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Influenza Virus Vaccines: Lessons from the 2009 H1N1 pandemic

Andrew J. Broadbent^a and Kanta Subbarao^{a,*}

^aLaboratory of Infectious Diseases, NIAID, NIH, Bethesda, MD 20892, USA

Abstract

Reflecting on the 2009 H1N1 pandemic, we summarize lessons regarding influenza vaccines that can be applied in the future. The two major challenges to vaccination during the 2009 H1N1 pandemic were timing and availability of vaccine. Vaccines were, however, well-tolerated and immunogenic, with inactivated vaccines containing 15µg of HA generally inducing antibody titers $\geq 1:40$ in adults within 2 weeks of the administration of a single dose. Moreover, the use of oil-in-water adjuvants in Europe permitted dose- reduction, with vaccines containing as little as 3.75 or 7.5µg HA being immunogenic. Case-control studies demonstrated that monovalent 2009 H1N1 vaccines were effective in preventing infection with the 2009 H1N1 virus, but preliminary data suggests that it is important for individuals to be re-immunized annually.

Keywords

2009 H1N1; pandemic; influenza; vaccines

Introduction

Influenza viruses are enveloped RNA viruses belonging to the *Orthomyxoviridae* family. There are three types of influenza viruses that cause disease in people: A, B and C. Type A viruses infect a wide range of hosts and pose the most significant public health risk. They are further subtyped based on the antigenicity of the hemagglutinin (HA) and neuraminidase (NA) surface proteins. Sixteen HA and nine NA subtypes have been identified in the natural hosts, waterfowl (order *Anseriformes*) and shorebirds (order *Charadriiformes*) and several subtypes have transmitted to domestic poultry and mammalian species including pigs, horses, and humans [1,2].

The genome is comprised of 8 segments of negative-stranded RNA that encode at least 11 proteins. Reassortment of gene segments between influenza viruses is a frequent occurrence in nature. This may lead to the emergence of a novel subtype of influenza capable of infecting humans. There have been three influenza pandemics in the 20th century. The first, caused by an H1N1 virus, occurred in 1918. The virus was maintained in humans until reassortment events led to the emergence of an H2N2 pandemic virus in 1957, which circulated until it was replaced by an H3N2 virus in 1968 [1,2]. In 1977, H1N1 viruses re-

*Corresponding Author: Kanta Subbarao, MD, MPH, Chief, Emerging Respiratory Viruses Section, Laboratory of Infectious Diseases, NIAID, NIH, Bldg 33, Room 3E13C.1, 33 North Drive, MSC 3203, Bethesda, MD 20892-3203, Phone: +1 301-451-3839, Fax: +1 301-480-4749, ksubbarao@niaid.nih.gov.

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emerged in humans and co-circulated with H3N2 viruses, causing seasonal influenza epidemics until 2009, when a new H1N1 virus emerged (2009 H1N1) (reviewed by [3]). In addition to these pandemics, people have also been infected with H5, H7 and H9 viruses. Since 2003, almost 500 cases of human infection with highly pathogenic avian influenza (HPAI) H5N1 viruses have been reported, mostly due to direct transmission from poultry to humans. Although there has not yet been sustained transmission from person to person, there is concern that continued transmission from birds to humans may increase the probability of an H5N1 virus emerging with the capacity to transmit efficiently.

Vaccination remains the primary strategy for the prevention and control of influenza [4,5] and both inactivated and live attenuated vaccines are licensed. Vaccine production begins with the generation of vaccine reference strains, reassortant viruses with the HA and NA derived from the vaccine target, combined with internal protein genes from a strain which, for inactivated vaccines, specifies high yield in eggs (A/Puerto Rico/8/34; PR8) [6] and, for live attenuated influenza vaccines (LAIV), confers temperature sensitivity, cold-adaptation (ca) and attenuation that limits viral replication to the cooler, upper respiratory tract (A/Ann Arbor/6/60 ca; A/Leningrad/47/57 ca) [7].

