6 (7.3–10.0)	13.7 (11.7–17.8)	8.7 (7.5–10.2)
A/Finland/464/2014	3C.2a	7.6 (6.7–8.7)	12.3 (10.8–15.0)	9.3 (8.1–10.7)
B (Yamagata)				
B/Massachusetts/02/2012[a]	Clade 2	19.8 (16.3–24.5)	37.1 (31.2–44.3)	30.3 (24.7–37.1)
B/Wisconsin/01/2010[d]	Clade 3	19.1 (15.8–23.2)	34.0 (28.4–40.7)	30.0 (25.1–35.7)

CI: confidence interval.

One dose of non-adjuvanted trivalent 2013/14 seasonal influenza vaccine was administered intramuscularly to Finnish healthcare workers. Day 0 refers to serum samples collected before vaccination.

[a] Vaccine strain, northern hemisphere season 2013/14 and 2014/15.

[b] Haemagglutination inhibition test with 20nM oseltamivir carboxylate (Roche).

[c] Vaccine strain, southern hemisphere season 2015.

[d] Vaccine strain, northern hemisphere season 2012/13.

differences was estimated using Student's t-test (paired, two-tailed), with a significance level of $p < 0.05$.

For all virus strains tested, there was a significant ($p < 0.01$) increase in the GMTs of the antibody response three weeks after TIV vaccination (Table 2). At six months, the GMTs decreased by 39.4–46.2%, 24.4–42.3% and 11.9–18.4% for influenza A(H1N1)pdm09, A(H3N2) and B viruses, respectively. The decrease was significant ($p < 0.05$ to $p < 0.001$) for both types influenza A viruses.

The baseline seroprotection rate for A(H1N1)pdm09 viruses was 57.0–58.2% (Figure 2). Three weeks after vaccination, the GMTs were higher for the recently circulating A/Finland/420/2014 strain than for the vaccine strain ($p < 0.05$). Post-vaccination seroprotection rates were 89.6% and 85.7% for A/Finland/420/2014 and A/California/07/2009 viruses, respectively.

The seroprotection rate for A(H3N2) vaccine virus A/Texas/50/2012 was 60.8% before vaccination and 87.0% three weeks after it. Three weeks post-vaccination, the GMTs were somewhat weaker for A/Finland/385/2013, a Finnish representative of group 3C.3 virus strains, than to the vaccine strain ($p < 0.01$). Significantly lower GMTs were detected for the group 3C.3a strain A/Switzerland/9715293/2013 as well as the Finnish group 3C.3a strain A/Finland/428/2014 and recently circulating group 3C.2a strain A/Finland/464/2014 compared with the vaccine strain ($p < 0.0001$).

For drifted Finnish 3C.3a and 3C.2a viruses, baseline seroprotection rates were low (8.9% and 1.3%, respectively) and fivefold reductions in GMTs (for both) were detected three weeks after vaccination, compared with the vaccine strain. The reduction in GMTs for A/Finland/428/2014 (group 3C.3a) was in line with recently reported HI and neutralisation levels [8,9].

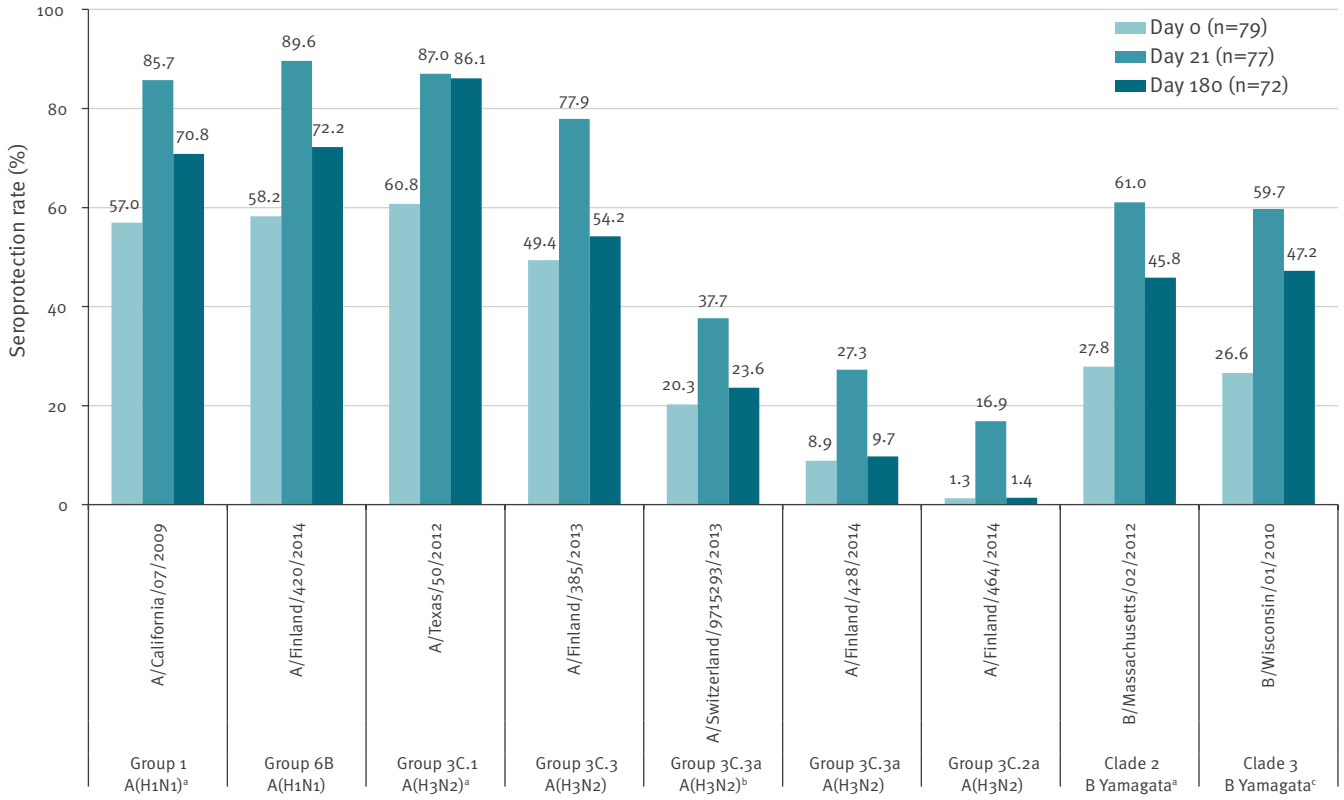
The baseline seroprotection rate for influenza B viruses was 26.6–27.8%. Three weeks after vaccination, very similar seroprotection rates were seen for vaccine strains B/Massachusetts/2/12 (61.0%) and B/Wisconsin/01/2010 (59.7%), which both represent the Yamagata-lineage viruses but belong to different clades.

Discussion

During the 2013/14 season, the relative prevalence of A(H1N1)pdm09, A(H3N2) and B influenza viruses varied from one European country to another [10]. In Finland, A(H1N1)pdm09 viruses predominated but A(H3N2) viruses were also detected. Most of the circulating A(H1N1)pdm09 and A(H3N2) viruses corresponded well with those included in the seasonal TIV vaccine for that season. Viruses from patients requiring intensive care

Figure 2

Seroprotection rates determined by haemagglutination inhibition test before and after vaccination of 79 healthcare workers with 2013/14 trivalent influenza vaccine, Finland



Seroprotection rate was defined as the percentage of participants with a haemagglutination inhibition titre ≥ 40. Day 0 refers to serum samples collected before vaccination.

[a] Vaccine strain, northern hemisphere season 2013/14 and 2014/15.

[b] Vaccine strain, southern hemisphere season 2015.

[c] Vaccine strain, northern hemisphere season 2012/13.

were not proven genetically different from other circulating viruses [11].

Representatives of influenza A(H3N2) groups 3C.2 and 3C.3 were found in Europe in the 2013/14 season and since February 2014, two new genetic subgroups, 3C.2a and 3C.3a, emerged in these clusters [8,10]. Both of these genetic subgroups contain viruses that show antigenic drift from the vaccine virus [1]. In Finland, infections caused by A(H3N2) genetic subgroup 3C.3a viruses were detected between February and April 2014. Genetic subgroup 3C.2a viruses, in contrast, did not circulate in Finland during the 2013/14 season but only emerged in the 2014/15 season.

Drifted influenza A(H3N2) viruses have been circulating in the countries of the European Union and European Economic Area in the 2014/15 season. The majority of genetically characterised viruses belong to group 3C.2a although 3C.3a viruses have also been detected [1]. In the United States (US), the Centers for Disease Control and Prevention has issued a health advisory notice regarding the circulation of drifted influenza A(H3N2) viruses in the US [12]. Early estimates of the current seasonal influenza vaccine effectiveness from the US and Canada suggest low effectiveness against circulating A(H3N2) viruses [13,14].

New antigenic A(H3N2) clusters appear on average every 3.3 years [15]. Seven amino acid locations have been shown to be responsible for the major antigenic changes in A(H3N2) viruses [16]. Subgroup 3C.2a and 3C.3a viruses carry specific amino acid substitutions that drifted from the corresponding main groups. Both subgroups have a substitution at position 159, which has shown to be one of seven positions responsible for the major antigenic changes between 1968 and 2003 A(H3N2) viruses [16].

In our analysis of antibody response, GMTs against the circulating A/Finland/428/2014 virus (a group 3C.3a A(H3N2) virus) were found to be significantly lower

than GMTs against the homologous A/Texas/50/2012 vaccine virus. These results are in line with those from Finnish A(H3N2) variant strains tested in WHO Collaborating Centre for Reference and Research on Influenza, in London, United Kingdom, using HI and virus neutralisation assays [9] and previous serological studies [8]. The pre-vaccination seroprotection rate of the HCWs we tested for this virus variant was only less than 10%. Even at three weeks after vaccination, the cross-protection rate was only less than 30% and decreased to less than 10% within 6 months.

The GMTs were found to be significantly lower against the currently circulating subgroup 3C.2a A(H3N2) virus A/Finland/464/2014 than against the homologous A/Texas/50/2012 vaccine virus. Only one of the 79 HCWs tested had pre-existing seroprotective antibody levels against this virus variant. Three weeks after vaccination, the cross-protection rate was 16.9% and decreased to less than 2% within six months. Subgroup 3C.2a viruses have also shown to have poor reactivity with post-infection ferret antisera against vaccine virus A/Texas/50/2012 [17].

Although influenza A(H1N1)pdm09 viruses have undergone genetic changes from the A/California/07/2009 strains present in the vaccine, the majority of epidemic viruses in Europe have been antigenically similar to the vaccine virus [2,10]. Our serological results indicate a strong vaccine-induced seroprotection rate against A(H1N1)pdm09 viruses. Consistent with this, more than half of the Finnish HCWs tested had pre-existing immunity against A(H1N1)pdm09 viruses. This may be due to the history of sequential TIV vaccinations in the study group or natural infections by A(H1N1)pdm09 viruses.

We acknowledge at least a few limitations in our serological analysis. First, the number of HCWs included in the study was limited. Secondly, the HCWs we tested did not represent all age groups: thus the results do not necessarily apply to children or elderly individuals. Antibody responses to influenza A(H1N1)pdm09 vaccination are age dependent [18] and low vaccine effectiveness against A(H3N2) has been reported among elderly persons [19]. For influenza B viruses, the overall impact of lineage-level mismatch between vaccine and circulating strains has been shown to be considerable, especially among children and adolescents [20]. Thirdly, HCWs are often vaccinated more regularly than others (in Finland, influenza vaccination is recommended for all HCWs who come in contact with patients) and they are also at higher risk of contracting influenza virus. The impact of repeated vaccination on vaccine effectiveness against influenza is still under investigation and discussion [21,22].

