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Protecting the very young against pertussis – cough, costs and cocooning

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The conclusions of the paper by Lim et al., in this issue of *Eurosurveillance*, assessing the relevance of a pertussis cocoon immunisation strategy in Ontario, are somewhat disappointing [1]. The authors estimate the number of persons that need to be vaccinated (NNV) in order to prevent an infant pertussis hospitalisation or death at 12,000 to 63,000 and 1.1 million to 12.8 million, respectively and conclude to the 'inefficiency inherent to this approach'. These high NNV seem little debatable – the analysis is well conducted. Furthermore, the authors have explored in their sensitivity analysis various assumptions and values that could have led them to overestimate the NNV in the base-case analysis. Their conclusions are robust and in line with a similar study conducted in Quebec and British Columbia for the years 2005 to 2009, which also concluded that 'in the context of low pertussis incidence, the parental cocoon strategy is inefficient and resource intensive' [2].

The idea behind the cocoon strategy is attractive in principle. Available pertussis vaccines and vaccination strategies do not allow the elimination of pertussis. Even a combination of very high vaccination coverage in infants, with additional boosters later in life, does not induce sufficient herd immunity to prevent the circulation of the bacteria in adults. This leaves us with a persisting burden of the disease and the resulting mortality is almost exclusively concentrated in the first weeks or months of life. This is due to a combination of two factors. Firstly, even though pertussis can be severe at all ages, it is most severe in infants and may lead to pulmonary and neurological complications and death. Secondly, newborns remain susceptible to pertussis until they receive at least the first dose of a pertussis-containing vaccine, which in all European Union Member States is given at two or three months of age. In the absence of pertussis vaccines for use in the neonatal period, young infants can thus only be indirectly protected. This can be achieved through vaccinating those who are most likely to contaminate them. This seems to make sense, given that very young infants have limited social contacts and the potential main contaminators are probably not too numerous and easy to identify. Indeed, countries having embarked on such a strategy mainly target household members for vaccination and such choice is supported by data. A recent review has concluded that, when the source of pertussis transmission to infants less than six months old is identified, parents contribute with between 39% and 57% and siblings with between 16% and 42% [3].

In this context, what conclusions can be inferred from Lim's results? The answer is not straightforward and needs further consideration.

Lim et al. cautiously point out that their conclusions stand for Ontario, which has a very low burden of severe pertussis in infants, and may not be relevant to contexts with different pertussis epidemiology. In addition to this, one needs to keep in mind the cyclical pattern of pertussis epidemiology. What is true for a period of low incidence may not apply during periods of resurgence of the disease. In France, 10 infant deaths were registered in 2000 and 2005, at the peak of epidemic cycles, and in the United Kingdom, 14 infant deaths were registered during the 2012 pertussis resurgence, making protection of young infants worth considering [4-5].

In their paper, Lim et al. measure the relevance of the immunisation strategy through the NNV. This metric represents the ratio of newly vaccinated individuals to cases prevented. It is defined as the reciprocal of the product of the vaccine effectiveness by the attack rate in the unvaccinated. Putting aside the imperfect effectiveness of a vaccine, the NNV is a reflection of the risk of the disease in the absence of vaccination. While this approach is definitively very informative, it only reflects the epidemiological benefit of the vaccination. Vaccination decisions usually also consider the risk-benefit balance and the cost-effectiveness ratio. If a vaccine is perfectly tolerated, with no detrimental indirect effect, the risk-benefit balance may be in favour of vaccination, even with a high NNV. In adults primed with pertussis whole-cell vaccines, the addition of an acellular pertussis component does not modify the safety profile of the Tetanus-Diphtheria (Td) boosters, at least for the first doses [6]. If a vaccine is cheap and adds only little to the costs of a programme, the cost-effectiveness ratio may again be favourable even with

a high NNV. Two Dutch studies have looked at the cost-effectiveness ratio of a pertussis cocooning strategy [7-8]. They come to conflicting conclusions in the base-case analysis with cost-effectiveness ratios of 4,600 and 89,000 EUR, respectively, per quality-adjusted life year (QALY) gained as compared to the Dutch (unofficial) cost-effectiveness threshold of 20,000 to 50,000 EUR/QALY gained [7]. The differences mainly reflect diverse assumptions regarding the level of underreporting and duration of the disease. However, in the sensitivity analysis of the least favourable study, the combination of the most favourable assumptions of the variables leads to a cost-effectiveness ratio of only 21,000 EUR/QALY gained. In contrast to these positive results, an Italian study has, similarly to Lim et al., concluded that cocooning would be poorly efficient, based on costs of over 100,000 EUR per hospitalisation avoided [9].

In addition to considering costs, it is important to look at feasibility and acceptability of vaccination strategies. The pertussis cocooning immunisation requires administering unneeded diphtheria and tetanus boosters, as there is no monovalent pertussis vaccine available to date. Moreover, it does not easily fit into existing vaccination practices as it cannot rely on an already implemented vaccination strategy targeting members of a family. One would imagine that the acceptability of household members for a vaccination aiming at preventing risk of a severe disease for the young infant in the happy context of a future or recent birth, should be high. However, such an optimistic view remains to be confirmed in real life. The recommendation of an adult pertussis booster dose that relies on the simple substitution of a pertussis-containing vaccine (Tdap) at the occasion of a planned Td, as implemented in Ontario and elsewhere, appears easier to implement than the cocooning strategy. However, had solely the NNV to prevent a hospitalisation or a death been used to decide on this strategy, it would probably not have been implemented. Hospitalisations and deaths due to pertussis in adults are respectively very rare and almost inexistent. At the same time, such a strategy can only impact on severe cases in infants when the vaccine is given by chance to an adult who is or will be in the next years in regular close contact with an infant. Only if regular boosters were proposed, could a significant impact on infant pertussis be expected through herd immunity. Still, the NNV to prevent a pertussis case in adults is currently challenged by the likely shorter than expected duration of protection conferred by boosters, as suggested by recent data in adolescents [10-11].

There are nevertheless some new approaches that give hope for improving the protection of the very young such as the promising preliminary results of the temporary maternal pertussis immunisation strategy implemented in England since 2012 [12]. Pertussis is the single 'old' vaccine-preventable disease that resists vaccination and even mocks us with its resurgence observed in many places in the recent years, despite high vaccine coverage in children and booster doses added in the immunisation schedules at different ages. Even if adults will probably continue to suffer from exhausting coughs due to pertussis for many more years, we may soon have new vaccination strategies at hand, namely maternal or neonatal vaccinations, to eliminate infant pertussis deaths. These are of course subject to the absence of any safety issue and to high acceptability of the strategy and to the various NNV. New calculations, new debates to come…

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Interim estimates of 2013/14 vaccine effectiveness against influenza A(H1N1)pdm09 from Canada's sentinel surveillance network, January 2014

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The 2013/14 influenza season to date in Canada has been characterised by predominant (90%) A(H1N1) pdm09 activity. Vaccine effectiveness (VE) was assessed in January 2014 by Canada's sentinel surveillance network using a test-negative case–control design. Interim adjusted-VE against medically-attended laboratory-confirmed influenza A(H1N1) pdm09 infection was 74% (95% CI: 58–83). Relative to vaccine, A(H1N1)pdm09 viruses were antigenically similar and genetically well conserved, with most showing just three mutations across the 50 amino acids comprising antigenic sites of the haemagglutinin protein.

Background

Since the 2009 pandemic, influenza A(H1N1)pfdm09 viruses have comprised a small proportion (<20%) of seasonal influenza virus detections each year in Canada [1]. However, A(H1N1)pdm09 activity has recently resurged in North America, comprising more than 90% of detected influenza strains in both Canada and the United States (US) to mid-January of the 2013/14 season [1,2]. This profile is in contrast to that of the same period last season in Canada, when 90% of detected strains instead belonged to the A(H3N2) subtype [3].

The 2013/14 trivalent influenza vaccine (TIV) for the northern hemisphere retains the same A(H1N1)pdm09 (A/California/07/2009-like) strain recommended since 2009 by the World Health Organization (WHO) [4]. In response to substantial A(H1N1)pdm09 resurgence, interim 2013/14 vaccine effectiveness (VE) was assessed in January 2014 using Canada's sentinel surveillance network. VE estimates are discussed in the context of antigenic and genetic characterisation of circulating A(H1N1)pdm09 viruses.

Estimating influenza vaccine effectiveness

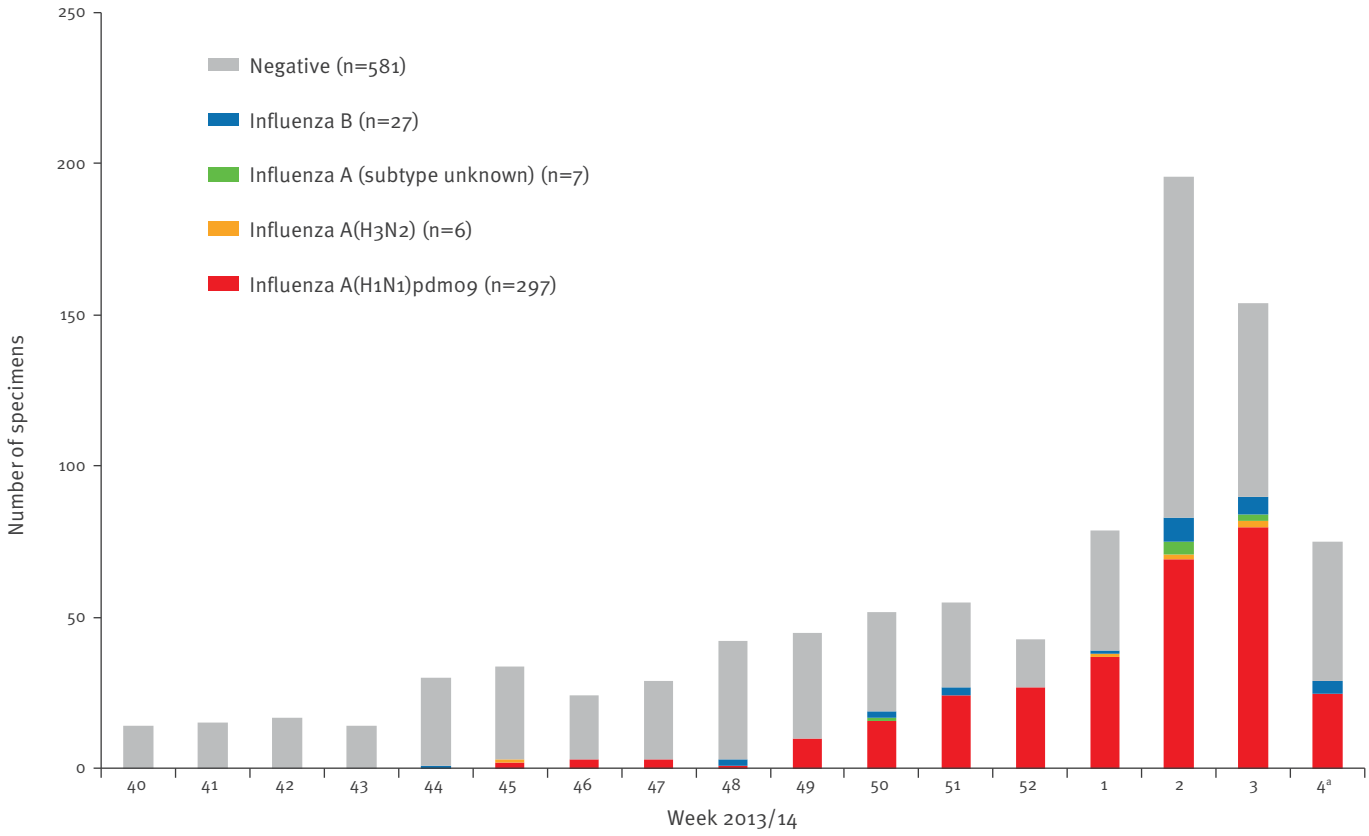
As previously described [3,5-12], a test-negative case–control design was used to estimate VE. Patients presenting with influenza-like illness (ILI) and testing positive for influenza viruses were considered cases, and those testing negative were considered controls.

Community-based practitioners at sentinel surveillance sites across participating provinces (British Columbia, Alberta, Manitoba, Ontario and Quebec) may offer nasal or nasopharyngeal swabbing to any patient presenting within seven days of symptom onset of ILI, defined as acute onset of respiratory illness with fever and cough and one or more of the following: sore throat, arthralgia, myalgia or prostration.

The analysis period included specimens collected from 1 November 2013 (week 44: 27 October 2013–2 November 2013) to 23 January 2014 (week 4: 19–25 January 2014), selected to account for influenza activity beginning in early November (Figure 1) and immunisation campaigns typically commencing in October. Epidemiological information was obtained from consenting patients or their parents/guardians using a standard questionnaire at specimen collection. Ethics review boards in participating provinces approved this study.

Figure 1

Laboratory detection of influenza by week and virus subtype, 2013/14 sentinel surveillance system, Canada, 29 September 2013–23 January 2014 (n=918)[a]



Of 1,200 nasal or nasopharyngeal specimens collected between 29 September 2013 (week 40: 29 September–5 October 2013) and 23 January 2014 (week 4: 19–25 January 2014), we excluded from the epidemic curve specimens from the following patients: those failing to meet the influenza-like illness (ILI) case definition or for whom it was unknown (n=50), those whose specimens were collected more than seven days after symptom onset or for whom the interval was unknown (n=169), those whose age was unknown or less than one year (n=10), those with unknown comorbidity status (n=80), and those for whom influenza test results were unavailable or indeterminate (n=10). Specimens were included regardless of the patient's vaccination status or timing of vaccination. Excluded specimens may have more than one exclusion criterion that applies. Counts for each criterion will sum to more than the total number of specimens excluded. Missing collection dates were imputed as the laboratory accession date minus two days, the average time period between collection date and laboratory accession date for records with valid data for both fields.

[a] Week 4 is based on partial week.

Specimens were tested for influenza A (by subtype) and B viruses at provincial reference laboratories using real-time RT-PCR. Odds ratios (OR) for medically-attended, laboratory-confirmed influenza were estimated by multivariable logistic regression. VE was calculated as (1–OR)x100%. Patients for whom comorbidity status was unknown or for whom the timing of vaccination was unknown or less than two weeks before symptom onset were excluded from the primary analysis but explored in sensitivity analyses. Age-stratified analysis and a study period beginning from week 49 (1–7 December 2013) to allow for additional vaccine uptake were also explored.