We are now in the post-pandemic period following the 2009 H1N1 virus outbreaks. Reflecting on the pandemic, this review summarizes lessons regarding influenza vaccines that can be applied in the future.

The 2009 H1N1 Pandemic

The 2009 pandemic H1N1 virus first emerged in Mexico and California in April and quickly spread globally. On June 11, 2009, a pandemic was declared by the World Health Organization [8]. The virus was antigenically unrelated to previously circulating seasonal H1N1 viruses, and molecular studies revealed that it had gene segments from several viruses that were circulating in pigs: the North American H3N2 triple-reassortant and the Eurasian 'avian-like' swine H1N1 viruses [9–11].

The majority of cases occurred in younger age-groups, with the median age estimated to be 12–17 years in Canada, USA, Chile, Japan and the UK [3]. In most individuals, infection led to a mild, self-limiting, upper respiratory tract illness, but 2–5% of cases required hospitalization [12] and pregnant women were at a higher risk for severe disease [13–15]. The overall case-fatality rate was 0.15–0.25%, and deaths mostly occurred in middle-aged adults (median age 40–50 years) [3]. Deaths amongst hospitalized patients mostly occurred in those with underlying health conditions, but nearly one third who died were previously healthy [3].

National authorities identified the following high-priority groups for vaccination: health care workers, pregnant women, young children, and individuals with underlying cardiovascular or respiratory medical conditions, autoimmune disorders and diabetes. Production of seasonal influenza vaccine for the 2009–2010 season was, however, already under way (reviewed in [16]) and the decision was made to produce a separate monovalent pandemic H1N1 vaccine.

A major challenge to pandemic vaccine production was timing. In spite of a rapid response, vaccines were neither available for widespread use during the 2009 winter season in the Southern Hemisphere [3], nor until after the autumn/winter wave had nearly peaked in the Northern Hemisphere [17] (Figure 1). Another challenge was availability. As of June 2009, the global capacity for seasonal vaccine production was only 876 million doses a year, with 7 manufacturers providing 64% of this capacity [3]. In addition, pandemic vaccine virus yields in eggs and cell culture were lower than expected (reviewed by [16,18]), the optimal

quantity of HA per dose was unknown, nor was it clear whether adjuvants should be utilized for dose sparing, as had been demonstrated for H5N1 vaccines (reviewed in [19]). It was also assumed that two doses of vaccine would be needed to induce protection, as clinical trials conducted in 1977 indicated that two doses of vaccine were needed to successfully immunize naive individuals against a novel virus [20–24].

Since then, new manufactures have emerged in China, India, Thailand and South America and there are now 26 manufacturers making pandemic 2009 H1N1 vaccines [3] using various platforms in many countries, but challenges remain for vaccinating people living in under-resourced countries that lack the infrastructure for mass vaccination.

Immunogenicity

All registered pandemic H1N1 vaccines were evaluated for safety and immunogenicity in clinical trials and met criteria required for licensure of seasonal influenza vaccines by regulatory authorities, including the Committee for Medicinal Products for Human Use (CHMP) in Europe and the Food and Drug Administration (FDA) in the United States [25]. This includes an assessment of the proportion of participants with either seroconversion or significant increase in antibody titer, the fold-increase in geometric mean antibody titer (GMT) and the proportion of participants with antibody titers of 1:40 or greater, which is associated with a 50% reduction in the risk of illness in a susceptible adult population [26]. Typically hemagglutination inhibition (HAI) antibody titers are determined as a surrogate for neutralizing antibodies.