In conclusion, our serological data suggest that although the 2013/14 and 2014/15 TIV would protect against A(H1N1)pdm09 viruses, the protection against influenza A(H3N2) 3C.2a and 3C.3a virus variants would be suboptimal. The current epidemic situation in the northern hemisphere underlines the need to change the A(H3N2) component of the 2015/16 vaccine to a virus that represents one of the drifted groups. With minimal pre-existing immunity and a limited cross-protective effect from the TIV, the population in the northern hemisphere may be more susceptible to the new influenza A(H3N2) virus variants during the current 2014/15 season. However, influenza vaccination is strongly encouraged for HCWs, as well as for persons in risk groups, to reduce influenza disease burden and the spread of the epidemics.

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Conflicts of interest

None declared.

Authors' contributions

All authors have contributed to, seen and approved the manuscript. AH performed the serological data analysis and wrote the draft manuscript. NI made the genetic characterisations and participated in the writing of the manuscript. IJ, ER, AK and VJA were involved in the design of TIV vaccination study and sera collections. OL and HN provided their comments and participated in discussions. CSK was responsible for the viral laboratory facility and participated in the writing of the manuscript.

References

1. European Centre for Disease Prevention and Control (ECDC). Rapid risk assessment. Circulation of drifted influenza A(H3N2) viruses in the EU/EEA - 22 December 2014. Stockholm: ECDC; 2014. Available from: http://ecdc.europa.eu/en/publications/Publications/RRA-InfluenzaA-H3N2-Dec-2014.pdf
2. Broberg E, Snacken R, Adlhoch C, Beauté J, Galinska M, Pereyaslov D, et al. Start of the 2014/15 influenza season in Europe: drifted influenza A(H3N2) viruses circulate as dominant subtype. Euro Surveill. 2015;20(4):21023. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21023
3. World Health Organization (WHO). Recommended composition of influenza virus vaccines for use in the 2014-2015 northern hemisphere influenza season. Geneva: WHO; 20 Feb 2014. Available from: http://www.who.int/influenza/vaccines/virus/recommendations/2014_15_north/en/

4. World Health Organization (WHO). Recommended composition of influenza virus vaccines for use in the 2013-2014 northern hemisphere influenza season. Geneva: WHO; 21 Feb 2013. Available from: http://www.who.int/influenza/vaccines/virus/recommendations/2013_14_north/en/
5. Ikonen N, Haanpää M, Rönkkö E, Lyytikäinen O, Kuusi M, Ruutu P, et al. Genetic diversity of the 2009 pandemic influenza A(H1N1) viruses in Finland. PLoS ONE. 2010;5(10):e13329. http://dx.doi.org/10.1371/journal.pone.0013329 PMID:20975994
6. World Health Organization (WHO). Global influenza surveillance network. Manual for the laboratory diagnosis and virological surveillance of influenza. Geneva: WHO; 2011. Available from: http://www.who.int/influenza/gisrs_laboratory/manual_diagnosis_surveillance_influenza/en/
7. Committee for proprietary medicinal products (CPMP). Note for guidance on harmonisation of requirements for influenza vaccines. London: European Medicines Agency; 12 March 1997. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003945.pdf
8. World Health Organization (WHO). Recommended composition of influenza virus vaccines for use in the 2015 southern hemisphere influenza season. Geneva: WHO; 25 Sep 2014. Available from: http://www.who.int/influenza/vaccines/virus/recommendations/2015_south/en/
9. National Institute for Medical Research. WHO Influenza Centre London. Report prepared for the WHO annual consultation on the composition of influenza vaccine for the Southern Hemisphere 2015. 22 – 24 September 2014. London: WHO Collaborating Centre for Reference and Research on Influenza, National Institute for Medical Research; 2014. Available from: http://www.nimr.mrc.ac.uk/documents/about/NIMR-VCM-report-Sep-14-web.pdf
10. European Centre for Disease Prevention and Control (ECDC). Surveillance report. Influenza virus characterisation. Summary Europe, July 2014. Stockholm: ECDC; 2014. Available from: http://www.ecdc.europa.eu/en/publications/Publications/influenza-characterisation-report-july-2014.pdf
11. Ikonen N, Murtopuro S, Virtanen M, Jokinen J, Baum U, Syrjänen R, et al. Influenssakausi Suomessa, viikot 40/2013-20/2014 - Seurantaraportti. Työpaperi: 2014_025. [Surveillance report. Influenza season in Finland, weeks 40/2013-20/2014.] Tampere: Terveyden ja hyvinvoinnin laitos; 2014. [Finnish]. Available from: http://www.julkari.fi/bitstream/handle/10024/116267/URN_ISBN_978-952-302-261-4.pdf?sequence=1
12. Centers for Disease Control and Prevention (CDC). CDC Health Advisory Regarding the Potential for Circulation of Drifted Influenza A (H3N2) Viruses. Atlanta, GA: CDC; 3 Dec 2014. Available from: http://emergency.cdc.gov/han/han00374.asp
13. Flannery B, Clippard J, Zimmerman RK, Nowalk MP, Jackson ML, Jackson LA, et al. Early estimates of seasonal influenza vaccine effectiveness - United States, January 2015. MMWR Morb Mortal Wkly Rep. 2015;64(1):10-5. PMID:25590680
14. Skowronski DM, Chambers C, Sabaiduc S, De Serres G, Dickinson JA, Winter AL, et al. Interim estimates of 2014/15 vaccine effectiveness against influenza A(H3N2) from Canada's Sentinel Physician Surveillance Network, January 2015. Euro Surveill. 2015;20(4):21022. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21022
15. Smith DJ, Lapedes AS, de Jong JC, Bestebroer TM, Rimmelzwaan GF, Osterhaus AD, et al. Mapping the antigenic and genetic evolution of influenza virus. Science. 2004;305(5682):371-6. http://dx.doi.org/10.1126/science.1097211 PMID:15218094
16. Koel BF, Burke DF, Bestebroer TM, van der Vliet S, Zondag GC, Vervaet G, et al. Substitutions near the receptor binding site determine major antigenic change during influenza virus evolution. Science. 2013;342(6161):976-9. http://dx.doi.org/10.1126/science.1244730 PMID:24264991
17. European Centre for Disease Prevention and Control (ECDC). Influenza virus characterization. Summary Europe, November 2014. Stockholm: ECDC; 2014. Available from: http://www.ecdc.europa.eu/en/publications/Publications/ERLI-Net%20report%20November%202014.pdf
18. Strengell M, Ikonen N, Ziegler T, Kantele A, Anttila VJ, Julkunen I. Antibody responses against influenza A(H1N1)pdm09 virus after sequential vaccination with pandemic and seasonal influenza vaccines in Finnish healthcare professionals. Influenza Other Respi Viruses. 2013;7(3):431-8. http://dx.doi.org/10.1111/j.1750-2659.2012.00415.x PMID:22913369
19. Bragstad K, Emborg H, Fischer TK, Voldstedlund M, Gubbels S, Andersen B, et al. Low vaccine effectiveness against influenza A(H3N2) virus among elderly people in Denmark in 2012/13--a rapid epidemiological and virological assessment. Euro Surveill. 2013;18(6):20397. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20397 PMID:23410258
20. Heikkinen T, Ikonen N, Ziegler T. Impact of influenza B lineage-level mismatch between trivalent seasonal influenza vaccines and circulating viruses, 1999-2012. Clin Infect Dis. 2014;59(11):1519-24. http://dx.doi.org/10.1093/cid/ciu664 PMID:25139969
21. McLean HQ, Thompson MG, Sundaram ME, Meece JK, McClure DL, Friedrich TC, et al. Impact of repeated vaccination on vaccine effectiveness against influenza A(H3N2) and B during 8 seasons. Clin Infect Dis. 2014;59(10):1375-85. http://dx.doi.org/10.1093/cid/ciu680 PMID:25270645
22. Neuzil KM. How can we solve the enigma of influenza vaccine-induced protection? J Infect Dis. 2014;pii: jiu651. 10.1093/infdis/jiu651 PMID:25416811

Interim estimates of 2014/15 influenza vaccine effectiveness in preventing laboratory-confirmed influenza-related hospitalisation from the Serious Outcomes Surveillance Network of the Canadian Immunization Research Network, January 2015

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The 2014/15 influenza season in Canada has been characterised to date by early and intense activity dominated by influenza A(H3N2). A total of 99.0% (593/599) hospitalisations for laboratory-confirmed influenza with a known influenza virus type enrolled in sentinel hospitals of the Serious Outcomes Surveillance Network of the Canadian Immunization Research Network were due to influenza A. Of the 216 with a known subtype, influenza A(H3N2) accounted for 99.1% (n=214). Interim unmatched vaccine effectiveness (VE) estimates adjusted for age and presence of one or more medical comorbidities were determined by test-negative case–control design to be –16.8% (90% confidence interval (CI): –48.9 to 8.3) overall and –22.0% (90% CI: –66.5 to 10.7) for laboratory-confirmed influenza A(H3N2). Among adults aged under 65 years, the overall VE was 10.8% (90% CI: –50.2 to 47.0) while in adults aged 65 years or older, the overall VE was –25.4% (90% CI: –65.0 to 4.6).

Clinical trial registration number: NCT01517191.

Introduction

In the 2014/15 influenza season, Canada has to date experienced early, intense influenza activity, with record numbers of long-term care facility outbreaks and a seasonal peak number of influenza-related hospitalisations, laboratory detections of influenza virus, and outpatient consultations for influenza-like-illness occurring in week 53 (28 December 2014 to 3 January 2015) [1]. Antigenic and genetic characterisation of the circulating influenza A(H3N2) strain viruses in both Canada and the United States (US) has demonstrated antigenic drift from the vaccine strain in a majority of characterised isolates, raising concern that vaccine effectiveness (VE) might be suboptimal [1]. In the US, interim VE estimates demonstrate limited effectiveness of the 2014/15 vaccines in the prevention of laboratory-confirmed, medically attended acute respiratory illness in persons of all ages, with adjusted VE estimates of 24% (95% confidence interval (CI): 0 to 43) in children aged 6 months to 17 years, 16% (95% CI: –18 to 41) in adults aged 18 to 49 years and 23% (95% CI: –14 to 47) in adults aged 50 years and older [2]. In Canada, the Sentinel Physicians Surveillance Network recently published interim estimates of VE against laboratory-confirmed, medically attended influenza A and influenza A(H3N2) of –4% (95% CI: –45 to 25) and –8% (95% CI: –50 to 23), respectively [3]. Interim estimates for VE in the prevention of laboratory-confirmed, influenza-related hospitalisations have not yet been reported.

In Canada, annual influenza immunisation is recommended for all persons aged 6 months of age or older, with the primary goal of preventing influenza-associated hospitalisation and death [4]. The vast majority

of influenza vaccine deployed in Canada is intramuscular, non-adjuvanted, trivalent inactivated influenza vaccine. Most immunisation programmes begin in mid-October.