Genetic characterisation of sentinel influenza A(H1N1)pdm09 viruses

The haemagglutinin (HA) genes (HA1/HA2) from a convenience sample of sentinel influenza A(H1N1) pdm09 viruses from original patient specimens were sequenced for phylogenetic analysis and pair-wise amino acid (aa) identity based on antigenic maps spanning the 50 aa residues across HA1 antigenic sites Sa, Sb, Ca1, Ca2 and Cb [12,13]. Findings were expressed as percentage identity to vaccine, calculated as (1–(number of aa substitutions in antigenic sites)/(total antigenic site aa residues))x100%. After removal of the signal peptide (residues 1–17), the approximate likelihood method was used to generate the phylogenetic tree of aligned nucleotide sequences in FastTree [14], visualised in FigTree [15], including reference HA sequences shown in Table 1.

Interim estimates of influenza vaccine effectiveness

A total of 1,091 specimens were submitted between 1 November 2013 and 23 January 2014. After exclusion

Table 1a

Reference haemagglutinin sequences obtained from the EpiFlu database of the Global Initiative on Sharing Avian Influenza Data (GISAID) and used in phylogenetic analysis, 2013/14 sentinel surveillance network, Canada

Segment ID	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors
EPI499574	Netherlands	2013-Oct-14	A/Netherlands/2248/2013	Erasmus University of Rotterdam	National Institute for Medical Research	
EPI499572	France	2013-Dec-02	A/Lyon/2899/2013	CRR virus Influenza region Sud	National Institute for Medical Research	
EPI498900	United States	2013-Dec-16	A/Kansas/13/2013	Kansas Department of Health and Environment	Centers for Disease Control and Prevention	
EPI498897	United States	2013-Dec-17	A/Wisconsin/35/2013	Marshfield Clinic Research Foundation	Centers for Disease Control and Prevention	
EPI498865	United States	2013-Dec-01	A/Georgia/14/2013	Georgia Public Health Laboratory	Centers for Disease Control and Prevention	
EPI498639	China	2013-Nov-15	A/Chongqing-Yuzhong/SWL11676/2013	WHO Chinese National Influenza Center	WHO Chinese National Influenza Center	Yu Lan,Xiyan Li,Xiang Zhao,Yanhui Cheng,minju Tan,Weijuan Huang,Dayan Wang,Dexin Li,Yuelong Shu
EPI498543	China	2013-Nov-21	A/Jiangsu-Qinhuai/SWL11396/2013	WHO Chinese National Influenza Center	WHO Chinese National Influenza Center	Yu Lan,Xiyan Li,Xiang Zhao,Yanhui Cheng,minju Tan,Weijuan Huang,Dayan Wang,Dexin Li,Yuelong Shu
EPI498415	Spain	2013-Nov-14	A/Galicia/1484/2013	Instituto de Salud Carlos III	National Institute for Medical Research	
EPI498294	United States	2013-Dec-09	A/Montana/15/2013	Montana Public Health Laboratory	Centers for Disease Control and Prevention	
EPI498277	United States	2013-Nov-04	A/Arkansas/20/2013	Arkansas Department of Health	Centers for Disease Control and Prevention	
EPI498274	United States	2013-Dec-03	A/Indiana/30/2013	Indiana State Department of Health Laboratories	Centers for Disease Control and Prevention	
EPI498269	United States	2013-Dec-02	A/Delaware/15/2013	Delaware Public Health Lab	Centers for Disease Control and Prevention	
EPI498230	United States	2013-Dec-02	A/Arkansas/23/2013	Arkansas Department of Health	Centers for Disease Control and Prevention	
EPI498214	United States	2013-Nov-22	A/Alaska/30/2013	Alaska State Virology Lab	Centers for Disease Control and Prevention	
EPI498208	United States	2013-Dec-01	A/Idaho/08/2013	State of Idaho Bureau of Laboratories	Centers for Disease Control and Prevention	

ID: identification number; WHO: World Health Organization.

We acknowledge the authors, originating and submitting laboratories of the sequences from GISAID's EpiFlu Database on which this research is based. All submitters of data may be contacted directly via the GISAID website: www.gisaid.org.

Table 1B

Reference haemagglutinin sequences obtained from the EpiFlu database of the Global Initiative on Sharing Avian Influenza Data (GISAID) and used in phylogenetic analysis, 2013/14 sentinel surveillance network, Canada

Segment ID	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors
EPI499324	United States	2013-Dec-09	A/Nevada/18/2013	Southern Nevada Public Health Lab	Centers for Disease Control and Prevention	
EPI498191	United States	2013-Nov-18	A/Mississippi/29/2013	New York State Department of Health	Centers for Disease Control and Prevention	
EPI497986	Japan	2013-Nov-28	A/TOKYO/32432/2013	Tokyo Metropolitan Institute of Public Health	National Institute of Infectious Diseases (NIID)	Takashita,Emi; Fujisaki,Seiichiro; Itoh,Reiko; Miura,Mai; Ejima,Miho; Tashiro,Masato; Odagiri,Takato
EPI497984	Japan	2013-Nov-20	A/TOKYO/32417/2013	Tokyo Metropolitan Institute of Public Health	National Institute of Infectious Diseases (NIID)	Takashita,Emi; Fujisaki,Seiichiro; Itoh,Reiko; Miura,Mai; Ejima,Miho; Tashiro,Masato; Odagiri,Takato
EPI497756	Sweden	2013-Dec-06	A/Gothenburg/5/2013		Swedish Institute for Infectious Disease Control	
EPI497694	Norway	2013-Nov-28	A/Norway/3230/2013	Ostfold Hospital - Fredrikstad, Dept. of Microbiology	Norwegian Institute of Public Health	Dudman, SG;Waalen, K; Hungnes, O
EPI497692	Norway	2013-Dec-04	A/Norway/3234/2013	Oslo University Hospital, Ulleval Hospital, Dept. of Microbiology	Norwegian Institute of Public Health	Dudman, SD;Waalen, K; Hungnes, O
EPI497634	United States	2013-Oct-22	A/Texas/42/2013	Texas Department of State Health Services-Laboratory Services	Centers for Disease Control and Prevention	
EPI492859	United States	2013-Nov-01	A/Maine/01/2013	Maine Health and Environmental Testing Laboratory	Centers for Disease Control and Prevention	
EPI492816	United States	2013-Oct-23	A/New York/09/2013	New York State Department of Health	Centers for Disease Control and Prevention	
EPI492782	United States	2013-Nov-18	A/Texas/36/2013	Texas Department of State Health Services-Laboratory Services	Centers for Disease Control and Prevention	
EPI492779	United States	2013-Nov-21	A/Wyoming/09/2013	Wyoming Public Health Laboratory	Centers for Disease Control and Prevention	
EPI492861	United States	2013-Nov-06	A/Florida/61/2013	Florida Department of Health-Jacksonville	Centers for Disease Control and Prevention	
EPI492856	United States	2013-Oct-30	A/Arizona/06/2013	Arizona Department of Health Services	Centers for Disease Control and Prevention	

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Table 1c

Reference haemagglutinin sequences obtained from the EpiFlu database of the Global Initiative on Sharing Avian Influenza Data (GISAID) and used in phylogenetic analysis, 2013/14 sentinel surveillance network Canada

Segment ID	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors
EPI492852	United States	2013-Nov-06	A/Iowa/07/2013	Iowa State Hygienic Laboratory	Centers for Disease Control and Prevention	
EPI492244	Norway	2013-Nov-12	A/Norway/3073/2013	Oslo University Hospital, Ulleval Hospital, Dept. of Microbiology	Norwegian Institute of Public Health	Dudman SG, Waalen K, Hungnes O
EPI489358	United States	2013-Oct-23	A/California/25/2013	California Department of Health Services	Centers for Disease Control and Prevention	
EPI489328	United States	2013-Oct-12	A/Mississippi/09/2013	Mississippi Public Health Laboratory	Centers for Disease Control and Prevention	
EPI489322	United States	2013-Nov-04	A/Indiana/23/2013	Indiana State Department of Health Laboratories	Centers for Disease Control and Prevention	
EPI486613	United States	2013-Oct-25	A/Colorado/04/2013	Colorado Department of Health Lab	Centers for Disease Control and Prevention	
EPI486607	United States	2013-Oct-10	A/South Carolina/04/2013	South Carolina Department of Health	Centers for Disease Control and Prevention	
EPI486601	United States	2013-Oct-15	A/North Dakota/04/2013	North Dakota Department of Health	Centers for Disease Control and Prevention	
EPI486407	United States	2013-Oct-07	A/Maryland/08/2013	Maryland Department of Health and Mental Hygiene	Centers for Disease Control and Prevention	
EPI486401	United States	2013-Oct-06	A/Utah/09/2013	Utah Department of Health	Centers for Disease Control and Prevention	
EPI486389	United States	2013-Oct-10	A/Arizona/03/2013	Arizona Department of Health Services	Centers for Disease Control and Prevention	
EPI486379	United States	2013-Oct-07	A/Washington/09/2013	Washington State Public Health Laboratory	Centers for Disease Control and Prevention	
EPI485754	United States	2013-Oct-02	A/Pennsylvania/07/2013	Pennsylvania Department of Health	Centers for Disease Control and Prevention	
EPI485751	United States	2013-Oct-02	A/Mississippi/08/2013	Mississippi Public Health Laboratory	Centers for Disease Control and Prevention	
EPI326206	Hong Kong (SAR)	2011-Mar-29	A/Hong Kong/3934/2011	Government Virus Unit	National Institute for Medical Research	
EPI468476	Norway	2013-May-03	A/Norway/2417/2013	Stavanger Universitetssykehus, Avd. for Medisinsk Mikrobiologi	Norwegian Institute of Public Health	Dudman, SG; Waalen, K; Hungnes, O
EPI466545	Estonia	2013-Mar-13	A/Estonia/76677/2013	Health Protection Inspectorate	National Institute for Medical Research	

ID: identification number; WHO: World Health Organization.

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Table 1D

Reference haemagglutinin sequences obtained from the EpiFlu database of the Global Initiative on Sharing Avian Influenza Data (GISAID) and used in phylogenetic analysis, 2013/14 sentinel surveillance network, Canada

Segment ID	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors
EPI417158	Ukraine	2012-Dec-02	A/Ukraine/523/2012	Institute of Epidemiology and Infectious Diseases AMS of Ukraine	National Institute for Medical Research	
EPI407291	United Kingdom	2012-Oct-29	A/Scotland/124660532/2012	Centre for Infections, Health Protection Agency	Health Protection Agency	Ellis, J
EPI382424	Hong Kong (SAR)	2012-May-21	A/Hong Kong/5659/2012	Public Health Laboratory Services Branch, Centre for Health Protection	Public Health Laboratory Services Branch, Centre for Health Protection	Mak,G.C.;Lo,J.Y.C.
EPI417552	Norway	2012-Nov-26	A/Norway/2362/2012	Stavanger Universitetssykehus, Avd. for Medisinsk Mikrobiologi	Norwegian Institute of Public Health	Kilander, A.;Khider, M.;Waalen, K.;Dudman, S.;Hungnes, O.
EPI406039	United States	2012-Oct-22	A/South Carolina/19/2012	South Carolina Department of Health	Centers for Disease Control and Prevention	
EPI466588	Norway	2013-Mar-06	A/Norway/1675/2013	WHO National Influenza Centre	National Institute for Medical Research	
EPI418082	France	2012-Nov-29	A/Paris/1878/2012	Institut Pasteur	Institut Pasteur	Enouf, V; Briand, D; Benassaya, M; Garbarg-Chenon, A;
EPI454436	Kenya	2013-Feb-22	A/Kenya/104/2013	CDC-Kenya	Centers for Disease Control and Prevention	
EPI331061	Ghana	2011-May-13	A/Ghana/763/2011	University of Ghana	National Institute for Medical Research	
EPI319590	Russian Federation	2011-Feb-28	A/Astrakhan/1/2011	WHO National Influenza Centre	National Institute for Medical Research	
EPI278607	New Zealand	2010-Jul-12	A/Christchurch/16/2010	Canterbury Health Services	WHO Collaborating Centre for Reference and Research on Influenza	Deng,Y-M; Iannello,P; Caldwell,N; Leang,S-K; Komadina,N
EPI319447	Czech Republic	2011-Jan-18	A/Czech Republic/32/2011	National Institute of Public Health	National Institute for Medical Research	
EPI239901	United States	2009-Apr-09	A/California/07/2009 X-181		Centers for Disease Control and Prevention	
EPI257201	United States	2009-May-01	A/California/07/2009 X-179A		Centers for Disease Control and Prevention	
EPI176470	United States	2009-Apr-01	A/California/04/2009		Centers for Disease Control and Prevention	

ID: identification number; WHO: World Health Organization.

We acknowledge the authors, originating and submitting laboratories of the sequences from GISAID's EpiFlu Database on which this research is based. All submitters of data may be contacted directly via the GISAID website: www.gisaid.org.

criteria were applied (Figure 2), 792 specimens were included in the primary analysis.

As in previous seasons, adults 20–49 years old contributed the largest proportion of specimens (50%) (Table 2) [3,6-12]. However, compared with the 2012/13 mid-season publication [3], a greater proportion of cases in 2013/14 were adults aged 20–49 years (53% versus 42%; p<0.01) or 50–64 years (22% versus 17%; p=0.13) (p<0.01 combined); proportions were more comparable among controls (48% versus 43%; p=0.17 and 20% versus 21%; p=0.86, respectively) (Table 2). Conversely, individuals younger than 20 years (21% versus 32%; p<0.01) and those 65 years and older (4% versus 9%; p<0.01) comprised a smaller proportion of cases compared with 2012/13 (Table 2) [3]. Adults aged 20–49 years and 50–64 years also comprised a greater proportion of cases in 2013/14 compared with the 2009 monovalent influenza A(H1N1)pdm09 VE analysis (53% versus 46%; p=0.14 and 22% versus 10%; p<0.01, respectively) [10].

Of the 792 specimens tested to date and included in primary VE analysis, 325 (41%) were positive for influenza, and 287 of 318 typed/subtyped viruses (90%) were A(H1N1)pdm09 (Table 3; Figure 1). Overall, 155 of 487 controls (32%) and 41 of 332 cases (12%) reported receipt of 2013/14 TIV (p<0.01). After applying exclusions related to immunisation timing, 29% of controls and 10% of cases were considered immunised (p<0.01) (Table 2). The proportion of controls reporting TIV receipt in 2013/14 and earlier seasons was comparable to that reported in previous VE analyses and other community-based surveys in Canada (ca 30%) [3,7-9,11,12,16]. Proportions comparable to previous community surveys were also observed in 2013/14 for receipt of the 2009 monovalent A(H1N1)pdm09 vaccine (43% versus 41%) [17]. The proportion of participants with co-morbidity was comparable to previous Canadian estimates (15–20%) [3,6-12,18] (Table 2).

The majority of participants immunised in 2013/14 also reported prior immunisation: 30 of 31 cases (97%) and 103 of 119 controls (87%) were immunised in 2012/13 (p=0.11); 26 of 29 cases (90%) and 89 of 116 controls (77%) were immunised in both 2012/13 and 2011/12 (p=0.12); and 21 of 26 cases (81%) and 83 of 108 controls (77%) received the 2009 monovalent A(H1N1) pdm09 vaccine (p=0.67).