Drifted influenza A(H3N2) viruses were first detected in late March 2014, after the World Health Organization (WHO) recommendations for the 2014/15 northern hemisphere vaccine in mid-February [5]. The 2014/15 influenza vaccines used in Canada include A/California/7/2009 (H1N1)pdm09-like virus; A/Texas/50/2012 (H3N2)-like virus; and B/Massachusetts/2/2012-like virus, as recommended by WHO and Canada's National Advisory Committee on Immunization [4]. Here, we provide an interim estimate of overall and age-stratified 2014/15 influenza VE in the prevention of laboratory-confirmed influenza-related hospitalisation using a test-negative case–control design, based on patients who were admitted up to 10 January 2015 in the hospitals of the Serious Outcomes Surveillance (SOS) Network of the Canadian Immunization Research Network (CIRN).

Methods

Hospital-based surveillance

The CIRN SOS Network was established in 2009 to prospectively monitor annual seasonal influenza VE in the prevention of laboratory-confirmed influenza-related hospitalisation in adults hospitalised in Canada [6]. In this 2014/15 season, the network comprises 15 adult academic and community hospitals in five of the 10 Canadian provinces (namely New Brunswick, Nova Scotia, Quebec, Ontario, and British Columbia) accounting for about 9,000 adult acute-care hospital beds. There are no network hospitals in Canada's three territories. Beginning on 15 November 2014, trained SOS Network surveillance monitors enrolled all hospitalised cases of influenza diagnosed through routine testing occurring as part of usual standard of care. Active surveillance began the week in which two hospitalised influenza cases were identified in the local network hospital or the week when the local hospital or public health laboratory reported two of more positive influenza tests in one week. Active surveillance requires review of all daily admissions of adult patients (aged 16 years and older) to medical wards (e.g. internal medicine, geriatric medicine, family medicine, cardiology, pulmonology) and medical and coronary intensive-care units to identify eligible patients. Patients 16 years of age or older admitted with an acute respiratory illness (i.e. pneumonia, acute exacerbation of chronic obstructive pulmonary disease or asthma, unexplained sepsis, any other respiratory infection or diagnosis, or any respiratory or influenza-like symptom) were eligible for enrolment.

All eligible patients had a nasopharyngeal swab collected as part of routine clinical care or by the SOS Network monitor for testing for influenza by reverse-transcription polymerase chain reaction (RT-PCR) or viral culture in the local hospital or public health laboratory according to routine local testing procedures. Patients were considered immunised if they reported receipt of a 2014/15 influenza vaccine more than two weeks before onset of their symptoms. Self-reported immunisation history was verified with the immunisation provider or an immunisation registry, providing that information was available. Detailed demographic information, medical and surgical history, history of present illness and hospitalisation and outcome details were collected from the patient and their medical record.

The study was approved by the research ethics boards of participating institutions and consent procedures followed local research ethics board requirements (clinical trial registration number: NCT01517191).

Estimation of influenza vaccine effectiveness

Eligible hospitalised patients admitted between 15 November 2014 and 10 January 10 2015 for whom results of influenza testing and self-reported 2014/15 influenza immunisation status were available were included in this interim analysis of VE. Patients with a positive laboratory test for influenza were defined as cases, while those testing negative for influenza within seven days of symptom onset were defined as controls.

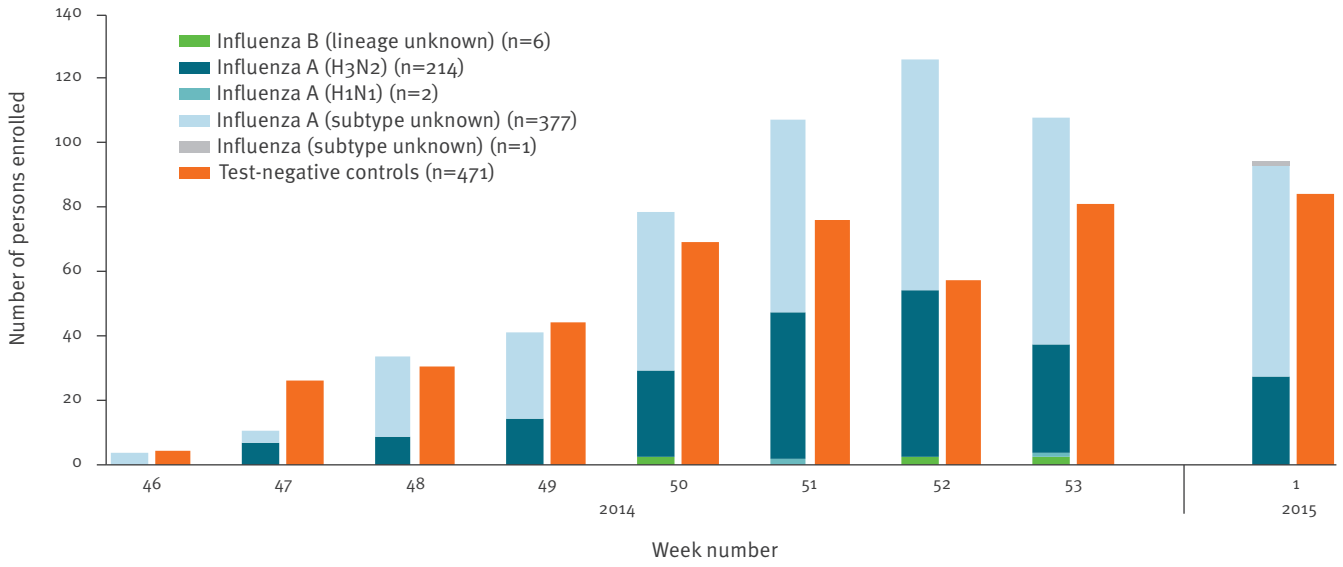
Odds ratios (OR) for influenza vaccination among cases and controls were calculated and VE was estimated as $(1 - OR) \times 100\%$ by logistic regression adjusting for age and presence of one or more medical comorbidities [4]. Overall adjusted VE and VE stratified by age (patients 65 years or older vs patients younger than 65 years) are presented.

In the current interim analysis, VE estimates are not adjusted for site of enrolment. However, it is important to note that the same protocol is used in all participating sites and all enrolment is done by study staff trained and monitored by the central study team. Enrolment criteria for cases and controls, as well as sampling procedures, are standardised across sites.

We have included 90% CIs, as we consider these more appropriate than 95% CIs for our purpose. The 90% CI is used to test our primary objective, which has a one-sided alternative rather than a two-sided alternative. More specifically, we are interested in testing the null hypothesis of VE ≤ 0 (vaccine is not protective) vs the alternative hypothesis of VE > 0 (vaccine is protective). We consider this is more appropriate than testing a null hypothesis of VE = 0 (vaccine will neither increase nor decrease the risk of acquiring influenza) vs the alternative hypothesis of VE < or > 0 (vaccine will either increase or decrease the risk of acquiring influenza). Since the first hypothesis has a one-sided alternative, only the lower bound of the CI matters. Since a 95% CI controls 2.5% type I error on each side, it will make our test a 2.5%-level test rather than a 5%-level test. To aid comparison with other studies, however, we have

Figure

Laboratory-confirmed influenza cases and test-negative controls admitted to hospitals of the Serious Outcomes Surveillance Network of the Canadian Immunization Research Network, by week and virus subtype, 15 November 2014–10 January 2015 (n=1,071)



also included 95% CIs in the table displaying the VE estimates.

Results

Interim estimates of influenza vaccine effectiveness

Between 15 November 2014 (week 46) and 10 January 2015 (week 1), 600 hospitalised influenza cases and 471 hospitalised test-negative controls were enrolled and included in the interim analysis. Laboratory-confirmed influenza cases and test-negative controls admitted to the SOS Network hospitals by week and virus subtype are shown in the Figure. Overall, 99.0% (593/599 cases with known subtype) of hospitalisations for laboratory-confirmed influenza enrolled in participating hospitals were due to influenza A; influenza A (H3N2) accounted for 99.1% (n=214) of the 216 cases with known subtype.

Hospitalised patients with laboratory-confirmed influenza were older than test-negative controls (mean age: 77.7 (standard deviation, SD: 15.2) years vs 70.9 (SD: 16.6) years, respectively; $p<0.001$); 68.8% (n=413) of cases were over 75 years of age compared with only 44.8% (n=211) of test-negative controls ($p<0.001$). The majority of both cases and controls were female (54.2% (n=325) and 52.7% (n=248), respectively; $p=0.62$) and had one or more underlying medical comorbidity (97.2% (512/527) and 97.0% (382/394), respectively; $p=0.85$) (Table 1). A total of 399 (66.5%) cases and 300 (63.7%) test-negative controls reported receipt of the 2014/15 influenza vaccine. Among those for whom outcome data were available, rates of admission to an intensive-care unit (10.1% (650/497) vs 11.1% (35/315); $p=0.64$), need for mechanical ventilation (4.2% (16/377) vs 4.6% (14/303); $p=0.85$), and death (7.9% (28/356) vs 9.7% (23/237); $p=0.46$) did not differ between patients with laboratory-confirmed influenza and test-negative controls.

The overall and age-stratified VE for the prevention of laboratory-confirmed influenza-related hospitalisation in the adults in our study are shown in Table 2. Overall interim VE of 2014/15 influenza vaccines in persons aged 16 years and older, adjusted for age and the presence of one or more medical comorbidities, was −16.8% (90% CI: −48.9 to 8.3). Among adults 65 years and older, the interim adjusted VE was −25.4% (90% CI: −65.0 to 4.6) and among adults under 65 years of age, the interim adjusted VE was 10.8% (90% CI: −50.2 to 47.0). Overall adjusted VE against confirmed influenza A(H3N2) was −22.0% (90% CI: −66.5 to 10.7). Among adults 65 years and older, the interim adjusted influenza A(H3N2) VE was −32.9% (90% CI: −90.0 to 7.0) and among adults younger than 65 years of age, the interim adjusted VE was 7.5% (90% CI: −78.3 to 52.0).

Discussion

The 2014/15 influenza season in Canada has been dominated by circulation of influenza A(H3N2) viruses [1] and this is consistent among hospitalised influenza cases admitted to SOS Network hospitals. Genetic and antigenic characterisation of circulating influenza strains by the National Microbiology Laboratory (NML) in Winnipeg, Manitoba, Canada has demonstrated a predominance of drifted influenza A(H3N2) strains, indicating a poor match between the circulating influenza A(H3N2) virus and the 2014/15 A(H3N2) northern hemisphere influenza vaccine strain [1,3]. Overall, less than 1% of viruses characterised were well matched to A/Texas/50/2012, the A(H3N2) component of the 2014/15 influenza vaccines. Of 55 A(H3N2) viruses

Table 1

Characteristics of laboratory-confirmed influenza cases (n = 600) and test-negative controls (n = 471) included in the interim analysis of 2014/15 influenza vaccine effectiveness, Serious Outcomes Surveillance Network of the Canadian Immunization Research Network, 15 November–10 January 2015

Characteristics	Cases n = 600 n (%)[a]	Controls n = 471 n (%)[a]	**Total n = 1,071 n (%)[a]**	P value[b]
Mean age (SD); range	77.7 (15.2) years; 18–105	70.9 (16.6) years; 19–101	**74.7 (16.2) years; 18–105**	<0.001
Age group				
16–49 years	42 (7.0)	55 (11.7)	**97 (9.1)**	<0.001
50–64 years	41 (6.8)	79 (16.8)	**120 (11.2)**	
65–75 years	104 (17.3)	126 (26.8)	**230 (21.5)**	
>75 years	413 (68.8)	211 (44.8)	**624 (58.3)**	
Sex				
Female	325 (54.2)	248 (52.7)	**573 (53.5)**	0.62
Inclusion criteria at enrolment				
Pneumonia	179 (29.8)	223 (47.3)	**402 (37.5)**	<0.001
Acute exacerbation of COPD or asthma	82 (13.7)	121 (25.7)	**203 (19.0)**	<0.001
Unexplained sepsis	15 (2.5)	25 (5.3)	**40 (3.7)**	0.02
Any other acute respiratory illness[c]	414 (69.0)	188 (39.9)	**602 (56.2)**	<0.001
Invasive pneumococcal disease	1 (0.2)	6 (1.3)	**7 (0.7)**	0.05
One or more medical comorbidities[d]	512/527 (97.2)	382/394 (97.0)	**894/921 (97.1)**	0.85
Received 2014/15 influenza vaccine[e]				
All age groups	399 (66.5)	300 (63.7)	**699 (65.3)**	0.37
16–49 years	13 (31.0)	22 (40.0)	**35 (36.1)**	0.40
50–64 years	21 (51.2)	44 (55.7)	**65 (54.2)**	0.70
65–75 years	70 (67.3)	86 (68.3)	**156 (67.8)**	0.88
>75 years	295 (71.4)	148 (70.1)	**443 (71.0)**	0.78
Course in hospital[d]				
Admitted to intensive-care unit	50/497 (10.1)	35/315 (11.1)	**85/812 (10.5)**	0.64
Required mechanical ventilation	16/377 (4.2)	14/303 (4.6)	**30/680 (4.4)**	0.85
Died in hospital	28/356 (7.9)	23/237 (9.7)	**51/593 (8.6)**	0.46

COPD: chronic obstructive pulmonary diseas; SD: standard deviation.

[a] Unless otherwise indicated.

[b] Cases vs controls.c Includes those with any other respiratory infection or diagnosis; or any respiratory or influenza-like symptom (e.g. dypsnoea, cough, sore throat, myalgia, arthralgia, fever).

[d] Data on medical comorbidities and course in hospital are reported as rates among those with available data. The denominator represents the number of patients from whom this data point was available.

[e] The denominators are the numbers in the respective age group.

tested by haemagglutinin inhibition assay, only one virus was antigenically similar to A/Texas/50/2012. Five viruses showed reduced antibody titres to A/Texas/50/2012 and 49 were antigenically similar to A/Switzerland/9715293/2013. Among 250 influenza A(H3N2) viruses genetically characterised by sequence analysis, 249 belonged to a drifted genetic group predicted to have reduced titres to the vaccine strain A/Texas/50/2012 [1].

Our interim VE estimates derived from influenza-related hospitalisations from 15 November 2014 to January 10, 2015 demonstrate overall lack of effectiveness of the 2014/15 influenza vaccine for the prevention of influenza-related hospitalisation in adults. While the relationship between VE and antigenic match is not always clear, and VE cannot be predicted directly from virological surveillance, our results might have been anticipated given that over 99% of circulating A(H3N2) strains characterised by the NML have been antigenically drifted from the A(H3N2) vaccine strain, and were similar to the antigenically distinct A/Switzerland/9715293/2013, which is the A(H3N2) component recommended for the 2015 southern hemisphere vaccine [1,7]. Our overall interim influenza A(H3N2) VE of –22% (90% CI: –66.5 to 10.7) is lower than the interim VE against influenza A(H3N2) laboratory-confirmed influenza associated with medically attended acute respiratory illness reported in the US (22% (95% CI: 5 to 35) [2] for a variety of reasons. Most importantly, although both Canada and the US have experienced early influenza seasons characterised by

Table 2

Interim estimates of 2014/15 influenza vaccine effectiveness in the prevention of laboratory-confirmed influenza-related hospitalisation in adults from the Serious Outcomes Surveillance Network of the Canadian Immunization Research Network, 15 November 2014–January 10, 2015 (n=1,071)

Cohort	Vaccine effectiveness estimate %	90% CI	95% CI
Unadjusted			
All strains			
Overall	−13.1	−39.9 to 8.5	−45.7 to 12.1
Age≥65 years	−5.7	−35.9 to 17.8	−42.6 to 21.6
Age<65 years	28.5	−13.7 to 55.1	−24.3 to 58.9
Confirmed A(H3N2)			
Overall	−14.8	−52.8 to 13.8	−61.4 to 18.4
Age≥65 years	−9.6	−53.2 to 21.6	−63.4 to 26.5
Age<65 years	17.6	−53.3 to 55.7	−72.7 to 60.7
Adjusted[a]			
All strains			
Overall	−16.8	−48.9 to 8.3	−56.0 to 12.5
Age≥65 years	−25.4	−65.0 to 4.6	−73.8 to 9.5
Age<65 years	10.8	−50.2 to 47.0	−66.0 to 52.1
Confirmed A(H3N2)			
Overall	−22.0	−66.5 to 10.7	−76.8 to 15.9
Age≥65 years	−32.9	−90.0 to 7.0	−103.5 to 13.2
Age<65 years	7.5	−78.3 to 52.0	−102.2 to 57.7

CI: confidence interval.

[a] Adjusted for age and presence of one or more medical comorbidities [4].

dominant circulation of influenza A(H3N2), only approximately two thirds of circulating A(H3N2) viruses in the US are genetically and antigenically drifted from the 2014/15 vaccine strain compared with more than 99% of circulating strains in Canada [1,2,8]. Both the US and Canadian interim VE estimates reported thus far have assessed VE against laboratory-confirmed medically attended acute respiratory illness in the community among both children and adults and thus might be predicted to be higher than our estimates of VE in the prevention of influenza-associated hospitalisation in predominantly elderly patients with medical comorbidities. While only 14% of cases included in the US VE analysis and 16% of cases in the Canadian Sentinel Physician Surveillance Network analysis were 65 years or older, 69% of our hospitalised cases were over the age of 75 years and 97% of adults in our study population had medical comorbidities, which put them at increased risk of influenza complications [2,3]. Point estimates of overall adjusted VE in adults younger than 65 years of age in our study were more comparable to those reported in the US (10.8% in our study vs 16% in 18–49 year-olds in the US) and in Canada (6% in 20–64 year-olds), although none of these estimates were statistically significant [2,3].

Canada last experienced an influenza A(H3N2)-dominant influenza season in 2012/13. During that season, only 47% of hospitalised laboratory-confirmed influenza patients in the SOS Network were over the age of 75 years and 92% had medical comorbidities, compared with 69% of cases over the age of 75 years and presence of comorbidities in 97% this year [9]. The percentage of patients in 2012/13 requiring admission to an intensive-care unit, requiring mechanical ventilation, or dying as a result of influenza was similar to, but marginally higher than, the current season (15% vs 11%; 9% vs 4%; and 9% vs 8%, respectively), possibly reflecting reduced intensity of care in the elderly individuals this season [9]. During the 2012/13 season, VE for the prevention of influenza A(H3N2)-associated hospitalisation was 38% [9]. While circulating A(H3N2) isolates during the 2012/13 season were antigenically similar to the A/Victoria/361/2011 vaccine strain, they were antigenically distinct from the egg-adapted vaccine strain used in vaccine production, potentially accounting for the observed suboptimal VE [10].

As hospital care for adults is provided in more than one hospital in most cities across Canada, the population-base, or catchment, for hospitals participating in the SOS Network cannot be readily assessed. We do, however, assess the representativeness of the cases admitted to SOS Network hospitals by comparing them to all hospitalised cases reported to the Public Health Agency of Canada through available surveillance mechanisms,

most notably, reporting from the provincial and territorial governments. Each season, the strain distribution of hospitalised cases enrolled across the SOS Network as well as the age distribution, immunisation coverage rates, and outcomes is comparable to that reported by the Public Health Agency of Canada in Canada's FluWatch, providing reassurance that the estimates of VE generated by the SOS Network should be generalisable in Canada.

Our findings are subject to several limitations. Because large numbers of patients are needed to demonstrate statistical significance when VE estimates are low, our estimates of VE in adults are imprecise and it is possible that low, but statistically significant, effectiveness of the 2014/15 vaccine is expected to be demonstrated as the accumulated sample size grows during the remainder of the influenza season. Because the influenza season thus far has been characterised by almost exclusive circulation of a drifted influenza A(H3N2) virus in Canada, it is possible that end-of-season VE estimates may differ if circulation of influenza A(H1N1) or influenza B viruses occurs later this season. Limited characterisation of influenza A(H1N1) and influenza B strains circulating in Canada thus far suggest a good match to vaccine strains [1]; thus vaccine recipients may still benefit from protection against these strains should they begin to circulate later in the season. In that case, fully adjusted end-of-season VE estimates may be higher than our interim estimates. Because estimates have not yet been fully adjusted for a variety of potential confounding factors particularly important for elderly patients (e.g. frailty) and a final analysis using a matched case–control design in which cases will be matched with controls by hospital site, age strata (<65 years vs ≥65 years) and date of admission has not yet been performed, the final matched and fully adjusted estimates might differ from the interim partially adjusted estimates presented here. Matching is not feasible at the stage of the interim analysis and is therefore conducted as an unmatched analysis. Finally, because for some subjects contributing to the current interim estimates, the self-reported influenza immunisation status could not be verified using the immunisation provider or an immunisation registry, it cannot be fully excluded that some misclassification may have occurred. However, based on our experiences in prior seasons, there is high concordance between self-report and provider-reported immunisation status, so the expected impact of misclassification is expected to be very low.

Using data from 15 November to 10 January 2015, the demonstrated lack of effectiveness of the 2014/15 seasonal influenza vaccines for the prevention of influenza-associated hospitalizations in adults, particularly in adults over the age of 65 years, highlights the importance of employing additional strategies to control and prevent the spread of influenza, such as frequent hand cleansing, encouraging people to stay home when sick and encouraging proper cough etiquette. Furthermore, it is critical that healthcare providers consider a diagnosis of influenza in all patients presenting with acute respiratory illness irrespective of immunisation history and test patients for influenza as appropriate. Healthcare providers should be aware that hospitalised adults with laboratory-confirmed influenza frequently do not present with influenza-like-illness [11]. Thus, they should test for influenza and implement contact and droplet precautions for all patients being admitted to hospitals with an acute respiratory illness during the influenza season while awaiting results, in order to minimise nosocomial influenza transmission.

Appropriate use of antiviral medication in the treatment of suspected cases of influenza is critical in seasons characterised by a low VE, such as the current season in Canada. In order to reduce severe complications such as hospitalisation and potential death among vulnerable individuals, the Association of Medical Microbiology and Infectious Disease Canada recommends the prompt use of neuraminidase inhibitors (oseltamivir or zanamivir) in hospitalised patients, patients with progressive, severe or complicated disease, and patients at high risk of complications from influenza regardless of their vaccination status [12]. Because the benefit of treatment with antiviral medications is maximal when treatment is started early in the course of illness, ideally within 48 hours of symptom onset, healthcare providers should maintain a high index of suspicion of influenza in patients presenting with acute respiratory illness, irrespective of their immunisation status, and should start antivirals empirically while awaiting influenza testing [12].