The adjusted VE estimate for any influenza, driven predominately by A(H1N1)pdm09, was 71% (95% CI: 54–81), and for A(H1N1)pdm09 alone was 74% (95% CI: 58–83) (Table 4). In sensitivity analyses, VE estimates remained within 1–7% of primary analysis.

Virus characterisation

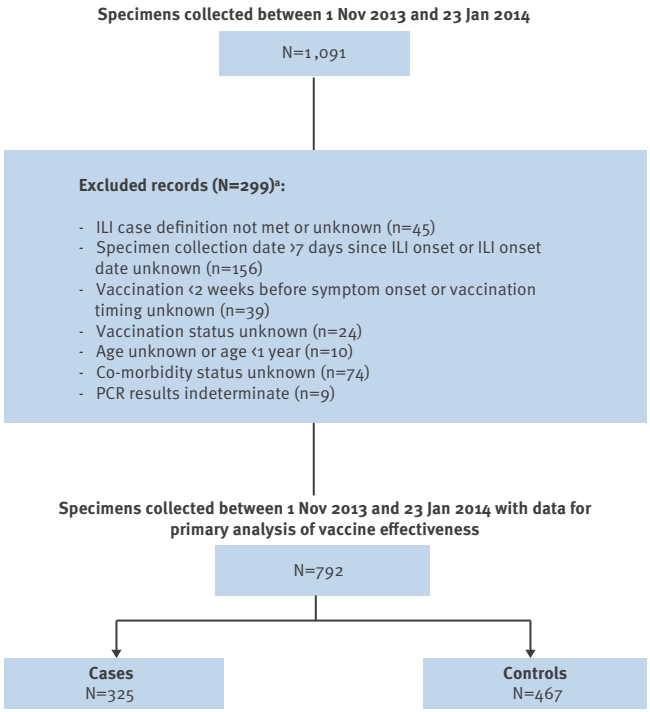
All A(H1N1)pdm09 isolates from Canada this season through week 4 (n=473, including 84 sentinel submissions) were identified by haemagglutination inhibition (HI) assay as antigenically similar to the A/California/07/2009 reference virus [1]. Only two A(H1N1)pdm09 isolates and none of the tested sentinel viruses, showed eightfold or higher reduction in HI titres against the reference strain, signalling sporadic antigenic change in only a very small proportion (<0.5%) [1,19].

HA1/HA2 sequences of a subset of 76 of 287 (26%) sentinel A(H1N1)pdm09 viruses were also assessed, including four collected in November, 45 in December and 27 in January (Figure 3; Table 5). All 76 sequences clustered within the European Centre for Disease Prevention and Control (ECDC)-described clade 6B (Figure 3) [20], representing a switch from clade 6C viruses that predominated among A(H1N1)pdm09 viruses during the 2012/13 season, albeit at substantially lower levels than A(H3N2) viruses [21].

Figure 3. Phylogenetic tree of influenza A(H1N1) pdm09 viruses, 2013/14 sentinel surveillance network, Canada, 1 November 2013–23 January 2014 (n=76)

Figure 2

Specimen exclusion, interim 2013/14 influenza vaccine effectiveness evaluation, Canada, 1 November 2013–23 January 2014 (n=1,091)



ILI: influenza-like illness.

[a] Excluded specimens may have more than one exclusion criterion that applies. Counts for each criterion will sum to more than the total number of specimens excluded. Missing collection dates were imputed as the laboratory accession date minus two days, the average time period between collection date and laboratory accession date for records with valid data for both fields.

Table 2

Profile of participants included in primary analysis, interim 2013/14 influenza vaccine effectiveness evaluation, Canada, 1 November 2013–23 January 2014 (n=792)

Characteristics	Test-positive: cases (n=325)	Test-negative: controls (n=467)	**Total (n=792)**	p value[a]
	n (%)	n (%)	**n (%)**	
Age group in years				<0.01
1–8	39 (12)	44 (9)	**83 (10)**	
9–19	30 (9)	50 (11)	**80 (10)**	
20–49	172 (53)	224 (48)	**396 (50)**	
50–64	71 (22)	95 (20)	**166 (21)**	
≥65	13 (4)	54 (12)	**67 (8)**	
Median age in years (range)	37 (1–81)	38 (1–93)	**37 (1–93)**	0.09
Female sex[b]	197 (61)	296 (64)	**493 (63)**	0.39
Co-morbidity[c]				0.09
No	263 (81)	354 (76)	**617 (78)**	
Yes	62 (19)	113 (24)	**175 (22)**	
Received 2013/14 TIV[d,e,f,g]				
≥2 weeks before symptom onset	34 (10)	135 (29)	**169 (21)**	<0.01
Among those				
without co-morbidity	19 (7)	84 (24)	**103 (17)**	<0.01
with co-morbidity	15 (24)	51 (45)	**66 (38)**	<0.01
Among those				
aged 1–8 years	3 (8)	7 (16)	**10 (12)**	0.32
aged 9–19 years	0 (0)	8 (16)	**8 (10)**	0.02
aged 20–49 years	16 (9)	58 (26)	**74 (19)**	<0.01
aged 50–64 years	10 (14)	34 (36)	**44 (27)**	<0.01
aged ≥65 years	5 (38)	28 (52)	**33 (49)**	0.39
Received prior influenza vaccine				
2012/13 TIV[h]	60/302 (20)	165/425 (39)	**225/727 (31)**	<0.01
2011/12 TIV[i]	60/284 (21)	159/414 (38)	**219/698 (31)**	<0.01
2009 A(H1N1)pdm09 vaccine[j]	91/265 (34)	156/366 (43)	**247/631 (39)**	0.04
Collection interval (days)				<0.01
≤4	264 (81)	329 (70)	**593 (75)**	
5–7	61 (19)	138 (30)	**199 (25)**	
Median interval in days (range)	3 (0–7)	3 (0–7)	**3 (0–7)**	0.04

TIV: trivalent inactivated vaccine.

[a] Differences between cases and controls were compared using the chi-squared test, Fisher's Exact test, or Wilcoxon rank-sum test.

[b] Patient's sex was missing for four specimens.

[c] Chronic co-morbidities that place individuals at higher risk of serious complications from influenza as defined by Canada's National Advisory Committee on Immunization (NACI) [43], including heart, pulmonary (including asthma), renal, metabolic (such as diabetes), blood, cancer, immune comprising conditions or those that compromise the management of respiratory secretions and increase the risk of aspiration, or morbid obesity. Questionnaire was answered as 'yes,' 'no,' or 'unknown' to any of these conditions without specifying.

[d] Vaccination status was based on self/parent/guardian report. Detail related to special paediatric dosing requirements was not sought.

[e] Immunised participants were predominantly offered split (non-adjuvanted) 2013/14 trivalent inactivated influenza vaccine during the regular autumn immunisation campaign. In British Columbia and Quebec, influenza vaccine is provided free of charge to high-risk groups [43]. Others are encouraged to receive vaccine but must purchase it. In Ontario, Alberta and Manitoba, the vaccine is provided free of charge to all residents aged six months or older.

[f] In Canada, live-attenuated vaccine for nasal administration is approved for those aged two to 59 years [43] but its use remains infrequent. For the 2013/14 season (as of 23 January 2014), of 169 participants reporting vaccine receipt at least two weeks before symptom onset in this study, 149 reported this was given through muscular injection and five through nasal spray (of whom four were individuals younger than 20 years); route of administration was unspecified for 15 participants.

[g] In Canada, MF59-adjuvanted vaccine is approved for people aged 65 years and older [43]. For the 2013/14 season (as of 23 January 2014), of the 33 people aged 65 years and older who were immunised at least 2 weeks before symptom onset in this study, eight reported they had received the adjuvanted vaccine and 13 did not know, while 12 received the non-adjuvanted formulation.

[h] Participants with unknown 2012/13 vaccine receipt and children younger than two years in 2013/14 were excluded from 2012/13 vaccine uptake analysis. Children younger than two years may not have been eligible for vaccination during the fall 2012/13 immunisation campaign on the basis of age under six months.

[i] Participants with unknown 2011/12 vaccine receipt and children younger than three years in 2013/14 were excluded from 2011/12 vaccine uptake analysis. Children younger than three years may not have been eligible for vaccination during the fall 2011/12 immunisation campaign on the basis of age under six months.

[j] Participants with unknown 2009 vaccine receipt and children younger than five years in 2013/14 were excluded from monovalent A(H1N1)pdm09 vaccine uptake analysis. Children younger than five years may not have been eligible for vaccination during the fall 2009 immunisation campaign on the basis of age under six months. More than 95% of the monovalent A(H1N1)pdm09 vaccine administered in Canada during the 2009 campaign was AS03-adjuvanted product [10].

Table 3

Laboratory profile of specimens included in primary analysis, interim 2013/14 influenza vaccine effectiveness evaluation, Canada, 1 November 2013–23 January 2014 (n=792)

Specimen included	Alberta (n=256)	British Columbia (n=149)	Manitoba (n=38)	Ontario (n=187)	Quebec (n=162)	**Total (n=792)**
	n (%)	n (%)	n (%)	n (%)	n (%)	**n (%)**
Influenza-negative	166 (65)	95 (64)	25 (66)	113 (60)	68 (42)	**467 (59)**
Influenza-positive	90 (35)	54 (36)	13 (34)	74 (40)	94 (58)	**325 (41)**
A-positive	89 (99)	53 (98)	12 (92)	73 (99)	73 (78)	**300 (92)**
B-positive	1 (1)	1 (2)	1 (8)	1 (1)	21 (22)	**25 (8)**
Influenza A-positive						
(H1N1)pdm09	88 (99)	48 (91)	9 (75)	69 (95)	73 (100)	**287 (96)**
H3N2	1 (1)	4 (8)	0 (0)	1 (1)	0 (0)	**6 (2)**
Subtype unknown	0 (0)	1 (2)	3 (25)	3 (4)	0 (0)	**7 (2)**

Two egg-adapted A/California/07/2009 seed strains, NYMC X-179A and X-181, have been available to manufacturers for vaccine production since 2009, both identical in their antigenic site aa sequence to the WHO-recommended A/California/07/2009 reference strain (with a single substitution in a non-antigenic site (N129D) in X-181). Of the publicly supplied TIV in Canada, 70% was derived from X-179A and 30% from X-181. Sentinel viruses shared 90%-94% aa identity with the vaccine across antigenic sites, the majority showing 94% identity with the vaccine. All 76 sentinel sequences had the same three antigenic site mutations: K163Q (site Sa), a clade 6B marker, as well as S185T (site Sb) and S203T (site Ca1), both of which were also identified among dominant circulating A(H1N1) pdm09 viruses of the past two seasons [12,21]. Five of 76 sequences bore a fourth aa substitution unique to each virus, and one Quebec sequence bore five substitutions (Table 5). Other than S185T, present in all 76 sequences, A186T, present in the single Quebec sequence, and possibly N156K and S157L [22], each present in a single and different Alberta sequence, none of the other substitutions were located within or adjacent to the receptor-binding site. With the exception of the single Quebec sequence, antigenic site mutations R205K, A141T, and A186T, which are located close to the receptor-binding site [22-25] and which occurred in 37%, 30% and 14%, respectively, of sentinel sequences during the 2012/13 season [21], were not evident in 2013/14.

Discussion

To date, the 2013/14 influenza season in North America has been characterised by substantial A(H1N1)pdm09 activity. This dramatic resurgence after only low-level circulation in the years since the 2009 pandemic has raised questions about possible virus evolution (i.e. antigenic drift) and reduced VE (i.e. vaccine failure). Our interim 2013/14 virological and VE analysis provides timely reassurance against both of these concerns. We show that circulating A(H1N1)pdm09 viruses are well-conserved based on genotypic and phenotypic characterisation, and that vaccine protection is substantial, reducing the risk of medically-attended laboratory-confirmed A(H1N1)pdm09 illness by about three quarters.

Our point estimate of ca 75% VE for the 2013/14 non-adjuvanted TIV against influenza A(H1N1)pdm09 is comparable, if not exceeding, 2009 estimates for non-adjuvanted formulations of the monovalent pandemic vaccine used in the US (ca 60%) [26,27], albeit lower than the 93% VE estimated by our sentinel system for the 2009 AS03-adjuvanted pandemic vaccine used in Canada [10]. The 2013/14 mid-season VE estimate against influenza A(H1N1)pdm09 of ca 75% is in the upper range of recent seasons' VE estimates for non-adjuvanted TIV against A(H1N1)pdm09 reported since 2010 from Canada [11,12,21], Europe [28-32] and the US [33-35], which span ca 60–80%. With several times more influenza A(H1N1)pdm09 cases already contributing thus far in 2013/14 than in previous seasons in Canada, we are likely to converge upon a more stable and accurate estimate of TIV protection against A(H1N1) pdm09 infection this season.

Although a switch from clade 6C to clade 6B* occurred between the 2012/13 and 2013/14 seasons [21], A(H1N1) pdm09 viruses remain genetically and antigenically similar to the A/California/07/2009 vaccine strain, a somewhat surprising finding given that this virus has circulated globally since 2009. Historically, however, H1N1 compared with H3N2 subtype viruses generally have shown a slower pace of HA antigenic change, judging at least by the recommended updates to vaccine composition made by the WHO between 1990/91 and 2008/09 (five H1N1 versus 11 H3N2 vaccine strain switches), with two H1N1 (but no H3N2) strains retained as TIV components for at least seven consecutive years during that period [4,36]. Genetic conservation of A(H1N1)pdm09 viruses may also be surprising in the context of population-level immune pressure. A

Table 4

Interim 2013/14 influenza vaccine effectiveness evaluation, influenza A(H1N1)pdm09 and influenza (any), Canada, 1 November 2013–23 January 2014 (n=792)

Analysis scenarios	A(H1N1)pdm09[a]		Influenza (any)	
	VE (95% CI)	Number Total (Cases; Vac) [Controls; Vac]	VE (95% CI)	Number Total (Cases; Vac) [Controls; Vac]
Primary analysis[b]				
Crude (unadjusted)	73 (59–83)	754 (287; 28) [467; 135]	71 (57–81)	792 (325; 34) [467; 135]
Age (1–8, 9–19, 20–49, 50–64, ≥65 years)	71 (55–82)		69 (53–80)	
Comorbidity (yes/no)	73 (58–83)		71 (56–81)	
Province (AB, BC, MB, ON, QC)	72 (56–82)		68 (52–79)	
Specimen collection interval (≤4/5–7 days)	73 (58–82)		71 (56–80)	
Week of illness onset	76 (62–85)		74 (61–83)	
Age, comorbidity, province, interval, week	74 (58–83)		71 (54–81)	
Sensitivity analysis[c]				
Restricted to specimens collected from 1 Dec 2013 to 23 Jan 2014 (week 49, 2013 to week 4, 2014)				
Crude	78 (65–86)	639 (279; 28) [360; 120]	76 (63–84)	674 (314; 34) [360; 120]
Adjusted	76 (60–85)		73 (57–83)	
Vaccination defined without regard to vaccination timing (i.e. any immunisation)				
Crude	72 (58–81)	780 (293; 34) [487; 155]	70 (56–79)	819 (332; 41) [487; 155]
Adjusted	71 (56–81)		68 (52–79)	
Restricted to patients with no comorbidities				
Crude	79 (63–89)	587 (233; 14) [354; 84]	75 (58–85)	617 (263; 19) [354; 84]
Adjusted[d]	81 (64–90)		76 (58–86)	
Restricted to participants with specimen collection interval ≤4 days				
Unadjusted	75 (58–85)	566 (237; 21) [329; 92]	74 (58–84)	593 (264; 24) [329; 92]
Adjusted[e]	76 (58–86)		74 (57–85)	
Restricted to participants aged 20–49 years				
Unadjusted	74 (50–86)	378 (154; 13) [224; 58]	71 (47–84)	396 (172; 16) [224; 58]
Adjusted[f]	75 (51–88)		71 (46–85)	
Restricted to participants aged 50–64 years				
Unadjusted	73 (36–89)	156 (61; 8) [95; 34]	71 (35–87)	166 (71; 10) [95; 34]
Adjusted[f]	80 (49–92)		77 (45–90)	
Restricted to participants aged 20–64 years				
Unadjusted	73 (56–84)	534 (215; 21) [319; 92]	70 (53–82)	562 (243; 26) [319; 92]
Adjusted[f]	76 (59–86)		73 (55–84)	

AB: Alberta; BC: British Columbia; CI: confidence interval; MB: Manitoba; ON: Ontario; QC: Quebec; Vac: vaccinated, i.e. number of (cases) or [controls] vaccinated; VE: vaccine effectiveness.

[a] Those with influenza A of H3N2 or unknown subtype or with influenza B were excluded from the A(H1N1)pdm09 analysis.

[b] For primary analysis, those with unknown comorbidity and those immunised less than two weeks before symptom onset or with unknown interval between immunisation and symptom onset were excluded but explored in sensitivity analysis as shown.

[c] Adjusted for age, comorbidity, province, specimen collection interval, and week of illness onset, unless otherwise specified.

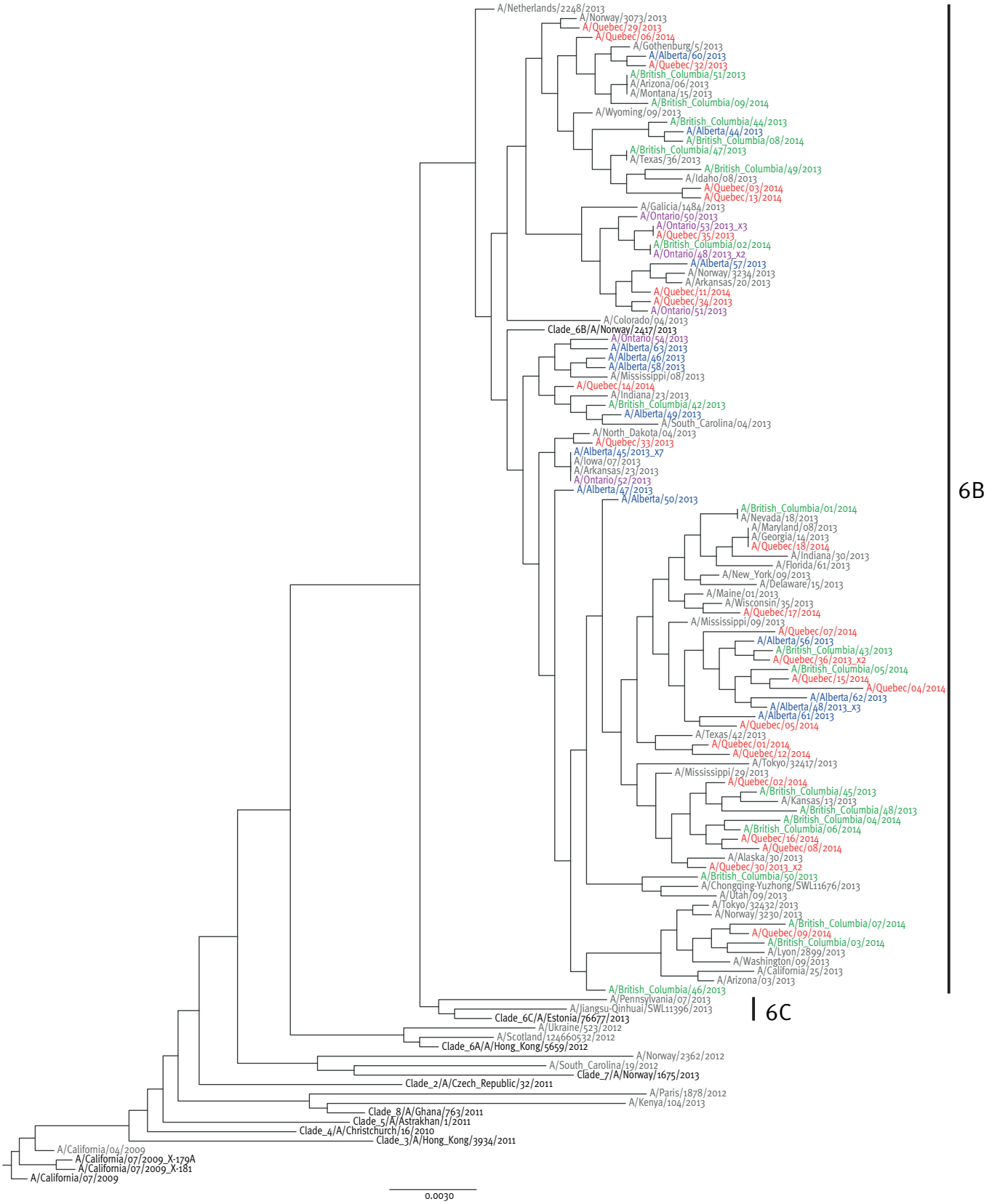
[d] Adjusted for age, province, specimen collection interval, and week of illness onset.

[e] Adjusted for age, comorbidity, province, and week of illness onset.

[f] Adjusted for comorbidity, province, specimen collection interval, and week of illness onset.

Figure 3

Phylogenetic tree of influenza A(H1N1)pdm09 viruses, 2013/14 sentinel surveillance network, Canada, 1 November 2013–23 January 2014 (n=76)



The phylogenetic tree was created by aligning the 76 Canadian sentinel sequences (colour-coded green for British Columbia, blue for Alberta, purple for Ontario and red for Quebec) against sequences representative of emerging viral clades as described by the European Centre for Disease Prevention and Control (ECDC) [20] (n=9), a random selection of A(H1N1)pdm09 sequences collected globally between 1 October 2013 and 21 January 2014 and obtained from the Global Initiative on Sharing Avian Influenza Data (GISAID) (n=43), and recent vaccine reference and egg-adapted seed strains (n=3).

Table 5

Amino acid changes in the haemagglutinin (HA1) genes (antigenic regions)[a] of a subset of 2013/14 Canadian sentinel influenza A(H1N1)pdm09 strains relative to vaccine reference strains[b], Canada, 1 November 2013–23 January 2014 (n=76)

Antigenic site		Cb	Sa				Ca1	Sb		Ca1
Amino acid number HA1		71	156	157	162	163	168	185	186	203
A/California/07/2009		S	N	S	S	K	D	S	A	S
A/California/07/2009 (X-179A)		S	N	S	S	K	D	S	A	S
British Columbia	n									
A/British Columbia/42/2013	16					**Q**		**T**		**T**
A/British Columbia/43/2013	1	**P**				**Q**		**T**		**T**
A/British Columbia/48/2013	1					**Q**	**N**	**T**		**T**
A/British Columbia/05/2014	1				**N**	**Q**		**T**		**T**
Alberta	n									
A/Alberta/44/2013	20					**Q**		**T**		**T**
A/Alberta/49/2013	1		**K**			**Q**		**T**		**T**
A/Alberta/62/2013	1			**L**		**Q**		**T**		**T**
Ontario	n									
A/Ontario/48/2013	9					**Q**		**T**		**T**
Quebec	n									
A/Quebec/29/2013	25					**Q**		**T**		**T**
A/Quebec/17/2014	1				**R**	**Q**		**T**	**T**	**T**

[a] Antigenic regions Sa, Sb, Ca1, Ca2 and Cb comprise 50 amino acid residues [12,13]. Only the nine positions in those 50 residues showing mutations in the present study are displayed.

[b] The northern hemisphere influenza A(H1N1)pdm09 vaccine reference strain since 2009, including the current 2013/14 season, is A/California/07/2009. The two egg-adapted seed strains available to manufacturers for vaccine production (NYMC X-179A and NYMC X-181) are both identical in their antigenic site amino acid sequences to the A/California/07/2009 reference strain recommended by the World Health Organization.

Bold font signifies amino acid substitution compared with the 2013/14 northern hemisphere vaccine reference strain.

All sequences were deposited into GenBank (accession numbers: KJ395993–KJ396037, KJ406381-KJ406387, KJ406507-KJ406528).

recent serosurvey conducted in May 2013 in Canada showed that levels of seroprotective antibody to A/California/07/2009 were high among school-aged children and the elderly; however, seroprotection was lower among very young children and adults between 20 and 69 years of age [37]. These findings may explain why conserved A(H1N1)pdm09 viruses resurged in 2013/14 and why there has been an apparent shift in the age distribution toward 20–64 year-old adults among medically-attended laboratory-confirmed influenza cases identified through the sentinel surveillance network this season. Such a demographic shift in disease burden toward adults following the 2009 pandemic was previously predicted in mathematical models from Canada [38] and warrants further empiric evaluation in additional surveillance datasets.

Limitations of the Canadian sentinel surveillance network for VE estimation have been described previously [3,5-12]. Although the validity of VE estimates derived by the test-negative approach has been demonstrated theoretically and in relation to randomised clinical trial analysis [39,40], the design remains observational, and bias and confounding cannot be ruled out. VE estimates for 2013/14 may vary at the end of the season, particularly since A(H1N1)pdm09 activity is still peaking in some regions of Canada [1]. However, end-of-season estimates for the 2012/13 VE differed by less than 5% from interim results presented in mid-season, even though the number of contributing cases increased by more than one third [3,21]. Ongoing monitoring is nevertheless warranted for changes in virus and/or VE with further time across the season. Variable

efficacy of repeated immunisation has previously been described, with differential effects depending upon the antigenic distance between successive vaccine components and circulating strains [41]. In that context, as in previous years, we emphasise that a substantial proportion of our immunised participants are repeat recipients of unchanged A(H1N1)pdm09 vaccine antigen. Generalisability to regions with a different profile of vaccine uptake may be limited on that basis. In recent analyses, we [12] and others [29,30,42] have noted a trend toward improved VE with recurrent receipt of the A(H1N1)pdm09 antigen, although other studies have reported contrary findings [28,31,35]. Assessment of these effects may benefit from the additional power available in end-of-season analysis.

In summary, our interim findings indicate that the 2013/14 TIV provides substantial protection against resurgent but conserved A(H1N1)pdm09 viruses circulating in Canada during the 2013/14 season, reducing the risk of medically-attended laboratory-confirmed A(H1N1)pdm09 illness by about three quarters. Neither antigenic drift nor homologous vaccine failure can account for resurgent A(H1N1)pdm09 activity this season in Canada. Other factors involved in agent–host interaction, including pre-existing antibody, should be considered in explaining the current epidemiology of this virus.

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Conflict of interest

Within 36 months of manuscript submission, GDS received research grants from GlaxoSmithKline (GSK) and Sanofi Pasteur for unrelated vaccine studies and travel fee reimbursement to attend an ad hoc GSK Advisory Board, without honorarium. JG has received research grants from GSK and Hoffmann-LaRoche for antiviral resistance studies. MK has received research grants from Roche, Merck, Gen-Probe and Siemens. SMM has received research grants from GSK, Sanofi Pasteur and Pfizer. SMM is a Canada Research Chair in Pharmaco-epidemiology and Vaccine Evaluation. SS and TLK are funded by the Canadian Institutes of Health Research Grant (TPA-90193). The other authors declare that they have no competing interests to report.

Authors' contributions

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

*Authors' correction:

On request of the authors, this passage was changed on 7 February 2014 from "a switch from clade 6B to clade 6C occurred" to "a switch from clade 6C to clade 6B occurred".

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Prevalence of antibodies against influenza A and B viruses in children in Germany, 2008 to 2010

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The prevalence of influenza A and B virus-specific IgG was determined in sera taken between 2008 and 2010 from 1,665 children aged 0–17 years and 400 blood donors in Germany. ELISA on the basis of whole virus antigens was applied. Nearly all children aged nine years and older had antibodies against influenza A. In contrast, 40% of children aged 0–4 years did not have any influenza A virus-specific IgG antibodies. Eighty-six percent of 0–6 year-olds, 47% of 7–12 year-olds and 20% of 13–17 year-olds were serologically naïve to influenza B viruses. By the age of 18 years, influenza B seroprevalence reached approximately 90%. There were obvious regional differences in the seroprevalence of influenza B in Germany. In conclusion, seroprevalences of influenza A and influenza B increase gradually during childhood. The majority of children older than eight years have basal immunity to influenza A, while comparable immunity against influenza B is only acquired at the age of 18 years. Children aged 0–6 years, showing an overall seroprevalence of 67% for influenza A and of 14% for influenza B, are especially at risk for primary infections during influenza B seasons.

Introduction

Influenza is a major public health threats worldwide with approximately 1 billion of the total population infected annually, resulting in 5 million serious diseases and 500,000 deaths [1]. Children are one of the most vulnerable groups since they are often immunologically naïve when placentally transferred antibodies have disappeared after ca one year of life [2], and the contacts made by children favour infections by the respiratory route [3]. Thus, influenza has been shown to be an important cause of morbidity during childhood, with attack rates ranging from 20% to 30% during epidemics [4]. Furthermore, infants and children with underlying disease are at increased risk of severe influenza and influenza-associated mortality [5-7]. Several studies have shown that influenza in childhood has considerable socioeconomic impact on the children's households [4,8,9] because children are regarded as the main and most efficient transmitters for spreading influenza in the community.