While the relationship between vaccine strain and circulating strain mismatch is not fully understood and variable effectiveness by match has been observed in past seasons, the suboptimal VE observed in the current season and in prior seasons with significant mismatch between circulating viruses and vaccine strains reflects, at least in part, the challenge in current vaccine technologies, which require determination of the vaccine composition months ahead of the influenza season and highlights the urgent need for the continued development of new vaccine technologies [7]. Stakeholders must continue to refine key elements that must be considered to optimise vaccine strain selection and vaccine manufacturers should strive to improve vaccine formulations to optimise cross-protection, particularly for influenza A(H3N2) viruses [13]. While influenza vaccination remains the most important means of preventing influenza, ongoing assessment of VE and provision of mid-season VE estimates for the prevention of influenza-related hospitalisation in adults is critical to understanding the periodic impact of circulating and vaccine strain mismatch on vaccine performance and to inform public health communication with respect to adjunctive preventive strategies, particularly in years of suboptimal VE.

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Conflict of interest

FH and RS are employed by the GlaxoSmithKline Group of Companies. VS reports that he was employed by GSK Vaccines at the time of the study and reports ownership of stock options and/or restricted shares in the GlaxoSmithKline Group of Companies; GDS is employed by Business and Decision Life Sciences (on behalf of GlaxoSmithKline Vaccines); SAM reports research grants from GlaxoSmithKline, Pfizer, Sanofi Pasteur, personal fees from Merck, Pfizer; JEM personal fees from GlaxoSmithKline, Sanofi Pasteur; TFH reports research grants from GlaxoSmithKline, Pfizer; MKA reports research grants from GlaxoSmithkline, Pfizer; JL reports research grants from GlaxoSmithKline, Pfizer.

Authors' contributions

SAM, MKA, LY, FH, TFH, ME, AA, AM, JEM, ML, DMC, VS were involved in the conception and design of the study; SAM, MKA, AM, JEM, ML were responsible for acquisition of data; TFH, ME conducted/supervised the CIRN SOS Network central laboratory; SAM, MKA, LY, FH, DMC, AM, JEM, ML, RS, GDS analysed and interpreted the data; SAM drafted the manuscript; all authors revised the manuscript critically for important intellectual content; all authors reviewed and approved the final draft of the manuscript

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References

1. Public Health Agency of Canada (PHAC). FluWatch report: January 4 to January 10, 2015 (Week 1). Ottawa: PHAC; 2015. Available from: http://www.phac-aspc.gc.ca/fluwatch/14-15/w01_15/index-eng.php
2. Flannery B, Clippard J, Zimmerman RK, Nowalk MP, Jackson ML, Jackson LA, et al. Early estimates of seasonal influenza vaccine effectiveness - United States, January 2015. MMWR Morb Mortal Wkly Rep. 2015;64(1):10-5. Available from: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6401a4.htm?s_cid=mm6401a4_w PMID:25590680
3. Skowronski DM, Chambers C, Sabaiduc S, De Serres G, Dickinson JA, Winter AL, et al. Interim estimates of 2014/15 vaccine effectiveness against influenza A(H3N2) from Canada's Sentinel Physician Surveillance Network, January 2015. Euro Surveill. 2015;20(4):pii=21022. Available from: http://www.eurosurveillance.org/Viewarticle.aspx?ArticleId=21022
4. National Advisory Committee on Immunization. Statement on seasonal influenza vaccine for 2014-2015. Ottawa: Public Health Agency of Canada; 2014. Available from: http://www.phac-aspc.gc.ca/naci-ccni/assets/pdf/flu-grippe-eng.pdf
5. Centers for Disease Control and Prevention (CDC). CDC health advisory regarding the potential for circulation of drifted influenza A(H3N2) viruses. Centers for Disease Control and Prevention Health Alert Network. Atlanta, GA: CDC: 3 Dec 2014. Available from: http://emergency.cdc.gov/HAN/han00374.asp
6. McNeil S, Shinde V, Andrew M, Hatchette T, Leblanc J, Ambrose A, et al. Interim estimates of 2013/14 influenza clinical severity and vaccine effectiveness in the prevention of laboratory-confirmed influenza-related hospitalisation, Canada, February 2014. Euro Surveill. 2014;19(9):20729. http://dx.doi.org/10.2807/1560-7917.ES2014.19.9.20729. PMID:24626207
7. Kelly HA, Sullivan SG, Grant KA, Fielding JE. Moderate influenza vaccine effectiveness with variable effectiveness by match between circulating and vaccine strains in Australian adults aged 20-64 years, 2007-2011. Influenza Other Respi Viruses. 2013;7(5):729-37. http://dx.doi.org/10.1111/irv.12018. PMID:23078073
8. Centers for Disease Control and Prevention (CDC). FluView. 2014-2015 Influenza Season Week 2 ending January 17, 2015. U.S. Virologic Surveillance, Influenza Virus Characterization. Atlanta, GA: CDC. [Accessed 31 Jan 2015]. Available from: http://www.cdc.gov/flu/weekly
9. McNeil SA, Ambrose A, Andrew M, Diaz Mitoma F, Boivin G, Bowie W, et al. Effectiveness of 2012/13 seasonal influenza vaccines in the prevention of influenza-related hospitalization in Canadian adults: A Public Health Agency of Canada/Canadian Institutes of Health Research (PCIRN) Serious Outcomes Surveillance Network Study. Abstract P2-511. Presented at Options for the Control of Influenza VIII, Cape Town, South Africa, 5-10 September, 2013. Available from: http://optionsviii.controlinfluenza.com/optionsviii/assets/File/Options_VIII_Abstracts_2013.pdf
10. Skowronski DM, Janjua NZ, De Serres G, Sabaiduc S, Eshaghi A, Dickinson JA, et al. Low 2012-13 influenza vaccine effectiveness associated with mutation in the egg-adapted H3N2 vaccine strain not antigenic drift in circulating viruses. PLoS ONE. 2014;9(3):e92153. http://dx.doi.org/10.1371/journal.pone.0092153. PMID:24667168
11. McNeil SA, Krajden O, Gajic S, Elsherif M, Green K, McGeer A, et al. Performance characteristics of influenza-like-illness case definitions in predicting influenza in hospitalized Canadian adults. Presented at IDWeek 2012, 17-21 Oct 2012, San Diego, CA. Available from: https://idsa.confex.com/idsa/2012/viewsessionpdf.cgi
12. Aoki FY, Allen UD, Stiver HG, Evans GA. The use of antiviral drugs for influenza: Guidance for practitioners 2012/2013. Can J Infect Dis Med Microbiol. 2012;23(4):e79-92. PMID:24294283
13. World Health Organization (WHO). 3rd WHO informal consultation for improving influenza vaccine virus selection. 1 to 3 April 2014, Geneva, Switzerland. Geneva: WHO. [Accessed 15 Jan 2015]. Available from: http://www.who.int/influenza/vaccines/virus/3rdmtg_improve_vaccine_virus_selection/en/

Typing of *Chlamydia psittaci* to monitor epidemiology of psittacosis and aid disease control in the Netherlands, 2008 to 2013

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A simple genotyping method was developed and validated for all known *Chlamydia psittaci* reference genotypes. *C. psittaci* is currently divided into nine genotypes (A-F, E/B, M56 and WC), all more or less associated with the preferred avian host. This method targeting variable domain 4 of the *ompA* gene has a lowest detection limit of 1 to 10 copies per PCR and was applied to 69 human samples collected in nine hospitals in the Netherlands from September 2008 until the end of October 2013. Genotype A was the most prevalent genotype. In addition, genotypes B, C, a new genotype, and *C. abortus* were found. A *C. caviae* infection was detected as a result of extension of this surveillance study to the national level. The sensitivity of this method compared with our real-time diagnostic PCR was 0.98 (66/67 typable samples). Specificity was 1.0 based on 33 commonly encountered bacterial and yeast species and 20 human respiratory samples. This typing method could help monitor *C. psittaci* infections in humans and provides insight into the relationships between notified human 'psittacosis' cases and the probable avian and other animal sources. When needed, a result can be obtained within 24 hours.

Introduction

Psittacosis, a notifiable disease in many countries, is caused by *Chlamydia psittaci* (also known as *Chlamydophila psittaci*). Clinical signs of psittacosis range from none to life-threatening disease requiring admission to intensive care. In 1999, Everett et al. proposed splitting the single genus *Chlamydia* into two genera, *Chlamydia* and *Chlamydophila*, based on clustering analyses of the 16S rRNA and 23S rRNA genes [1]. However, taxonomic separation of the genus based on ribosomal sequences is not consistent with the natural history of the organism as revealed by recent genome comparisons. Consequently, the proposal was made to reunite the *Chlamydia* in a single genus [2]. The single genus nomenclature was published in the latest edition of the Bergey's Manual of Systematic Bacteriology [3]. *C. psittaci* is currently divided into nine genotypes (A-F, E/B, M56 and WC), all more or less associated with the preferred avian host in each case.

The genotypes WC and M56 have been found in cattle and a muskrat, respectively, and are probably not associated with birds [4]. Genotype A is associated with *Psittaciformes* (cockatoos, parrots, parakeets, lories), B with *Columbiformes* (doves and pigeons), C with *Anseriformes* (mainly ducks and geese) and D with turkeys. Genotype E is the most diverse; ca 20% of strains were isolated from pigeons, but genotype E has also been found in ratites. Genotype E/B is mainly associated with ducks. Genotype F is encountered rarely in *Psittaciformes* and Turkeys [5]. *C. psittaci*, together with the closely related *C. abortus*, has also been found in cattle [6]. The role of these mammals as vectors of zoonotic *Chlamydia* spp. infections still needs to be established.

For epidemiologic purposes, characterisation of *C. psittaci* in human samples provides knowledge on the most prevalent genotypes in human infections, infers probable avian sources and aids in the process of notification, surveillance and outbreak management. For decades, the diagnosis has been based on serological tests. In the past decade, diagnostic *C. psittaci* PCR assays were developed and introduced in the clinical setting. In the Netherlands this aided the diagnostic process for suspected psittacosis cases [7,8]. One of the advantages of the PCR approach over serological testing is the presence of *C. psittaci* DNA in these clinical samples. These samples are therefore suitable for further genotyping assays. Genotyping

can be done by real-time PCR with competitor probes [9], melting curve analysis [10], MLVA [11], MLST [4], microarray or other sequence analysis [12]. Previously, a sequencing-based approach aimed at the outer membrane protein gene (ompA) was successful on human clinical samples, but the method proved laborious and was not very sensitive, mainly owing to the relatively long PCR product and abundant side products [13].

Culturing of *C. psittaci* could provide sufficient DNA for more in-depth typing methods such as MLVA or MLST. However, culturing is hampered by limited sensitivity, previous antibiotic use and the necessarily strict biosafety regulations. When culturing *C. psittaci*, biosafety level 3 precautions are needed. Nowadays, *C. psittaci* is only cultured in a few specialised laboratories.

None of the above typing methods has been evaluated on more than a handful of human samples. Even in a recently described outbreak of psittacosis in Sweden, only four of 12 available human samples could be typed [14]. In this study, we describe a new simple typing method for *C. psittaci* based on variable domain 4 (VD4) of the *ompA* gene. It does not require any specialised equipment other than a real-time PCR cycler and a (remote) sequencing facility. This method was applied directly on human samples positive for *C. psittaci*.