The most effective existing intervention to prevent morbidity and mortality of children due to influenza is the annual vaccination against seasonal influenza [10]. A study in Germany has shown that 50% of severe influenza cases in paediatric intensive care units might have been prevented if the current recommendations for vaccination, which only include risk groups of children, had been followed [6]. As demonstrated in several randomised clinical trials, intranasal live attenuated influenza vaccine has higher efficacy than the standard inactivated split vaccine and may improve vaccination in children [11,12]. However, owing to variations in circulating virus strains and in children's immune systems, current influenza vaccines are not fully protective [13]. Immunologically naïve younger children may be at increased risk of severe influenza disease and will therefore benefit from vaccination more than older children who have already had one or more influenza virus infections. This may explain why hospitalisation rates related to influenza virus infections are high in young children and comparable to those observed in adults over 60 years [14-16]. Therefore, seroepidemiological data on influenza A and B during childhood as a surrogate for type-specific priming are a fundamental prerequisite for the development of efficacious

Table 1

Prevalence of IgG antibodies against influenza A virus in children (aged 0–17 years, n=1,664[a]) and adults (blood donors aged 18–65 years, n=400), Germany, 2008–11

Age group in years	Male		Female		Male and female	
	Number of positive samples/total number	Percentage (95% CI)	Number of positive samples/total number	Percentage (95% CI)	Number of positive samples/total number	Percentage (95% CI)
Infants, children and adolescents						
0–2	71/150	47.3 (39.1–55.6)	55/121	45.4 (36.4–54.8)	126/271	46.5 (40.4–52.6)
3–4	81/104	77.9 (68.7–85.4)	59/71	83.1 (72.3–91.0)	140/175	80.0 (73.3–85.7)
5–6	64/74	86.5 (76.6–93.3)	71/77	92.2 (83.8–97.1)	135/151	89.4 (83.4–93.8)
7–8	86/89	96.6 (90.5–99.3)	82/86	95.3 (88.5–98.7)	168/175	96.0 (91.9–98.4)
9–10	95/97	97.9 (92.8–99.8)	95/95	100.0 (96.2–100.0)	190/192	99.0 (96.3–99.9)
11–12	100/100	100.0 (96.4–100.0)	101/101	100.0 (96.4–100.0)	201/201	100.0 (98.2–100.0)
13–14	112/112	100.0 (96.8–100.0)	111/112	99.1 (95.1–100.0)	223/224	99.6 (97.5–100.0)
15–17	113/113	100.0 (96.8–100.0)	162/162	100.0 (97.8–100.0)	275/275	100.0 (98.7–100.0)
Total	**722/839**	**86.1 (83.5–88.3)**	**736/825**	**89.2 (86.9–91.2)**	**1,458/1,664**	**87.6 (85.9–89.2)**
Adults (blood donors)						
18–29	78/79	98.7 (93.3–100.0)	72/72	100.0 (95.0–100.0)	150/151	99.3 (96.4–100.0)
30–45	67/72	93.1 (84.5–97.7)	52/52	100.0 (93.2–100.0)	119/124	96.0 (90.8–98.7)
46–65	71/78	91.0 (82.4–96.3)	45/47	95.7 (85.5–99.5)	116/125	92.8 (86.8–96.7)
Total	**216/229**	**94.3 (90.5–96.9)**	**169/171**	**98.8 (95.8–99.9)**	**385/400**	**96.3 (93.9–97.9)**

[a] For one patient, there was only enough serum to perform the test against influenza B.

vaccination policies for children. To date, the available data are scarce, and restricted to regional sampling points [2] or virus strain-specific tests [17,18]. The seroprevalence against influenza A and B, determined by sensitive and specific type-specific ELISA, may be a good surrogate marker for immunological priming since strain-specific assays such as the haemagglutination inhibition test depend on carefully selected panels of virus antigens and may underestimate the true seroprevalence.

Here, we describe a multicentre seroepidemiological study to determine influenza A and B antibody prevalence in infants, children and adolescents in Germany. Sera were obtained between 2008 and 2010 from nine paediatric and diagnostic centres throughout Germany. To compare these data with the influenza seroprevalence in adults, sera from blood donors were included. For the determination of influenza A- and B-specific IgG antibodies, type-specific ELISA with high sensitivity and specificity was used.

Methods

Patients and serum samples

A total of 1,665 sera from children aged one month to 17 years and 400 sera from blood donors aged 18 to 65 years were included. Sera of children were collected primarily between 2008 and 2010 from eight German paediatric primary care hospitals (Bremen, Berlin, Krefeld, Wuppertal, Erfurt, Würzburg, Mannheim, Munich) and one diagnostic institute (Ulm) for seroprevalence studies of pandemic influenza A [19]. Children with an illness impeding an adequate immune response were excluded. Some 15.8% (220/1,396) of children had been vaccinated against seasonal influenza and 7.1% (99/1,396) against pandemic influenza A between 2008 and 2010, but the reasons were unknown. This means that the vaccination was carried out in the same year or one year before the sample was taken. Sera of blood donors aged 18 to 65 years were collected anonymously between 2010 and 2011 mainly in North-Rhine Westphalia (German Red Cross blood donation centre Muenster, 337/400, 84.3%) and in Lower Saxony (German Red Cross blood donation centre Springe, 63/400, 15.7%) and there was no information about the donors' vaccination status against influenza. However, an average vaccination rate of 15–20% can be assumed [20].

In accordance to recommendations of the Central Ethical Committee of Germany [21], patient consent is not required for studies on anonymised residual samples. The Ethical Committee of the Jena University approved the study protocol.

Testing of sera

Sera were stored in aliquots at –20 °C without interruption until testing. All sera were brought to room temperature immediately before testing. Antibody testing was carried out blindly in groups of 90 serum samples. Sera were tested in parallel using influenza virus A IgG

Table 2

Regional distribution of influenza A and B seroprevalence in children (aged 0–17 years, n=1,665) of nine German paediatric or diagnostic centres, 2008–10

Center (Number of sera)	Mean age in years	Seroprevalence in percentage (95% CI)	
		Influenza A	Influenza B
Wuppertal (366)	9.6	90.7 (87.3–93.5)	49.5 (44.2–54.7)
Bremen (268)	8.3	81.7 (76.6–86.2)	34.7 (29.0–40.7)
Ulm (278)	8.2	81.7 (76.6–86.2)	51.4 (45.4–57.5)
Mannheim (225)	8.4	89.3 (84.6–93.1)	48.4 (41.8–55.2)
Würzburg (140)	9.9	97.9 (93.9–99.6)	64.3 (55.8–72.2)
Krefeld (111)	11.2	98.2 (93.6–99.8)	46.9 (37.3–56.6)
Erfurt (108)	5.8	75.7 (66.5–83.5)	25.9 (18.0–35.3)
Berlin (85)	8.5	90.6 (82.3–95.9)	52.9 (41.8–63.4)
Munich (84)	9.1	89.3 (80.6–95.0)	50.0 (38.9–61.1)

ELISA (IBL International, Hamburg, Germany) and influenza virus B IgG ELISA (Euroimmun, Lübeck, Germany). These two ELISAs had been selected as the most sensitive and specific tests after comparing different commercially available ELISAs for influenza A and B IgG. Testing of defined serum samples from children [2], newborns and their mothers [22] by several commercial ELISAs, including the haemagglutination inhibition assay [23], revealed sensitivities ≥97% and no cross-reactivities between influenza A and B virus or to other viral pathogens for the ELISAs used in this study. Both ELISAs were carried out manually and used for qualitative and semi-quantitative antibody testing. All samples were tested twice on different days, and sera with the same qualitative results were included in this study without retesting. Samples with discordant qualitative results were retested twice, and the most frequent result, including the original test result, was accepted.

The influenza virus A IgG ELISA used whole inactivated influenza virus A Sydney/5/97 (H3N2) and Bejing/262/95 (H1N1), and the influenza B IgG ELISA whole inactivated influenza virus B Hongkong/5/72 as antigens in pre-coated microtitration strips. The antigen solutions contained high amounts of conserved influenza type-specific nucleo- and matrix proteins. Testing of sera was carried out at the dilution of 1:100 according to the manufacturer's instructions. Results were assessed on the basis of a standard curve calculated from three to four calibrators including positive and negative controls. In the influenza A IgG ELISA, samples were considered positive if the antibody concentration was calculated as ›12 U/mL, a range of 8–12 U/mL was considered equivocal and ‹8 U/mL was interpreted as negative. For the influenza B IgG ELISA, samples were considered positive if the antibody concentration was calculated as ≥22 relative units (RU) per mL, a range of ≥16 to ‹22 RU/mL was considered equivocal, and ‹16 RU/mL was interpreted as negative.

Statistical analysis

A sample size of about 150 subjects per pre-defined age group was planned to assure that a single two-sided 95% confidence interval (CI) for the prevalence of influenza A and B IgG antibodies would deviate at most 8% from the observed value for a prevalence range of 5% to 95%. When regional differences were analysed, less precise seroprevalences obtained from single paediatric centres resulted from small sample size. Antibody prevalence was calculated using the number of seropositive cases divided by the number of all subjects tested. Assuming binominal distribution, the two-sided exact 95% CI was calculated. The Cochran–Armitage test for trend [24] was used to examine the increase of antibody prevalence by age. Age-adjusted sex differences in antibody prevalence were investigated by the Mantel–Haenszel test [25]. Logistic regression odds ratios evaluated by the Wald statistics were used to compare age-specific prevalence of the children with the prevalence of the adult group as the whole.

Age- and sex-specific antibody concentrations were described by mean and standard deviation (SD). The association of age and sex, and the concentration of antibodies were analysed using linear multiple regression. Antibody concentrations of the different age groups of children were compared with the corresponding data of the whole adult group by the Dunnett test [26]. For both analyses, antibody concentration was transformed by the common logarithm. The level of significance was 0.05 (two-sided). The SAS V9.2 software was used for statistical analyses.

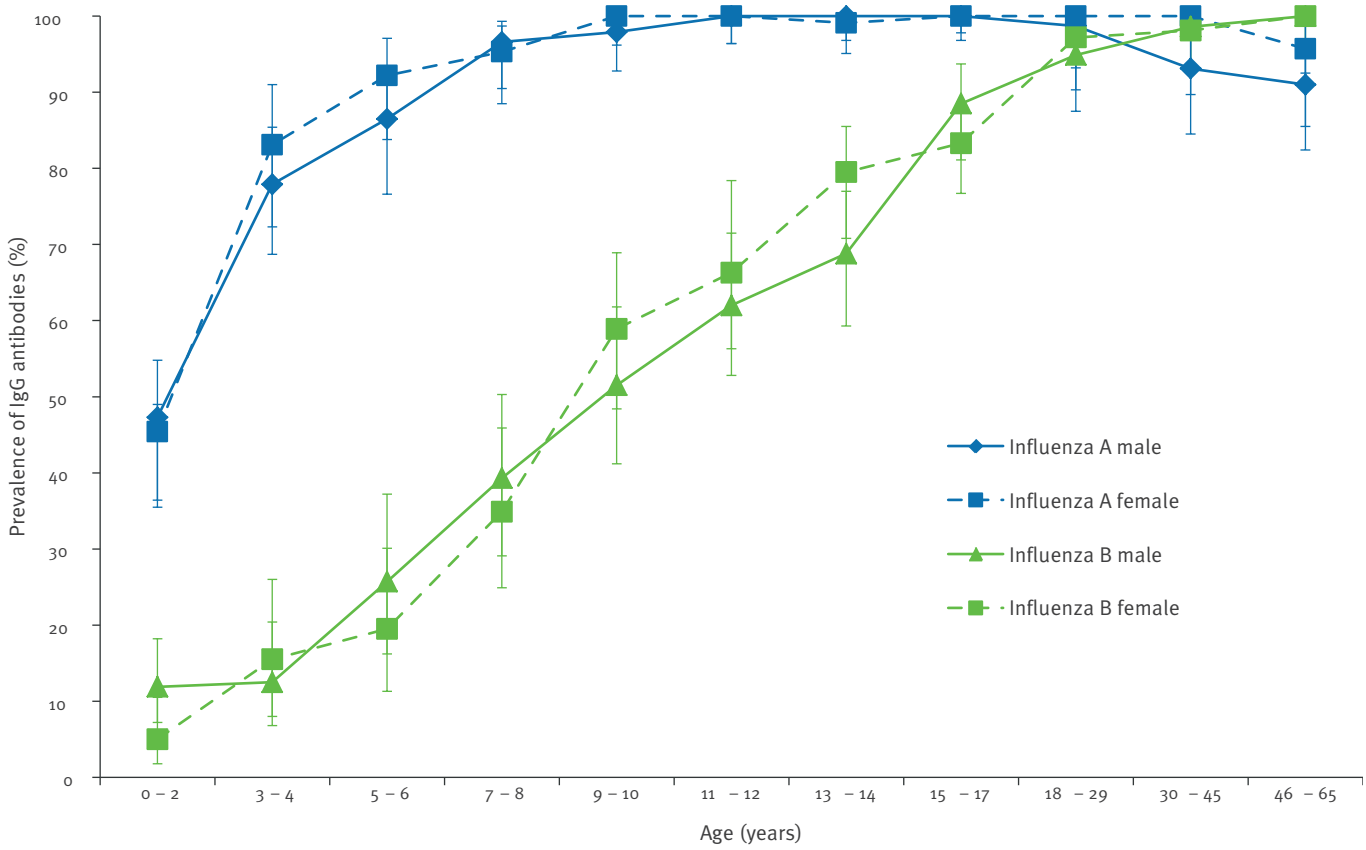
Results

Prevalence and concentrations of influenza A virus IgG

The prevalence of IgG antibodies against influenza A virus in the samples tested is shown in Table 1. The overall prevalence of antibodies against influenza A virus was 87.6% (95% CI: 85.9–89.2) in the tested children aged 0–17 years; among the tested blood donors,

Figure 1

Age- and sex-specific prevalence of IgG antibodies against influenza A and B virus in children (aged 0–17 years) and adults (blood donors aged 18–65 years), Germany, 2008–11 (n=2,065)



The bars show the 95% confidence intervals for the point of estimates.

the overall prevalence of influenza A IgG antibodies was 96.3% (95% CI: 93.9–97.9).

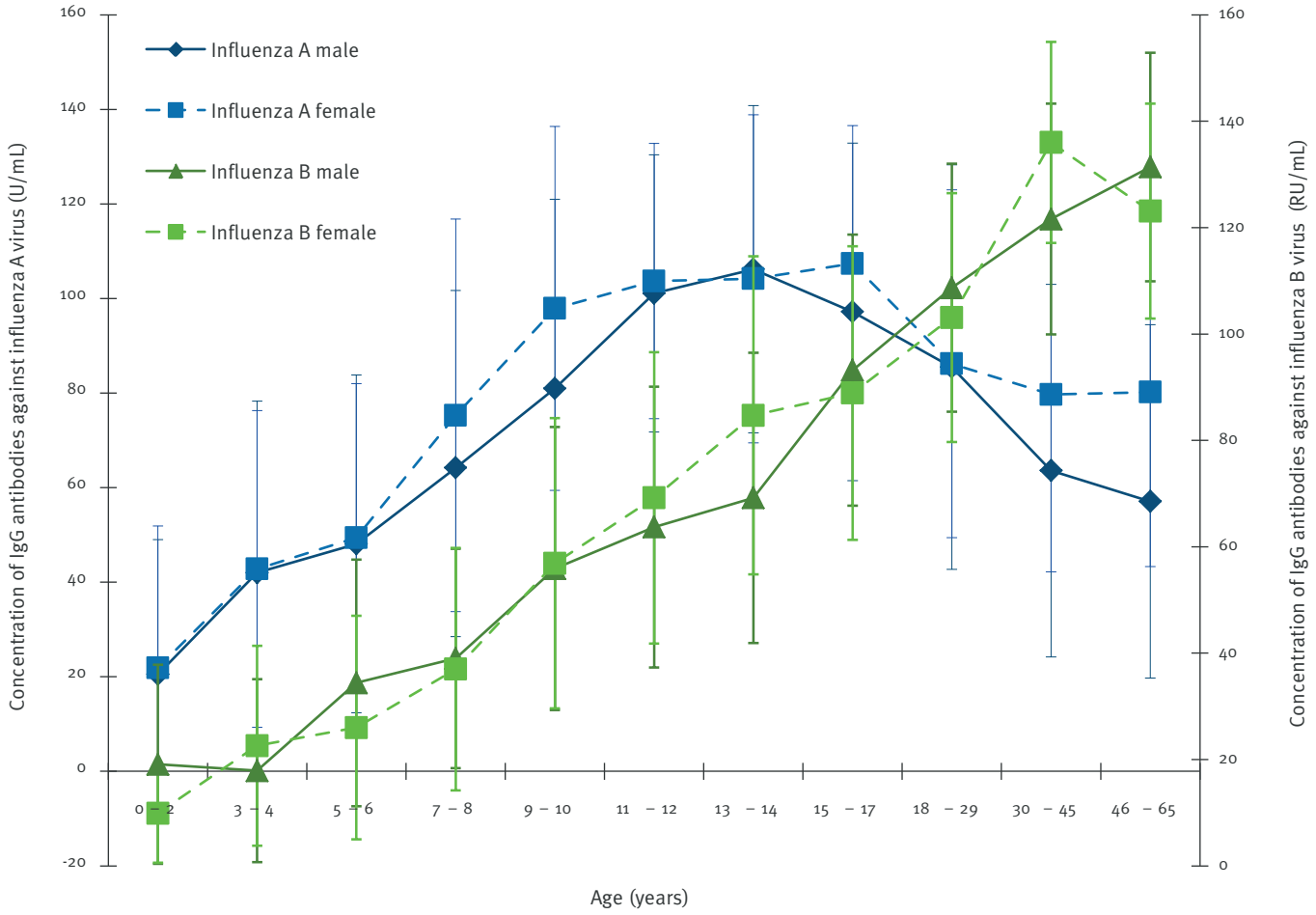
The regional distribution of the influenza A seroprevalence in children among the nine centres included in this study is shown in Table 2. The prevalence of antibodies ranged from 75.7% (Erfurt) to 97.9% (Würzburg); mean age of the children differed between the centres.

Figure 1 shows the age- and sex-specific prevalence of influenza A IgG in the tested children and adults (blood donors). Statistical analysis demonstrated that the antibody prevalence against influenza A virus increased significantly with age in children ($p<0.001$) and decreased with age in adults ($p=0.004$). Adjusted to age, there were no significant differences between the prevalence of antibodies among boys and girls ($p=0.576$), but in blood donors a significantly higher prevalence was detected in women than in men ($p=0.031$). Children up to the age group of five to six years had a significantly lower prevalence of antibodies than the adult controls (0–2 and 3–4 years: $p<0.001$, 5–6 years: $p=0.003$). In children vaccinated against seasonal ($p=0.002$) or pandemic influenza ($p=0.01$), the number of positives was significantly higher (seasonal: 209/220, 95.0%; pandemic: 98/99, 99.0%) than in non-vaccinated children (seasonal: 1,029/1,176, 87.5%; pandemic: 1,140/1,296, 88.0%).

In the study group of children, the mean concentration of antibodies against influenza A virus was calculated as 59.95 U/mL (SD: 46.04), and the adult controls had a mean antibody concentration of 72.02 U/mL (SD: 39.90). Figure 2 shows the concentrations of antibodies against influenza A virus by age and sex of the tested children and adults (blood donors). The antibody concentrations increased significantly with age during childhood ($p<0.001$) and declined with age in adults ($p=0.041$). In young adults of 18–29 years, the mean antibody concentration was 85.75 U/mL (SD: 40.33) compared with 66.06 U/mL (SD: 38.67) in older adults of 46–65 years. Adjusted to age, no significant differences could be found between boys and girls ($p=0.426$) nor between men and women ($p=0.300$). Children up to the age group of five to six years had significantly lower concentrations of IgG antibodies against influenza A virus than the control group of adults ($p<0.001$), whereas the antibody concentrations

Figure 2

Age- and sex-specific distribution of IgG antibody concentrations against influenza A and B virus in children (aged 0–17 years) and adults (blood donors aged 18–65 years), Germany 2008–11 (n=2,065)



The bars show the standard deviation for the point of estimates.

were significantly higher among the 11–12 (p<0.001), 13–14 (p=0.001) and 15–17 (p<0.001) year-olds than among the adults. These data were not adjusted for vaccination status.

Prevalence and concentrations of influenza B virus IgG

Table 3 shows the prevalence of antibodies against influenza B virus in the samples tested. In children, the overall prevalence was 47.0% (95% CI: 44.6–49.5), and in the blood donors, it was 98.0% (95% CI: 96.1–99.1). The regional distribution of influenza B seroprevalence in the nine centres is shown in Table 2. The lowest prevalence of antibodies was found with 25.9% in the Erfurt group and the highest with 64.3% in the Würzburg group, and these prevalences were related to the mean age of the children in the different centres. The age- and sex-specific prevalences of influenza B IgG in the tested children and adults (blood donors) are shown in Figure 1. Statistical analysis demonstrated that the prevalence of antibodies against influenza B increased significantly with age in children (p<0.001) and adults (p=0.018). Adjusted to age, there were no significant differences between the prevalence of antibodies as a function of sex for children (p=0.977) and adults (p=0.635). In all age groups of children, significantly lower prevalence of antibodies was measured than in the adult controls (p<0.001). In the group of children vaccinated against seasonal influenza, the number of positives (146/220, 66.4%) was significantly higher than in the group of non-vaccinated children (497/1,176, 42.3%, p<0.001).

The mean antibody concentrations against influenza B virus were estimated as 54.67 RU/mL (SD: 55.30) in the study group of children and as 119.31 RU/mL (SD: 44.97) in the control group of adults (blood donors). Figure 2 shows the concentrations of influenza B-specific antibodies depending on age and sex of the tested children and adults (blood donors). The concentrations of antibodies increased with age up to the 15–17 year-olds (p<0.001), but there were no significant differences between the three age groups of adults (p=0.228). The antibody concentrations were not dependent on sex neither in the children (p=0.317) nor in the adults (p=0.892). For all age groups of children, significantly

Table 3

Prevalence of IgG antibodies against influenza B virus in children (aged 0–17 years, n=1,665) and adults (blood donors aged 18–65 years, n=400), Germany, 2008–11

Age group in years	Male		Female		Male and female	
	Number of positive samples/total number	Percentage (95% CI)	Number of positive samples/total number	Percentage (95% CI)	Number of positive samples/total number	Percentage (95% CI)
Infants, children and adolescents						
0–2	18/151	11.9 (7.2–18.2)	6/121	5.0 (1.8–10.5)	24/272	8.8 (5.7–12.8)
3–4	13/104	12.5 (6.8–20.4)	11/71	15.5 (8.0–26.0)	24/175	13.7 (9.0–19.7)
5–6	19/74	25.7 (16.2–37.2)	15/77	19.5 (11.3–30.1)	34/151	22.5 (16.1–30.0)
7–8	35/89	39.3 (29.1–50.3)	30/86	34.9 (24.9–45.9)	65/175	37.1 (30.0–44.8)
9–10	50/97	51.5 (41.2–61.8)	56/95	58.9 (48.4–68.9)	106/192	55.2 (47.9–62.4)
11–12	62/100	62.0 (51.8–71.5)	67/101	66.3 (56.3–75.4)	129/201	64.2 (57.1–70.8)
13–14	77/112	68.8 (59.3–77.2)	89/112	79.5 (70.8–86.5)	166/224	74.1 (67.9–79.7)
15–17	100/113	88.5 (81.1–93.7)	135/162	83.3 (76.7–88.7)	235/275	85.5 (80.7–89.4)
Total	**374/840**	**44.5 (41.1–48.0)**	**409/825**	**49.6 (46.1–53.0)**	**783/1,665**	**47.0 (44.6–49.5)**
Adults (blood donors)						
18–29	75/79	94.9 (87.5–98.6)	70/72	97.2 (90.3–99.7])	145/151	96.0 (91.6–98.5)
30–45	71/72	98.6 (92.5–100.0)	51/52	98.1 (89.7–100.0)	122/124	98.4 (94.3–99.8)
46–65	78/78	100.0 (95.4–100.0)	47/47	100.0 (92.5–100.0)	125/125	100.0 (97.1–100.0)
Total	**224/229**	**97.8 (95.0–99.3)**	**168/171**	**98.2 (95.0–99.6)**	**392/400**	**98.0 (96.1–99.1)**

lower antibody concentrations were measured compared with the control group of adults (p<0.001).

Discussion

In this study, it was of particular interest to determine influenza seroprevalence of children up to the age of 17 years in Germany. To obtain data most widely representative for the whole population of children in Germany, nine different German regions were included. A recently published study on the influenza seroprevalence in Germany included only sera from children who lived in the German federal state Thuringia [2], and the results were not regarded as representative for the entire country. Furthermore, as that study used other serological methods than ours, the results are only comparable to a limited extent.

To date, the haemagglutination inhibition test or microneutralisation assays are the gold standards to determine IgG antibodies to influenza viruses in seroprevalence studies, and the only current correlate of immunity to influenza A and B is based on haemagglutination inhibition titre [17,18,27]. However, these assays are even virus strain- or lineage- and subtype-specific, and studies carried out with a limited or poorly chosen panel of viral antigens may underestimate the true seroprevalence [28]. Furthermore, these assays are not suitable for large-scale studies because they are labour-intensive, time-consuming, not amenable to automation and not commercially available. Thus, sensitive and specific ELISAs mainly targeting conserved type-specific antibodies against the influenza virus nucleo- and matrix proteins have been used successfully in several studies for the determination of influenza seroprevalence in humans as well as pigs [2,22,28]. An essential prerequisite is, however, that the ELISAs used are evaluated for their performance characteristics. The ELISAs in the present study used inactivated influenza A and B viruses containing high amounts of conserved influenza type-specific nucleo- and matrix proteins and were selected because of their high sensitivity and specificity. Higher antibody titres measured in the vaccinated group suggest that the assays detected also IgG antibodies induced by vaccination against seasonal and pandemic influenza. That is why the vaccination coverage was analysed, but the proportion of vaccinated children was equally low in all regions, and the vaccination rate in adults could be assumed to be low [20]. Vaccination coverage was therefore not of significance for our findings. A limitation of this study is that while the ELISAs indicate a previous infection, they provide no information about the time of infection. Antibodies to influenza virus nucleo- and matrix protein antigens fail to contribute to protection, but they indicate the presence of subtype-independent T-cell-mediated protection [29].

In children, the overall prevalence of antibodies to influenza A was 87.6%, reflecting the epidemiological dominance of seasonal and pandemic influenza A over influenza B between 2007 and 2010 in Germany [30-32], and assuming that antibodies persist at least six months after infection or vaccination [23]. The seroprevalence showed an age-dependent increase until

the age of nine to 10 years, when nearly all children had developed antibodies against influenza A virus. These data correlate well with the results published recently for children in the Netherlands [18]. The latter study was carried out with the haemagglutination inhibition test and showed that all children seven years and older had antibodies to at least one of six representative influenza A(H3N2) and six representative influenza A(H1N1) virus strains selected for serological testing. Our data demonstrate that children, starting with the age of nine years, have a good basal immunity to influenza A. Thus, these children are at lower risk for potentially severe primary influenza infections and do not need a second dose of the vaccine against influenza A as long as they have received the first vaccine dose. This is in agreement with findings published previously [33]. In contrast to the influenza seroprevalence in older children, nearly 40% of children under the age of four years had no influenza A virus-specific IgG antibodies. These children are immunologically naïve, and therefore have to be regarded as susceptible to potentially severe primary influenza A infection. Accordingly, Bodewes et al. [18] found the highest attack rates with primary influenza A infections, calculated on the basis of antibody prevalence, in children two and three years of age. In addition, children up to the age of six years in our study had a significantly lower influenza A seroprevalence and significantly lower antibody concentrations against influenza A virus than adults. This is most likely due to the lower number of boosting influenza A infections in their lifetime [2]. Adolescents at the age of 13 to 14 years had significantly higher influenza A seroprevalence, and 11 to 17 year-olds had significantly higher antibody concentrations to influenza A virus than adults. These data, which correspond to results reported previously [2], may suggest that these age groups have the highest attack rates of influenza A reinfections or more frequent silent boosting. However, the lower seroprevalence in adults might also be due to faster waning of immunity in older people. Among the adults, the antibody prevalence against influenza A virus was significantly higher in women than in men. These findings may be associated with the high incidence of influenza A during childhood since care of children in Germany is generally undertaken by women [34]. Since there was no information about the influenza vaccination status of our study participants, it remained unclear whether the higher prevalence of anti-influenza A antibodies may reflect a difference in vaccination status.

A different pattern was observed for the seroprevalence of influenza B. The overall prevalence of antibodies to influenza B in children was 47%. Approximately 60–70% of all children up to the age of 12 years were serologically naïve and have to be considered susceptible to influenza B. By the age of 18 years, an influenza B seroprevalence of approximately 90% was reached. The considerably lower seroprevalence rate of 25% among 12 year-old children reported in our recent study [2], can only be interpreted in the context of the single test population from Thuringia and the different serological method used. In the present study, the group of all children and adolescents had significantly lower influenza B seroprevalences and significantly lower antibody concentrations against influenza B than adults. They seem to have had fewer infections during their lifetimes than adults [2]. It can be concluded from the current study that a natural immunity against influenza B at a level comparable to influenza A, is only established around the age of 18 years. Since children with incomplete specific immunity may be at risk for severe courses of influenza B [35,36] a seasonal vaccination against influenza B could benefit all children. In Germany, there are obvious regional differences in seroprevalences of influenza B. As the present study shows, the influenza B seroprevalences of distinct German regions differ in children with a mean age of eight to 10 years between approximately 35% and 65%, i.e. by as much as 30%. By contrast, the overall values of influenza A seroprevalence varied by as much as approximately 16% (range: 82–98%). This means that influenza B outbreaks, contrary to influenza A epidemics, may often be restricted to certain local regions. Multicentre studies are required to obtain representative seroprevalence data. Different rates of influenza vaccination may be of importance, but data on this are not available. Interestingly, the lowest seroprevalence of influenza B of this study was observed with 25.9% in the child population with a mean age of six years, recruited from the paediatric clinic in Erfurt, the capital of the German federal state Thuringia. Sera from children 18 years and younger in this region had been included in our previous study resulting in an overall influenza B seroprevalence of 9.6% [2]. Reasons for this variation can be differences between the serological methods used and the test populations. This means that the methods used in seroprevalence studies must be validated thoroughly.