Methods

Bacterial strains and control DNA

C. psittaci genotypes A-F, E/B, M56 and WC were used as positive controls. The following strains were used: Genotype A: Orni (human), Genotype B: CP3 (pigeon), Genotype C: GR9 (German duck), Genotype D: NJ1 (New Jersey turkey), Genotype E: CPMN (human), Genotype F: VS225 (parakeet), Genotype E/B: WS/RT/E30 (German duck), Genotype M56 (muskrat), Genotype WC (*Bos taurus*). A quantified (15,000 copies per µl) commercially available *C. psittaci* DNA (Amplirun *C. psittaci* genotype A control, Vircell, Granada, Spain) control was used for determination of the lower limit of detection. The new assay was evaluated with the Quality Control for Molecular Diagnostics (QCMD, Glasgow, Scotland) External Quality Assessment (EQA) pilot panel for *C. psittaci* 2013 (CPS13).

The following strains and samples were used for specificity testing: 27 ATCC (American type culture collection) strains, one NCTC (National Collection of Type Cultures, Public Health England) strain and three Dutch quality control (SKML; Dutch Foundation for Quality Assessment in Medical Laboratories) strains, as well as QCMD EQA *Mycoplasma pneumoniae*/*Chlamydia pneumoniae* panel CP.MP13 samples 09 and 05 containing *C. pneumoniae* and *M. pneumoniae* DNA (Table 1).

Clinical samples and DNA extraction

For specificity testing, 20 respiratory samples negative for *C. psittaci* DNA were tested with the newly developed typing method.

Clinical samples (sputa, bronchoalveolar lavage fluid, (naso)pharyngeal swabs and serum) positive in diagnostic *C. psittaci* PCRs were obtained from nine hospital laboratories from the Netherlands. Most of these laboratories use real-time PCRs that detect, but do not differentiate, at least *C. psittaci* and *C. abortus* and sometimes also *C. caviae* and *C. felis* [7,8]. This means that clinical samples could contain these very closely related species as well. Nucleic acid purification was performed at the nine Dutch laboratories with the Magnapure (Roche Diagnostics), EasyMag (BioMérieux) or Versant kPCR Molecular system (Siemens Healthcare Diagnostics). Two clinical samples were obtained from Scotland and related to a previously reported outbreak [15]. Clinical samples and/or eluates were sent to the Orbis Medical Centre in the Netherlands for further analysis. Archived samples were collected from 2008 to 2012. Since September 2012, the typing method has been implemented nationally and samples have prospectively been typed and reported to submitting laboratories and public health authorities.

For validation experiments, nucleic acids were purified with the Versant kPCR Molecular system using Sample Preparation (SP) Kit 1.0 with SP protocol 250 µl sample input and 100 µl eluate output (Siemens Healthcare Diagnostics). All positive control strains were spiked with a background of pooled *C. psittaci*-negative sputum samples to simulate the diagnostic setting as close as possible. QCMD samples were processed according to the accompanying instructions.

This research was submitted for consideration to our local accredited medical ethical research committee METC Atrium-Orbis-Zuyd. According to this committee, this research does not fall under the scope of the Medical Research Involving Human Subjects Act. All prospective samples were obtained for diagnostic use and handled accordingly. Retrospective samples were analysed anonymously to the extent reasonably possible.

PCR based on variable domain 4 of the *ompA* gene

With the aid of Primer3Plus, a new primer set was developed to include a variable part of the *ompA*, the VD4 domain [16]. The amplified VD4 sequence permits differentiation of at least nine *C. psittaci* genotypes (A-F and E/B) and the closely related *C. abortus*. The primer set was verified with in-silico amplification [17] and revealed positive results for available *C. psittaci* genomes and *C. abortus* S26/3 only (setting: 'maximum two mismatches allowed'). The primer set consisted of CPVDF 5'–GTC AAG AGC AAC TTT TGA TGC–3' and CPVDR 5'–ATT TTG TTG ATC TGA ATC GAA GC–3' (nucleotide positions CPVDF 897-917 and CPVDR

1,057-1,079 of the *ompA* gene of the *C. psittaci* VS1 strain, GenBank accession number AY762608). A fragment between 174 and 183 base pairs, depending on the genotype, is amplified. *C. caviae* primers were constructed by substituting five nucleotides in the above primer pair to obtain complete homology with the *C. caviae* VD4 sequence. CCVDF 5'–GTC CAG AGC TAC ATT TGA TGC–3' and CCVDR 5'–ATT TTG TTG ATT TGA AGC GAA GC–3'. *C. caviae* species confirmation was done by PCR high-resolution melting (HRM) curve analysis as described by Robertson et al., using DNA of the *C. caviae* reference strain (GPIC) as positive control [18].

Reactions for the VD4 PCR were performed in the Stratagene MX3005P QPCR system (incorporated in the Siemens Versant kPCR system). The uracil-N-glycosylase system (UNG) was used to prevent false-positive reactions due to amplicon carry over. After optimisation, the final reaction volume (25 µl) included 5 µl eluate, 12.5 µl (2x) Greenmaster qPCR mix with ROX reference dye, uracil-N-glycosylase (Jena biosciences, Jena, Germany) and 0.5 µM of each primer. The real-time PCR steps were as follows: 1) 50 °C for 2 min, 2) 95 °C for 3 min, 3) 40 cycles of 95 °C for 5 sec, and 60 °C for 60 sec. Fluorescence was detected in the FAM channel and normalised on the ROX signal. Subsequently, a dissociation curve was generated by continuous fluorescence acquisition from 60 to 95 °C to observe possible additional PCR products and establish the formation of the expected PCR amplicon by determining the melting temperature (Tm). At first, all reference genotypes were used as positive controls in each run. For ease of application, we decided later to use only three genotypes (A, C and D) as controls.

Validation of the VD4 PCR

Analytical sensitivity was determined by testing 10-fold dilutions of the commercially available Amplirun *C. psittaci* genomic DNA control starting from 10^4 genome equivalents per PCR reaction. Serial dilutions were prepared in Tris/EDTA buffer, pH 8.0, supplemented with 20 ng/µl salmon sperm DNA). Reactions were performed in triplicate. Limiting dilutions were tested with and without previous nucleic acid extraction. When applying nucleic acid extraction, a matrix of *C. psittaci* DNA-negative, pooled and liquefied sputum samples was used. Sequence analysis was performed only on the lowest positive dilution series to confirm the identity of the positive control strain. For comparison, all dilutions were also tested with the previously described full-length *ompA* PCR and the diagnostic PCR [7,13].

Specificity was determined on a panel of bacterial and yeast species commonly encountered in human (respiratory) specimens (Table 1). Strains were diluted to a 0.5 McFarland standard turbidity equivalent to ca 10^8 colony-forming units (CFU)/ml, and 250 µl of this suspension was subsequently subjected to nucleic acids purification. Diagnostic specificity was tested using 20 human respiratory samples, previously tested negative for *C. psittaci* DNA by our previously described *C. psittaci* PCR [7]. Clinical sensitivity was determined on all *C. psittaci* PCR-positive clinical samples sent to our laboratory with a request for genotyping.

Table 1

Strains used for specificity testing of the *Chlamydia psittaci* VD4 PCR (n=33)

Species	Strain[a]	VD4 PCR
Klebsiella pneumoniae	ATCC 700603	negative
Klebsiella pneumoniae	ATCC 13883	negative
Streptococcus pyogenes	ATCC 19615	negative
Streptococcus pneumoniae	ATCC 6303	negative
Streptococcus agalactiae	SKML 1905	negative
Staphylococcus aureus	ATCC 25923	negative
Staphylococcus aureus	ATCC 29213	negative
Staphylococcus epidermidis	ATCC 12228	negative
Staphylococcus aureus (meticillin-resistant)	ATCC 43300	negative
Enterococcus faecalis	ATCC 29212	negative
Enterococcus faecium	ATCC 35667	negative
Moraxella catarrhalis	SKML 967	negative
Haemophilus parainfluenzae	ATCC 7901	negative
Haemophilus influenzae	ATCC 35056	negative
Neisseria meningitidis	ATCC 13090	negative
Legionella pneumophila	SKML 2013	negative
Bacillus cereus	ATCC 11778	negative
Enterobacter aerogenes	ATCC 35028	negative
Proteus vulgaris	ATCC 13315	negative
Proteus mirabilis	NCTC 10975	negative
Escherichia coli	ATCC 25922	negative
Pseudomonas aeruginosa	ATCC 27853	negative
Burkholderia cepacia	ATCC 25416	negative
Bacteroides fragilis	ATCC 25285	negative
Peptostreptococcus anaerobius	ATCC 27337	negative
Prevotella melaninogenica	ATCC 25845	negative
Bacteroides thetaiotaomicron	ATCC 29741	negative
Candida albicans	ATCC 90028	negative
Candida tropicalis	ATCC 13803	negative
Candida krusei	ATCC 6258	negative
Candida glabrata	ATCC 90030	negative
Mycoplasma pneumoniae	QCMD CPMP13–09	negative
Chlamydia pneumoniae	QCMD CPMP13–05	negative

[a] ATCC: American Type Culture Collection; NCTC: national collection of type cultures; QCMD: external quality assessment samples; SKML: Dutch quality control assessment strains.

Sequence analysis

Sequence analysis was performed by an external Sanger sequencing facility (Baseclear BV, Leiden, the Netherlands). A 1:10 dilution of the amplification product in PCR-grade water was added to the forward or reverse primer with a final primer concentration

Table 2

Lower detection limit of the *Chlamydia psittaci* VD4 PCR and comparison with the diagnostic PCR and the previously described full-length *ompA* PCR

Copies/PCR	Diagnostic PCR	VD4 PCR	Full-length ompA PCR	Diagnostic PCR	VD4 PCR	Full-length ompA PCR
	without nucleic acid extraction			with nucleic acid extraction[a]		
10,000	3/3	3/3	3/3	Not done	Not done	Not done
1,000	3/3	3/3	3/3	3/3	3/3	3/3
100	3/3	3/3	3/3[b]	3/3	3/3	3/3[c]
10	3/3	3/3	0/3	1/3	2/3	0/3
1	3/3	2/3	0/3	0/3	0/3	0/3
0.1	0/3	0/3	Not done	0/3	0/3	Not done
Negative control	Negative	Negative	Negative	Negative	Negative	Negative

Results shown as number of positive samples vs number of samples tested.

[a] Nucleic acid extraction of the dilution series in a background of *C. psittaci* DNA-negative pooled and liquefied sputum.
[b] All three weak reactions.
[c] One of three was a weak reaction.

of 1 pmol/µl. Sequences were delivered by email as original peak plots. The forward and reverse overlapping sequences were edited to obtain the complete sequence. Alignment and calculation of a similarity index p-distance was done with MEGA 5.1 [19]. The newly discovered VD4 genotype was subjected to complete *ompA* sequencing as previously described [13]. A phylogenetic tree was constructed using the neighbor-joining method. Reference *ompA* genotype sequences A-F, E/B and *C. abortus* (strain S26/3) available in the GenBank database (accession numbers AY762608–12 and AF269261) were included in this analysis.

Data acquisition and descriptive statistics

Systematically collected data concerning notified human cases from September 2008 until the end of October 2013 were obtained anonymously from the national database for notifiable diseases at the Dutch Centre for Infectious Disease Control as far as possible. Age, sex, hospital admission, mortality, probable country of acquisition and suspected source of infection were noted. Occasionally, additional information was provided on submitted laboratory forms or provided via personal communication.