In conclusion, this study provides representative data of influenza A and B seroprevalences in children aged up to the age of 17 years in Germany. They may have implications for the development of vaccination strategies to protect children against influenza A and B.

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Conflict of interest

The co-author Ruprecht Schmidt-Ott is employed by GlaxoSmithKline Vaccines, Wavre, Belgium.

Authors' contributions

A Sauerbrei: author of the publication, also provided analysis and interpretation of data, responsible for study design. TL: co-author of the publication, carried out the ELISAs and one part of statistical analysis. AB: co-author of the publication, carried out the second part of statistical analysis. RS-O: co-author of the publication and responsible for study design. AK, HG, H-IH, PK, JL, A Streng, TN, JP, A Sauerbrey, HS, TT, SW and PW: co-authors, responsible for the collection of patients' samples. All authors have read and approved the final manuscript.

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A cocoon immunisation strategy against pertussis for infants: does it make sense for Ontario?

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Pertussis deaths occur primarily among infants who have not been fully immunised. In Ontario, Canada, an adult booster dose was recently added to the publicly funded immunisation programme. We applied number-needed-to-treat analyses to estimate the number of adults that would need to be vaccinated (NNV) to prevent pertussis disease, hospitalisation and death among infants if a cocoon strategy were implemented. NNV=1/(P_M X R) + 1/(P_F X R), where P_M,P_F (proportion of infants infected by mothers, fathers) were sourced from several studies. Rates of disease, hospitalisation or death (R) were derived from Ontario's reportable disease data and Discharge Abstract Database. After adjusting for under-reporting, the NNV to prevent one case, hospitalisation or death from pertussis was between 500–6,400, 12,000–63,000 and 1.1–12.8 million, respectively. Without adjustment, NNV increased to 5,000–60,000, 55,000–297,000 and 2.5–30.2 million, respectively. Rarer outcomes were associated with higher NNV. These analyses demonstrate the relative inefficiency of a cocoon strategy in Ontario, which has a well-established universal immunisation programme with relatively high coverage and low disease incidence. Other jurisdictions considering a cocoon programme should consider their local epidemiology.

Introduction

Pertussis is an infectious respiratory disease caused by *Bordetella pertussis*, typically presenting with a paroxysmal cough followed by a characteristic 'whoop' sound. Young infants, adolescents and adults are less likely to present with typical symptoms, which leads to under-diagnosis by physicians, who may fail to consider the diagnosis [1]. While disease occurs in all age groups, complications occur most frequently in infants too young to have begun or completed their primary immunisation series, particularly among those under four months of age [2]. The case–fatality ratio (CFR) among infants under one year of age is estimated to be 0.2% [3] in countries with low mortality, though it can reach 3% [4].

In Ontario, Canada, pertussis vaccines have been available since 1943 and are currently offered as combination vaccines. Diphtheria and tetanus toxoids, acellular pertussis, inactivated poliomyelitis and *Haemophilus influenzae* type b (DTaP-IPV-Hib) is administered as a primary series at 2, 4 and 6 months with a booster dose at 18 months of age. A second booster of DTaP-IPV is administered at 4–6 years. Since 2003, an adolescent acellular pertussis booster dose using the adolescent/adult formulation (Tdap) has also been offered at 14–16 years, with coverage among 17-year olds estimated at 67.7% [5]. On 8 August 2011, a single dose of Tdap vaccine (Adacel by Sanofi Pasteur or Boostrix by GlaxoSmithKline) was publicly funded for adults aged 19 to 64 years who had not previously received an adolescent booster.

Parents, siblings and other household contacts are frequently identified as the primary source of infection among infants with pertussis [6–16]. Cocooning refers to the vaccination of mothers and other contacts of newborns and infants in order to prevent the transmission of pertussis to infants who may not have completed their primary vaccination series. Since 2012 in the United States (US), the Advisory Council on Immunization Practices (ACIP) has recommended a dose of Tdap during every pregnancy, irrespective of previous vaccination history [17]. In Canada, the National Advisory Committee on Immunization (NACI) recommends a universal adult immunisation programme) [18]. New Brunswick is the only Canadian jurisdiction to recommend and to have implemented a cocoon programme where parents are entitled to receive publicly-funded Tdap vaccine since 1 January 2011.

We applied the concept of 'number needed to treat' to estimate the number of adults that would need to be vaccinated (NNV) to prevent one case of disease, hospitalisation and death among infants if a cocoon strategy were implemented in Ontario.

Methods

The number of mothers and fathers that would need to be vaccinated to prevent one case, hospitalisation or death due to pertussis among infants (defined as

children less than one year of age) was estimated using the following formula:

$$NNV_{total} = NNV_{mother} + NNV_{father}$$

$$= \frac{1}{AR_{mother} \times VE} + \frac{1}{AR_{father} \times VE}$$

$$= \frac{1}{\{Incidence \times \%Infected_{mother}\} \times VE} + \frac{1}{\{Incidence \times \%Infected_{father}\} \times VE}$$

where

- AR = attributable risk due to the mother or father, as specified
- VE = vaccine effectiveness
- incidence = rate of disease, hospitalisation or death among infants aged under one year
- % infected = the proportion of infants aged under one year who were infected by their mother or father, as specified.

Vaccine effectiveness (VE) was generally assumed to be 85% except where noted. Estimates of incidence varied depending on the outcome of interest. Rates of disease were based on confirmed cases of pertussis in infants under one year of age, as reported in Ontario's integrated Public Health Information System (iPHIS) between 1 January 2005 and 31 December 2009. In 2009, the case definition was changed so that clinically compatible illness, in addition to laboratory detection of pertussis, was required to meet the definition for a confirmed case. This more specific definition will have resulted in fewer confirmed cases. Mortality rates were determined by applying the CFR of 0.2% [3] to the rates of disease. Hospitalisation rates were determined using data from the Discharge Abstract Database maintained by the Canadian Institute for Health Information. Infants who were discharged between 2005 and 2009 were included in this analysis. Patients for whom pertussis was determined to have contributed most significantly to their hospitalisation (i.e. most responsible diagnosis) were identified by selecting a code of A37.0 under the Canadian Enhancement to the 10th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10-CA) [19]. Using methodology consistent with the Ontario Burden of Infectious Diseases (ONBOIDS) report, rates of disease (and consequently death) were inflated by a factor of 9.4 to adjust for the under-reporting of cases [4]. Since hospitalised cases and deaths were less likely to be under-reported, hospitalisation and mortality rates were only inflated by factors of 4.7 and 2.35, respectively (i.e. multiplying the original inflation factor of 9.4 by 0.5 and 0.5^2). To account for yearly fluctuations in incidence, the minimum, maximum and average rates during the study period were used. Demographic data from Statistics Canada, accessed through intelliHealth, were used to calculate incidence. As denominator data by month were not available for infants under one year of age, we assumed equal population distribution over the first year of life to calculate rates among infants less than four months of age. The introduction of real-time PCR testing in 2005 may have contributed to the increase in cases observed in subsequent years.

To determine the proportion of infants who were infected by their mother or father (% infected), several studies were reviewed. While numerous studies have been conducted to explore the role of adults and siblings in transmitting infection to infants in Australia [6,7], the Netherlands [8], Canada [9], the US [10–12], England [13] and France [14,15], including a recent review [16], only a few studies published the data necessary to determine the proportion of infants (including those for whom the source of infection was unknown) who were infected by the mother or father [6,8,10]. In an additional study [13], the required data were obtained from the author (data not shown). Table 1 presents the range of mother- and father-specific estimates from these studies, and shows the impact of including and excluding cases whose source of infection was unknown. When cases with unknown sources of infection were included, the proportion of infants who were infected by their mother and father ranged between 14% and 21%, and 6% and 11%, respectively. When unknowns were excluded, the proportion infected by their mother and father ranged between 20% and 41%, and 12% and 18%, respectively. Estimates from the Dutch study were excluded since the methodology used to determine sources of infection was not comparable to the other studies (no determination of a unique source of infection was made, whereas this was defined in the other studies).

Results

Between 2005 and 2009, 844 confirmed cases of pertussis among infants less than one year old were reported through iPHIS in Ontario. Of these, 49.2% (n=415) of cases occurred in infants less than four months old. The unadjusted incidence of disease ranged between 46.4 and 186.9 cases per 100,000 infants per year, while the hospitalisation rate ranged between 9.4 and 17.2 cases per 100,000 infants per year (Figure 1). Fluctuations observed in pertussis incidence rates were not reflected in the hospitalisation rates. Among infants less than four months old, disease incidence and hospitalisation rates ranged between 75.0 and 269.5, and 21.9 and 38.1 per 100,000 infants, respectively.

NNV estimates for a range of each outcome of disease, hospitalisation and death are provided in Tables 2, 3 and 4, respectively. In addition to the total number of parents that would need to be vaccinated (NNV_{total}), which takes into account the risk of infection by mothers and fathers combined, the number of women that would need to be vaccinated if the programme was targeted solely to mothers is also provided (NNV_{mother}),

Table 1

Summary of studies used to derive estimates of percentage of infection among infants less than one year of age

Country	Year	Study population	Source of infection			
			Mother		Father	
			Number	Percentage[a]	Number	Percentage[a]
Australia [6]	2009	Laboratory-confirmed outbreak cases < 12 months	13	14–20	8	8–12
Netherlands [8]	2006–2008	Laboratory-confirmed hospitalisations < 6 months	52	54	23	24
United States [10]	1999–2002	Notifications < 12 months	84	14–32	39	6–15
England[b]	1998–2000	Laboratory-confirmed hospitalisations < 5 months	7[c]	21–41	3	11–18

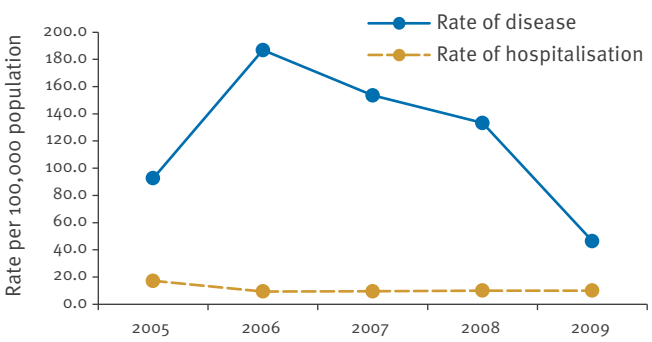
[a] Percentage range reflects percentage including unknowns to percentage excluding unknowns.
[b] Unpublished data.
[c] Includes one case whose source of infection was either the mother or father.

as some programmes have considered this strategy as mothers are often easier to reach. Because mothers are more frequently identified as the source of infection for an infant relative to the father (Table 1), estimates of NNV_{mother} were considerably smaller than estimates of NNV_{total}.

A range of estimates of pertussis incidence, hospitalisation and mortality rates among infants were assumed, and adjustments for under-reporting, as described in the methods, were made. As expected, the NNV to prevent a case of pertussis was less than that needed to prevent hospitalisation and death. For example, using the minimum inflated rates while assuming 85% VE and 14% and 6% of infants were infected by their mothers and fathers, respectively, the NNV_{total} estimate to prevent one pertussis case, hospitalisation and death was approximately 6,400, 63,000, and 12.8 million, respectively. For comparison, if VE was reduced to 80%, NNV_{total} estimates increased (6,800, 67,000, and 13.6 million respectively, data not shown), while increasing VE to 90% reduced NNV_{total} estimates (6,100, 60,000, and 12.1 million respectively, data not shown). If no adjustments were made for under-reporting, the NNV increased by the magnitude of the inflation factor. Conversely, as the mother- and father-specific estimates of risk increased, the NNV estimates decreased. These estimates of risk of infection were influenced by the inclusion or exclusion of cases with an unknown source of infection in the denominator. Excluding these cases inflated the mother- and father-specific risks, which resulted in a decrease in the corresponding NNV estimates.

Figure 1

Unadjusted rates of disease and hospitalisation due to pertussis among infants less than one year of age, Ontario, 2005–2009



Using the average inflated rates observed between 2005 and 2009, between 800 and 2,400 individuals would need to be vaccinated to prevent one case of pertussis; 18,000 and 53,000 individuals would need to be vaccinated to prevent one hospitalisation; 1.6 and 4.9 million individuals would need to be vaccinated to prevent a death. The estimates varied according to the proportion of infants infected by a mother or father assumed, and whether unknown sources of infection were included. Further limiting the analysis to prevent a case, hospitalisation or death in an infant less than four months old resulted in a reduction in NNV due to the increased frequency of outcomes in this younger age group (600–1,600, 7,000–21,000 and 1.1–3.3 million, respectively, data not shown).

Comparisons of the proportion of infants infected by parents in Table 1 yielded a ratio of 1.7–2.3 to 1 for mothers to fathers. Therefore, assuming a 2:1 ratio (i.e. infants are twice as likely to be infected by a mother than a father), Figure 2 illustrates the relationship

Table 2

Estimated number needed to vaccinate to prevent one pertussis case among infants less than one year of age, Ontario

Incidence rate per 100,000 population	Unknown sources included				Unknown sources excluded			
	Mother (%)[a]	Father (%)[a]	NNV_{total}[b]	NNV_{mother}[c]	Mother (%)[a]	Father (%)[a]	NNV_{total}[b]	NNV_{mother}[c]
Unadjusted rates								
46.4 (minimum)	14	6	60,345	18,104	20	12	33,793	12,672
	14	11	41,144		20	18	26,753	
	21	6	54,311	12,069	41	12	27,303	6,182
	21	11	35,110		41	18	20,262	
122.6 (average)	14	6	22,855	6,857	20	12	12,799	4,800
	14	11	15,583		20	18	10,133	
	21	6	20,570	4,571	41	12	10,341	2,341
	21	11	13,298		41	18	7,674	
186.9 (maximum)	14	6	14,986	4,496	20	12	8,392	3,147
	14	11	10,218		20	18	6,644	
	21	6	13,488	2,997	41	12	6,780	1,535
	21	11	8,719		41	18	5,032	
Inflated rates[d]								
436.3 (minimum)	14	6	6,420	1,926	20	12	3,595	1,348
	14	11	4,377		20	18	2,846	
	21	6	5,778	1,284	41	12	2,905	658
	21	11	3,735		41	18	2,156	
1,152.1 (average)	14	6	2,431	729	20	12	1,362	511
	14	11	1,658		20	18	1,078	
	21	6	2,188	486	41	12	1,100	249
	21	11	1,415		41	18	816	
1,757.0 (maximum)	14	6	1,594	478	20	12	893	335
	14	11	1,087		20	18	707	
	21	6	1,435	319	41	12	721	163
	21	11	928		41	18	535	

NNV: number needed to vaccinate.

[a] A range of the proportion of infants infected by mothers and fathers is provided based on estimates presented in Table 1.

[b] Total number needed to vaccinate, including mothers and fathers.

[c] Number needed to vaccinate, including mothers only.

[d] Inflated by factor of 9.4 to adjust for under-reporting.

between NNV_{total} and the proportion of infants infected by parents, for cases of disease, hospitalisations and deaths. This approach allows us to hypothesise about the overall parental risk, yet still account for mother- and father-specific estimates. Assuming 40% of infants are infected by parents and using the average inflated rate, the NNV_{total} estimates to prevent one case, hospitalisation and death due to pertussis is approximately 1,100, 25,000 and 2.3 million individuals, respectively.

Discussion

The practice of applying the concept of 'number needed to treat' for vaccine-preventable diseases [20–25] including pertussis [26,27] is not new. In the context of rabies, it was estimated that up to 2.7 million people would need to be vaccinated to prevent a single case of human rabies at associated costs of up to 2 billion CAD (1.3 billion EUR) [20]. The example of rabies illustrates how the context is important; the outcome of rabies infection is much more severe than pertussis with a CFR of 100%. In this analysis, we have demonstrated that NNV estimates for pertussis vary greatly depending on the frequency of the outcome including the target age group, the degree of under-reporting believed to be in existence, assumed VE and the estimated proportion of infants infected by the mother and father. In

Table 3

Estimated number needed to vaccinate to prevent one pertussis hospitalisation among infants less than one year of age, Ontario

Hospitalisation rate per 100,000 population	Unknown sources included				Unknown sources excluded			
	Mother (%)[a]	Father (%)[a]	NNV_{total}[b]	NNV_{mother}[c]	Mother (%)[a]	Father (%)[a]	NNV_{total}[b]	NNV_{mother}[c]
Unadjusted rates								
9.4 (minimum)	14	6	297,423	89,227	20	12	166,557	62,459
	14	11	202,788		20	18	131,858	
	21	6	267,681	59,485	41	12	134,566	30,468
	21	11	173,046		41	18	99,866	
11.2 (average)	14	6	249,771	74,931	20	12	139,872	52,452
	14	11	170,298		20	18	110,732	
	21	6	224,794	49,954	41	12	113,006	25,586
	21	11	145,321		41	18	83,866	
17.2 (maximum)	14	6	163,137	48,941	20	12	91,357	34,259
	14	11	111,230		20	18	72,324	
	21	6	146,824	32,627	41	12	73,810	16,712
	21	11	94,916		41	18	54,777	
Inflated rates[d]								
44.3 (minimum)	14	6	63,281	18,984	20	12	35,438	13,289
	14	11	43,146		20	18	28,055	
	21	6	56,953	12,656	41	12	28,631	6,482
	21	11	36,818		41	18	21,248	
52.7 (average)	14	6	53,143	15,943	20	12	29,760	11,160
	14	11	36,234		20	18	23,560	
	21	6	47,828	10,629	41	12	24,044	5,444
	21	11	30,919		41	18	17,844	
80.7 (maximum)	14	6	34,710	10,413	20	12	19,438	7,289
	14	11	23,666		20	18	15,388	
	21	6	31,239	6,942	41	12	15,704	3,556
	21	11	20,195		41	18	11,655	

NNV: number needed to vaccinate.

[a] A range of the proportion of infants infected by mothers and fathers is provided based on estimates presented in Table 1.
[b] Total number needed to vaccinate, including mothers and fathers.
[c] Number needed to vaccinate, including mothers only.
[d] Inflated by factor of 4.7 to adjust for under-reporting.

particular, due to the decreased frequency of infants whose source of infection was stated as the father, the inclusion of fathers resulted in a large increase in the NNV estimates. Although the concept is not new, there is no acceptable threshold for the NNV. It serves as an intuitive and simple measure that can be used to compare interventions in a limited way.

Regardless of the methodology or inflation factor used, our NNV analyses demonstrate that estimates vary greatly depending on the frequency of the outcome of interest. Therefore, the objectives of implementing a cocoon immunisation strategy must be carefully considered. If the objective of the programme is to prevent pertussis in the population in general, then a universal strategy should be considered. Otherwise, if the objective of the programme is to prevent deaths due to pertussis, a large number of adults would need to be vaccinated. Similarly, in order to prevent an infant case or infant hospitalisation due to pertussis, then regardless of the degree to which under-reporting is believed

Table 4

Estimated number needed to vaccinate to prevent one pertussis death among infants less than one year of age, Ontario

Hospitalisation rate per 100,000 population	Unknown sources included				Unknown sources excluded			
	Mother (%)[a]	Father (%)[a]	NNV_{total}[b]	NNV_{mother}[c]	Mother (%)[a]	Father (%)[a]	NNV_{total}[b]	NNV_{mother}[c]
Unadjusted rates								
0.093 (minimum)	14	6	30,172,592	9,051,778	20	12	16,896,652	6,336,244
	14	11	20,572,222		20	18	13,376,516	
	21	6	27,155,333	6,034,518	41	12	13,651,258	3,090,851
	21	11	17,554,963		41	18	10,131,122	
0.245 (average)	14	6	11,427,661	3,428,298	20	12	6,399,490	2,399,809
	14	11	7,791,587		20	18	5,066,263	
	21	6	10,284,895	2,285,532	41	12	5,170,320	1,170,638
	21	11	6,648,821		41	18	3,837,093	
0.374 (maximum)	14	6	7,493,214	2,247,964	20	12	4,196,200	1,573,575
	14	11	5,109,010		20	18	3,321,992	
	21	6	6,743,893	1,498,643	41	12	3,390,223	767,598
	21	11	4,359,688		41	18	2,516,014	
Inflated rates[d]								
0.218 (minimum)	14	6	12,839,401	3,851,820	20	12	7,190,065	2,696,274
	14	11	8,754,137		20	18	5,692,134	
	21	6	11,555,461	2,567,880	41	12	5,809,046	1,315,256
	21	11	7,470,197		41	18	4,311,116	
0.576 (average)	14	6	4,862,834	1,458,850	20	12	2,723,187	1,021,195
	14	11	3,315,569		20	18	2,155,857	
	21	6	4,376,551	972,567	41	12	2,200,136	498,144
	21	11	2,829,285		41	18	1,632,805	
0.878 (maximum)	14	6	3,188,602	956,581	20	12	1,785,617	669,606
	14	11	2,174,047		20	18	1,413,613	
	21	6	2,869,742	637,720	41	12	1,442,648	326,637
	21	11	1,855,187		41	18	1,070,644	

NNV: number needed to vaccinate.

[a] A range of the proportion of infants infected by mothers and fathers is provided based on estimates presented in Table 1.

[b] Total number needed to vaccinate, including mothers and fathers.

[c] Number needed to vaccinate, including mothers only.

[d] Inflated by factor of 2.35 to adjust for under-reporting.

to exist, up to 298,000 individuals would need to be vaccinated (this represents approximately 6.4% of the adult (20–44 years old) population in Ontario). Another Canadian study using different methodology also reported extremely high NNV to prevent deaths and serious outcomes, and also concluded that a parental cocoon programme was inefficient and resource intensive [27]. A similar conclusion was reached by authors of a study conducted in Italy, which also has a low incidence of disease [28]. It is important to note that our estimates were derived based on the epidemiology of pertussis in Ontario which has a well-established universal immunisation programme with relatively high coverage and low disease incidence. Other jurisdictions considering a cocoon programme should consider their local epidemiology.

Currently, data evaluating the effectiveness of a cocoon strategy are limited. Since the implementation of such a strategy in the US in 2006, data from

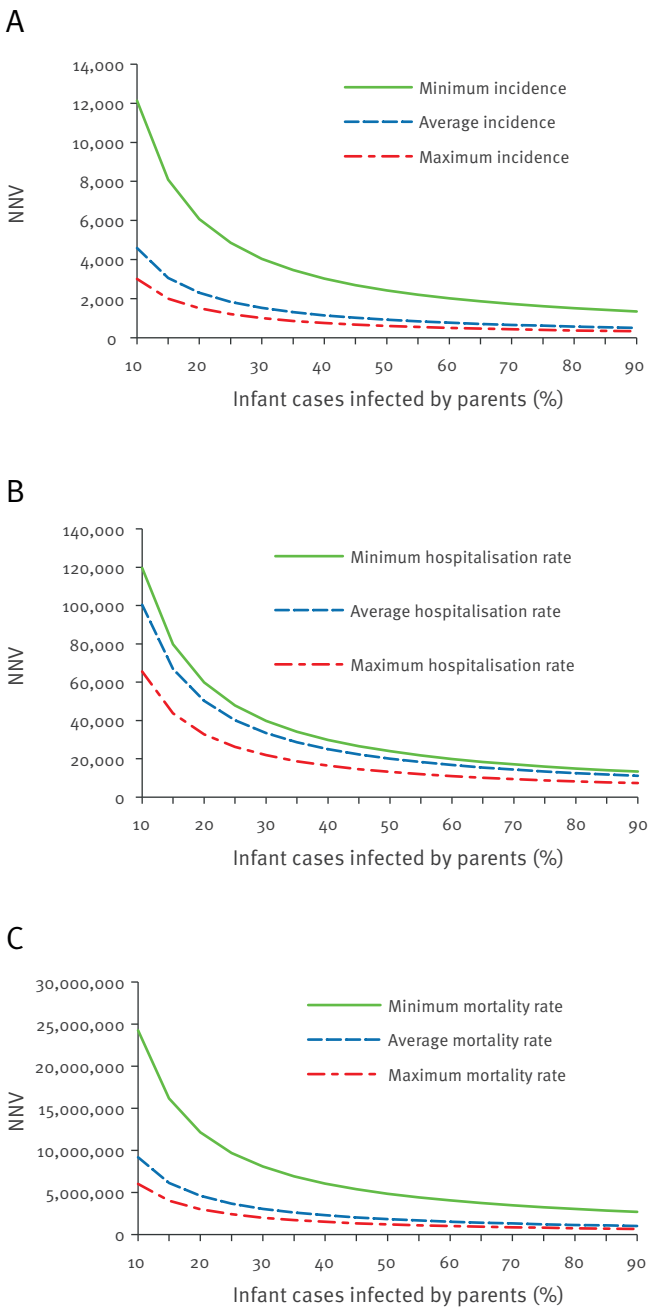
two small studies have been reported with conflicting results. One study documented a 50% decline in the incidence of pertussis in hospitals with a post-partum Tdap vaccination policy in 2006 (n=48), while a 20% increase was observed among hospitals that did not have such a policy (n=145) [29]. In contrast, Castagnini et al. [30] found no difference in the rates of illness, length of stay or mortality in infants under six months of age when post-partum women were vaccinated prior to discharge. The authors recommended that all household and key contacts of newborns should be immunised instead.

Additional factors that are important to consider with respect to the cocoon strategy include the feasibility of achieving satisfactory uptake using this approach, its cost-effectiveness, and impact on health equity. A cocoon strategy for mothers may offer benefits that accumulate through subsequent pregnancies depending on the duration of protection from the vaccine and may also result in greater uptake within this population due to the targeted nature of the programme and accessibility of the population. However the recent addition of an adult pertussis booster to the immunisation programme in Ontario has the added benefits of providing protection to other close contacts of infants, such as fathers, grandparents and other adult caregivers. There is also evidence that immunisation coverage of high-risk groups increase when vaccination programmes are universal rather than targeted [31,32]. A universal adult pertussis programme not only serves to decrease the overall risk of disease among infants (beyond that which might be achieved with a more focused cocoon strategy), but also to protect adults from the morbidity associated with the disease. Critical to the success of a universal programme is to ensure that adequate pertussis vaccine coverage is achieved. A comparison of various immunisation strategies suggests coverage of at least 40% within the adult population is required to achieve herd immunity [33]. Unfortunately in the absence of a comprehensive immunisation registry in Ontario, vaccine uptake since the implementation of the universal programme in 2011 is unknown. Routine adult immunisation has been observed to be more cost-effective than a cocoon programme targeting parents [34]. However compared to just an infant immunisation programme, Westra et al. from the Netherlands found that adding maternal immunisation or a cocooning programme for both parents was cost-effective and even cost-saving [35].

This analysis was limited by the sources of data that were available to estimate the overall incidence of disease, hospitalisation and deaths, including lack of age-specific denominator information by month for infants less than four months of age. Due to delays in reporting and under-reporting, the vital statistics database was not used to derive mortality estimates. However, for comparison, three deaths due to pertussis among infants less than one year old were reported in Canada using data from the Vital Statistics database [36].

Figure 2

Estimated number needed to vaccinate to prevent (A) one pertussis case, (B) one pertussis hospitalisation, (C) one pertussis death, in an infant less than one year of age, Ontario



NNV: number needed to vaccinate.

Adjustments for under-reporting were used and mothers were assumed to be twice as likely as fathers to infect the infant in this analysis.

Extrapolating this to the Ontario population would have resulted in an estimated CFR of 0.03%, whereas the unadjusted CFR estimate used in this analysis was 0.2%. Under-reporting of severe cases of pertussis and deaths has previously been reported [11,13]. Inflation factors were assumed to attempt to adjust for under-reporting, but true rates were unknown. Despite this, the provision of unadjusted rates in the sensitivity analyses provided a range of estimates for reference. In addition, estimates of the proportion of infants who were infected by the mother or father were derived from several studies using different methodologies. Although these estimates varied between studies, it was reassuring to observe that the relative proportion of infants infected by mothers versus fathers remained generally consistent at a ratio of 2:1.

Conclusion

This study demonstrates that NNV analyses can incorporate many assumptions and assist when considering implementation of a targeted or universal programme. The NNV estimates derived from this study suggest that a cocoon strategy may or may not be acceptable, depending on the objective of the pertussis vaccination programme. If the objective is to reduce morbidity in the general population, a universal programme might be the most efficient option available. In the current epidemiological situation where pertussis is increasing even in areas with high coverage, and where public health has easier access to parents than to the general adult population, cocooning may be the most feasible or even the only strategy we have to protect infants. What the NNV shows very clearly is the inefficiency inherent in any approach, with relatively large numbers of people to vaccinate in all scenarios. And finally, regardless of approach, a better vaccine is needed, with longer duration of protection that can protect the youngest infants more effectively, preferably through herd immunity.

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