Results

Validation of the VD4 PCR

The newly designed primer set allowed for amplification of *ompA* VD4 regions of all nine *C. psittaci* reference strains. The lowest detection limit was 1 to 10 copies per PCR (Table 2). In a background of sputum, sensitivity was slightly lower, but still 10 to 100 copies per PCR. Dissociation curves showed only one PCR amplification product for each genotype. The genotypes were visible as dissociation peaks with Tm's ranging from 79 to 83 °C. Several replicates of the reference genotypes showed overlap between the Tm of the different genotypes. It was not possible to separate each genotype solely by Tm. The CPS13 panel was tested during validation and all truly positive samples were detected, and all negative samples were correctly identified as well. Specificity was 100% based on 33 commonly encountered bacterial and yeast species and 20 human respiratory samples. Sixty-nine *C. psittaci* real-time PCR-positive samples from 66 human individuals were available for typing. The expected amplification product was obtained in 66 of 69 available human samples. The Ct values (quantification cycle) of these clinical samples ranged from 22 to 38 cycles. Three of 69 samples were negative in the VD4-PCR. Two of them contained *C. caviae* (see below), leaving only one of the remaining 67 samples untypable. Overall, the sensitivity of this method compared with our real-time diagnostic PCR was 98% (66 genotypes from 67 typable samples).

In our hands, the method (excluding technician costs) costs ca EUR 22 per sample. This includes four PCR controls per run, nucleic acid extraction, PCR reagent and two sequence reactions. When needed, a typing result can be obtained within 24 hours.

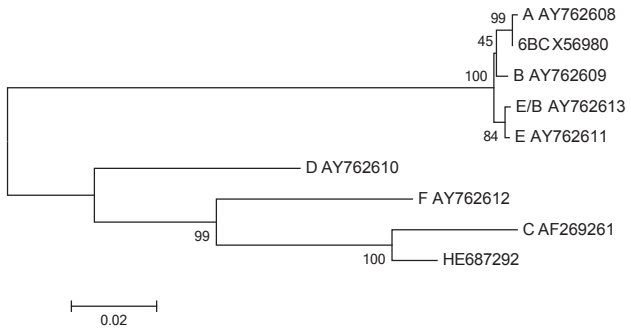
Sequence analysis and distribution of genotypes

During validation, the Amplirun *C. psittaci* genomic DNA control (only on the lowest positive dilution series), all reference strains and the CPS13 panel were successfully sequenced. All 66 VD4 PCR-positive clinical samples were sequenced revealing *C. psittaci* genotype A in 42 samples. Genotype B was found in 14 samples, E/B was found in two samples and genotype C in one sample.

Four samples revealed a new identical sequence type. Sequence analysis showed this strain to be a unique

Figure

Phylogenetic tree displaying the *Chlamydia psittaci ompA* sequence HE687292 in relation to reference genotypes A–F and E/B



Neighbor-joining method, Jukes-Cantor model, using 1,000 bootstraps created with MEGA.

ompA genotype with homology to the *ompA* of both genotype C and D. In the similarity calculation the sequence was most similar to reference genotype C strain (95% and 86% similarity to the *ompA* VD4 of genotype C and D respectively). These four samples were subjected to full-length *ompA* sequence analysis including all four variable domains. Full-length *ompA* could only be obtained for two of the four samples. These two full-length *ompA* sequences revealed an identical but new genotype in both samples. The sequence was submitted to the EMBL nucleotide sequence database and assigned reference number HE687292. The Figure shows the phylogentic relationships between the new genotype and genotypes A–F and E/B. BLAST search did not reveal an identical sequence. Phylogenetic analysis showed the *ompA* gene of this strain to be most similar to that of the *C. psittaci* genotype C (96% homologous to genotype C) but still discordant in 37 nucleotides resulting in nine different amino acids.

Chlamydia caviae and *Chlamydia abortus*

One sample contained *C. abortus*. Two VD4 PCR-negative samples from another patient were suspected to contain *C. caviae* DNA rather than *C. psittaci* DNA. This assumption was based on information from public health officials, who told us that the patient showed clinical symptoms after purchasing guinea pigs. *C. caviae* was indeed detected by use of *C. caviae*-specific primers and subsequent sequence analysis of the amplified *ompA* VD4 gene region. Both samples were also analysed by a PCR-HRM and confirmed positive for *C. caviae*.

Descriptive epidemiology

For 54 of the 66 PCR-positive patients, data were available in the national notification database.

Table 3

Characteristics of notified patients harbouring *Chlamydia psittaci* genotype A and B, the Netherlands, 2008 to 2013 (n = 50)

Genotype	A (n = 37)	B (n = 13)
Age in years: median (IQR)	63 (51 – 72)	70 (62 – 73)
Sex (male)	27	11
Deaths	1	0
Probable source of infection (location)[a]		
Home	21	10
Bird show	4	0
Bird dealer/bird dealing company	3	0
Pet shop	2	1
Public resort	1	0
Abroad (farm)	0	1
Unknown/not reported	10	2
Probable source of infection (type of bird)[a]		
Columbiformes	0	10
Captive	0	8
Wild	0	3
Psittaciformes[b]	8	0
Passeriformes[c]	5	0
Anseriformes (duck)	0	1
Galliformes (pheasant)	1	0
Unofficial bird groups	0	0
Poultry	3	2
Wild, free ranging birds	3	0
Aviary birds	6	0
Unknown/not reported	15	1
Date of disease onset		
First quarter	17	3
Second quarter	14	2
Third quarter	4	6
Fourth quarter	2	2

[a] More than one source could be noted.

[b] Four parakeets, one parrot, one cockatiel, one budgerigar, one unidentified parrot-like bird.

[c] Two zebra finches, one canary, one jay, one siskin.

Characteristics associated with infection with either genotype A or B are presented in Table 3.

Among all 54 cases, men predominated, one death was reported and all were admitted to hospital. One person probably acquired the infection outside of the Netherlands. Exposure to *Psittaciformes* and *Passeriformes* was reported for patients harbouring genotype A, while exposure to *Columbiformes* predominated among patients harbouring genotype B. Remarkable is the large proportion of genotype A cases in the first half of the year: 31 cases vs six in the second half (Table 3).

C. abortus was found in one patient. This patient, suffering from severe pneumonia requiring admission to an intensive care unit, was living on a farm raising sheep and goats [20]. The *C. caviae*-positive patient was only diagnosed because an extensive diagnostic investigation took place for sepsis of unknown origin, revealing psittacosis as the most likely diagnosis. The patient had recently purchased two young guinea pigs before becoming ill [21]. The new unique *ompA* sequence was found in four cases. In none of the four could a direct link to specific birds be found, although one of the infected people was a volunteer working with and exposed to many different kinds of birds. One case with a genotype C infection was detected. This case was related to a bird hospital.

Discussion

In this study, we present a simple, sensitive and cheap genotyping method to detect *C. psittaci* genotypes (A-F, E/B,WC,M56) and the closely related species *C. abortus*. The sensitivity of 98% compared with our real-time diagnostic PCR and a specificity of 100% were satisfactory. A result can be obtained in ca 24 hours. The lower detection limit of the VD4 PCR (at least 10 copies per reaction) is very sensitive and comparable to previously used *C. psittaci ompA* typing methods [9]. Geens et al. required separate PCR reactions for each genotype while our method is a singleplex format [9]. Although the VD4 PCR was validated in only one laboratory, we were still able to detect the genotype in 66 of 67 typable samples sent from across the Netherlands.

Although the dissociation curve analyses could roughly separate the *C. psittaci* reference strains used in this study, this method was not accurate enough on its own. In particular, overlap occurred in the Tm for genotypes A, B, E and E/B. Mitchell et al., using dedicated equipment for high-resolution melting curve analysis, also found 21% of their tested positive samples to be untypable owing to inconclusive melting curve data [10].

To the best of our knowledge, the current study characterises the largest series of human-derived psittacosis strains described to date. In the past we had analysed a limited number of 10 human strains originating from psittacosis outbreaks and sporadic cases. As in the present study, genotype A was the most prevalent strain [13]. Recently, an outbreak of psittacosis was described in Sweden. Twelve samples were available for *ompA* genotyping but sequencing of the *ompA* was successful in only four of them [14]. It should be noted that many *C. psittaci* typing methods described previously included hardly any human clinical samples [4,9-12]. They were validated mainly on bird samples or cultured strains. Validation on human samples is needed because the clinical matrix (for example sputum and bronchoalveolar lavage fluids) and the bacterial load can differ substantially between birds and humans.

Many of the genotype A-positive samples were obtained in spring 2011, during a period of increased psittacosis notifications. It seems that this temporary increase was in part due to these genotype A strains. A similar observation was described recently by Rehn et al. who reported a threefold increase in notified psittacosis cases from January to April 2013 [14]. A matched case–control study showed that cases were more likely than controls to have been cleaning bird feeders or were exposed to bird droppings in other ways.

Until now, a source for this temporary increase in the Netherlands of psittacosis notifications in Spring 2011 has not been found. This clearly emphasises the need for genotyping *C. psittaci* strains in human samples prospectively, as it could provide earlier information on probable avian sources, allowing for appropriate outbreak control measures. In the spring of 2013, the same effect was seen, albeit on a smaller scale. Within three months, eight people were infected with *C. psittaci* genotype A. Three of these eight were traced back to a bird show. Bird shows present a zoonotic risk. Visitors can be infected during their visit or afterwards as psittacine and passeriforme birds are often traded and disseminated at such events which are mainly held in the spring. In our dataset, genotype A strains were more prominently found in the first half of the year. Genotype A is most often associated with *Psittaciformes*. This genotype is highly virulent for these birds, which excrete the bacterium in large amounts for long periods of time [5]. This might be one of the reasons of the high virulence in humans. The high proportion of genotype A could possibly be related to more intensive exposure to the main bird source of these genotypes (*Psittaciformes*). These birds are frequently kept as pets inside the house, while birds harbouring the other genotypes more often live outside a person's home. Remarkable is the exposure to *Passeriformes* in patients harbouring genotype A strains, possibly also due to exposure to these birds as pets. Analysis of the fluctuations in genotypes and possible causes should be a subject of ongoing surveillance. Genotype B is mainly associated with *Columbiformes*. In this sample set, we found 14 such strains. Previous research also determined genotype B to be the second most prevalent genotype in humans [13].

Four patients were infected with a new *C. psittaci ompA* VD4 genotype, showing the highest *ompA* VD4 sequence homology with the genotype C GR9 strain. Full-length *ompA* could only be obtained for two of these four cases, underlining the lack of sensitivity of this typing method. The DNA and amino acid sequence of the full-length *ompA*/MOMP of this strain confirmed its unique sequence as we could not find a single match by BLAST. This raises the question of which birds or animals host these strains. Two of the samples were obtained in the context of a previously described outbreak in which a bird source could not be identified [15].

C. abortus was found in one patient. In the Netherlands, *C. abortus* is known to be endemic in sheep and goats [22]. Human *C. abortus* infections have been described. The infection can cause severe septic shock and fetal loss in pregnant women [23-25]. In most cases, testing for psittacosis is only performed when medical history reveals obvious bird contact. Therefore underestimation of these pulmonary *C. abortus* cases is quite likely. The same is true for the *C. caviae*-positive patient. It was only due to the sepsis of unknown origin that an extensive diagnostic investigation was done, revealing psittacosis as the most likely diagnosis based on a positive PCR of the conserved domain of *ompA* (which besides *C. psittaci*, also detects *C. felis*, *C. caviae*, and *C. abortus*). The discrepancy of this positive diagnostic PCR and the negative VD4 PCR led us to consider *C. caviae* as the cause of this infection, which was confirmed by molecular characterisation. Knowledge on the zoonotic potential of *C. caviae* is limited and until now, *C. caviae* has not been linked to fulminant sepsis in humans [26-28].

Human medicine should be aware of the zoonotic potential of *Chlamydia* as there is accumulating evidence that these species are more abundant in animals than previously assumed [6]. These cases also stress the need for close collaboration of physicians, medical microbiologists and public health officials involved in the notification process, as crucial information such as potential animal reservoirs with their associated *Chlamydia* can be missed. The distribution of genotypes in human hosts as found in this study should be carefully considered with respect to geographical location. The local fauna could be relevant when interpreting the results, and extrapolating them to other countries is probably premature. The interaction between human behaviour (urban vs rural) and the present wild bird species (tropical vs non-tropical) could influence local epidemiology. Accidental introduction of invasive, exotic pet bird species or invasion of foreign bird species could create a niche for certain genotypes, including genotypes not present in this study, and lead to unexpected increases in psittacosis cases [29].

In conclusion, this study shows that genotype A and B were the most prevalent causative strains of human psittacosis in the Netherlands. Psittacosis is a clinical syndrome caused by diverse *C. psittaci* genotypes, but typing results suggest that the clinical signs and symptoms are quite similar to closely related zoonotic *C. abortus* and *C. caviae* infections. The discovery of a unique *ompA* sequence points to currently unknown links between human cases and avian or other animal reservoirs.

Accession number

The EMBL accession number for the newly described *Chlamydia psittaci* genotype is HE687292.

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Conflict of interest

None declared.

Authors' contributions

Heddema: idea and design of the study, wrote the manuscript. Bongaerts: performed and analysed most of the typing results, revised the article draft and approved the final manuscript. Van Hannen, Ten Hove and De Wever: submitted archived samples, collected data, revised the article draft and approved the final manuscript. Dijkstra: revealed and analysed demographic and epidemiologic data, revised the article draft and approved the final manuscript. Vanrompay: Cultured and provided all control strains, analysed crucial samples (*C. caviae*), co-designed the study, revised the article draft substantially and approved the final manuscript.

References

1. Everett KD, Bush RM, Andersen AA. Emended description of the order Chlamydiales, proposal of Parachlamydiaceae fam. nov. and Simkaniaceae fam. nov., each containing one monotypic genus, revised taxonomy of the family Chlamydiaceae, including a new genus and five new species, and standards for the identification of organisms. Int J Syst Bacteriol. 1999;49(Pt 2):415-40. http://dx.doi.org/10.1099/00207713-49-2-415 PMID:10319462
2. Stephens RS, Myers G, Eppinger M, Bavoil PM. Divergence without difference: phylogenetics and taxonomy of Chlamydia resolved. FEMS Immunol Med Microbiol. 2009;55(2):115-9. http://dx.doi.org/10.1111/j.1574-695X.2008.00516.x PMID:19281563
3. Kuo CC, Stephens RS, Bavoil PM, Kaltenboeck B. Genus Chlamydia Jones, Rake and Stearns 1945, 55. In: Krieg NR, Staley JT, Brown DR, Hedlund BP, Paster BJ, Ward W, et al, editors. Bergey's manual of systematic bacteriology. 2nd ed; Vol 4. Heidelberg: Springer; 2011. p. 846-65.
4. Pannekoek Y, Dickx V, Beeckman DS, Jolley KA, Keijzers WC, Vretou E, et al. Multi locus sequence typing of Chlamydia reveals an association between Chlamydia psittaci genotypes and host species. PLoS ONE. 2010;5(12):e14179. http://dx.doi.org/10.1371/journal.pone.0014179 PMID:21152037
5. Harkinezhad T, Geens T, Vanrompay D. Chlamydophila psittaci infections in birds: a review with emphasis on zoonotic consequences. Vet Microbiol. 2009;135(1-2):68-77. http://dx.doi.org/10.1016/j.vetmic.2008.09.046 PMID:19054633
6. Reinhold P, Sachse K, Kaltenboeck B. Chlamydiaceae in cattle: commensals, trigger organisms, or pathogens?

Vet J. 2011;189(3):257-67. http://dx.doi.org/10.1016/j.tvjl.2010.09.003 PMID:20980178

7. Heddema ER, Beld MG, de Wever B, Langerak AA, Pannekoek Y, Duim B. Development of an internally controlled real-time PCR assay for detection of Chlamydophila psittaci in the LightCycler 2.0 system. Clin Microbiol Infect. 2006;12(6):571-5. http://dx.doi.org/10.1111/j.1469-0691.2006.01417.x PMID:16700707
8. van der Bruggen T, Kaan JA, Heddema ER, van Hannen EJ, de Jongh BM. [Rapid diagnosis of psittacosis using a recently developed real-time PCR technique]. Ned Tijdschr Geneeskd. 2008;152(34):1886-8. PMID:18788681
9. Geens T, Dewitte A, Boon N, Vanrompay D. Development of a Chlamydophila psittaci species-specific and genotype-specific real-time PCR. Vet Res. 2005;36(5-6):787-97. http://dx.doi.org/10.1051/vetres:2005035 PMID:16120253
10. Mitchell SL, Wolff BJ, Thacker WL, Ciembor PG, Gregory CR, Everett KD, et al. Genotyping of Chlamydophila psittaci by real-time PCR and high-resolution melt analysis. J Clin Microbiol. 2009;47(1):175-81. http://dx.doi.org/10.1128/JCM.01851-08 PMID:19005152
11. Laroucau K, Thierry S, Vorimore F, Blanco K, Kaleta E, Hoop R, et al. High resolution typing of Chlamydophila psittaci by multilocus VNTR analysis (MLVA). Infect Genet Evol. 2008;8(2):171-81. http://dx.doi.org/10.1016/j.meegid.2007.12.002 PMID:18242149
12. Sachse K, Laroucau K, Vorimore F, Magnino S, Feige J, Müller W, et al. DNA microarray-based genotyping of Chlamydophila psittaci strains from culture and clinical samples. Vet Microbiol. 2009;135(1-2):22-30. http://dx.doi.org/10.1016/j.vetmic.2008.09.041 PMID:18950965
13. Heddema ER, van Hannen EJ, Duim B, Vandenbroucke-Grauls CM, Pannekoek Y. Genotyping of Chlamydophila psittaci in human samples. Emerg Infect Dis. 2006;12(12):1989-90. http://dx.doi.org/10.3201/eid1212.051633 PMID:17326961
14. Rehn M, Ringberg H, Runehagen A, Herrmann B, Olsen B, Petersson AC, et al. Unusual increase of psittacosis in southern Sweden linked to wild bird exposure, January to April 2013. Euro Surveill. 2013;18(19):20478. PMID:23725809
15. McGuigan CC, McIntyre PG, Templeton K. Psittacosis outbreak in Tayside, Scotland, December 2011 to February 2012. Euro Surveill. 2012;17(22):20186. PMID:22687915
16. Untergasser A, Nijveen H, Rao X, Bisseling T, Geurts R, Leunissen JA. Primer3Plus, an enhanced web interface to Primer3. Nucleic Acids Res. 2007;35(Web Server issue):W71-4.
17. Bikandi J, San Millán R, Rementeria A, Garaizar J. In silico analysis of complete bacterial genomes: PCR, AFLP-PCR and endonuclease restriction. Bioinformatics. 2004;20(5):798-9. http://dx.doi.org/10.1093/bioinformatics/btg491 PMID:14752001
18. Robertson T, Bibby S, O'Rourke D, Belfiore T, Lambie H, Noormohammadi AH. Characterization of Chlamydiaceae species using PCR and high resolution melt curve analysis of the 16S rRNA gene. J Appl Microbiol. 2009;107(6):2017-28. http://dx.doi.org/10.1111/j.1365-2672.2009.04388.x PMID:19583801
19. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28(10):2731-9. http://dx.doi.org/10.1093/molbev/msr121 PMID:21546353
20. Rijksinstituut voor Volkgezondheid en Milieu. Gesignaleerd: Binnenlandse signalen Chlamydia abortus pneumonie [Signaled: Chlamydia abortus pneumonia]. Infectieziekten Bulletin. 2012;23(6):162. Dutch. Available from: http://www.rivm.nl/Documenten_en_publicaties/Algemeen_Actueel/Uitgaven/Infectieziekten_Bulletin/Jaargang_23_2012/Juli_2012/Inhoud_juli_2012/Gesignaleerd_tot_en_met_14_juni_2012
21. Teirlinck AC, van Asten L, Brandsema PS, Dijkstra F, Donker GA, Euser SM, et al. Psittacose. In: Jaarrapportage surveillance respiratoire infectieziekten 2013. [Psittacosis. In: Annual report on surveillance of respiratory infectious diseases 2013]. Bilthoven: Rijksinstituut voor Volksgezondheid en Milieu; 2014. Dutch. Available from: http://www.rivm.nl/bibliotheek/rapporten/150002006.pdf
22. van den Brom R, Lievaart-Peterson K, Luttikholt S, Peperkamp K, Wouda W, Vellema P. Abortion in small ruminants in the Netherlands between 2006 and 2011. Tijdschr Diergeneeskd. 2012;137(7):450-7. PMID:22900421
23. Meijer A, Brandenburg A, de Vries J, Beentjes J, Roholl P, Dercksen D. Chlamydophila abortus infection in a pregnant woman associated with indirect contact with infected goats. Eur J Clin Microbiol Infect Dis. 2004;23(6):487-90. http://dx.doi.org/10.1007/s10096-004-1139-z PMID:15141338
24. Pospischil A, Thoma R, Hilbe M, Grest P, Zimmermann D, Gebbers JO. [Abortion in humans caused by Chlamydophila abortus (Chlamydia psittaci serovar 1)]. Schweiz Arch Tierheilkd. 2002;144(9):463-6. http://dx.doi.org/10.1024/0036-7281.144.9.463 PMID:12677684
25. Kampinga GA, Schröder FP, Visser IJ, Anderson JM, Buxton D, Möller AV. [Lambing ewes as a source of severe psittacosis in a pregnant woman]. Ned Tijdschr Geneeskd. 2000;144(52):2500-4. PMID:11155507
26. Lutz-Wohlgroth L, Becker A, Brugnera E, Huat ZL, Zimmermann D, Grimm F, et al. Chlamydiales in guinea-pigs and their zoonotic potential. J Vet Med A Physiol Pathol Clin Med. 2006;53(4):185-93. http://dx.doi.org/10.1111/j.1439-0442.2006.00819.x PMID:16629952
27. Rohde G, Straube E, Essig A, Reinhold P, Sachse K. Chlamydial zoonoses. Dtsch Arztebl Int. 2010;107(10):174-80. PMID:20358033
28. Rodolakis A, Yousef Mohamad K. Zoonotic potential of Chlamydophila. Vet Microbiol. 2010;140(3-4):382-91. http://dx.doi.org/10.1016/j.vetmic.2009.03.014 PMID:19345022
29. Dickx V, Kalmar ID, Tavernier P, Vanrompay D. Prevalence and genotype distribution of Chlamydia psittaci in feral Canada geese (Branta canadensis) in Belgium. Vector Borne Zoonotic Dis. 2013;13(6):382-4. http://dx.doi.org/10.1089/vbz.2012.1131 PMID:23654298