The results of monovalent 2009 H1N1 vaccine trials are summarized in Table 1. A single dose of unadjuvanted inactivated split-virion 2009 H1N1 vaccine containing 15µg HA led to antibody titers $\geq 1:40$ in 95–98% of healthy adults [27–30]. In children, one study found that 92.5% of 6 month–9 year olds had antibody titers $\geq 1:40$ after a single dose of 15µg HA in an inactivated split-virion unadjuvanted 2009 H1N1 monovalent vaccine [31], however another study found that only 47–50% of 6–35 month-olds and 65–75% of 3–9 year olds achieved these titers [32]. After two doses, however, 90–100% children had titers $\geq 1:40$ in both studies [31,32] (Table 1). Therefore, a single dose of monovalent 2009 H1N1 vaccine was recommended in adults, but young children were recommended to receive 2 doses (reviewed by [3]). It is likely that a single dose was sufficient to induce immunity in adults because prior exposure to seasonal H1N1 viruses had immunologically primed the population. This hypothesis is supported by studies in a mouse model of infection, where prior infection with seasonal influenza virus or seasonal LAIV primed mice for a robust response to a single dose of pandemic LAIV while unprimed mice were only partially protected against 2009 H1N1 infection with a single dose of pandemic LAIV [33].

The use of oil-in-water adjuvants, MF59 (Focetria, Novartis Vaccines, Italy) and AS03 (GlaxoSmithKline (GSK) Biologicals, Dresden, Germany) in 2009 H1N1 vaccines was approved by the European Medicines Agency (EMA). The adjuvants had previously been approved in Europe for use in seasonal influenza vaccines and adjuvanted inactivated H5N1 vaccines had enabled dose sparing and induced broad cross-clade humoral responses [34,35]. Their mechanism of action remains incompletely understood, however they may amplify immune responses by enhancing antigen presentation and recruiting inflammatory cells to the area of antigen deposition (reviewed by [36]).

Adjuvanted monovalent 2009 H1N1 vaccines permitted dose sparing (Table 1). Antibody titers $\geq 1:40$ were induced in 92% of adults given a single dose of MF59-adjuvanted vaccine [37] and 94–96% given the AS03-adjuvanted vaccine each containing only 3.75µg HA [38,39]. In children, antibody titers $\geq 1:40$ were induced in 100% of 6–35 month olds given 2 doses of 7.5µg or 3.75µg HA [40,41] and 99.3% of 6 month–13 year olds given 1.875µg HA

in adjuvanted vaccine [42]. Moreover, in the UK, adjuvanted split-virion vaccines induced seroconversion and antibody titers $\geq 1:40$ in a larger percentage of adults and children than non-adjuvanted whole virion vaccines [38,42]. Adjuvanted influenza vaccines are, however, not licensed in the USA [43]. Fortunately, adjuvants were not necessary to achieve adequate immunity to the 2009 H1N1 virus, though they may prove to be important in the future.

Live attenuated influenza vaccine (LAIV) viruses were also licensed for use against the 2009 H1N1 virus, however, it was necessary to introduce point mutations in the HA to optimize the yield in eggs [44]. Intranasal vaccination with 2 doses of 10^7 fluorescent focus units of virus, 28 days apart, led to antibody titers $\geq 1:32$ in 26.4% children 2–17 years old and 13.5% of adults 18–49 years old (Table 1) [45]. While these responses were modest, HAI titers are not predictive of protective efficacy of LAIV and vaccine efficacy is seen in the absence of seroconversion [46,47]. Determining meaningful immune correlates of protection for LAIV is an active area of research.

Safety

The safety of the monovalent 2009 H1N1 vaccines was assessed in clinical trials (Table 1). All vaccines were well-tolerated and elicited only mild or moderate transient side-effects, such as local pain, swelling and redness at the injection site, temporary fever, headache, fatigue or myalgia. Moreover, in the USA, data from the Vaccine Adverse Event Reporting System (VAERS) indicated that the adverse event profile was consistent with that of seasonal influenza vaccines and Guillian-Barre syndrome, anaphylaxis and death were rare [48]. However, there are reports of narcolepsy being a rare adverse event following vaccination with an AS03-adjuvanted 2009 H1N1 influenza vaccine, Pandemrix [49–51]. At the time of writing, this is under investigation [52].

In the 2010–2011 influenza season, trivalent vaccines incorporated an A/California/07/2009 H1N1-like virus, an A/Perth/16/2009 (H3N2)-like virus and a B/Brisbane/60/2008-like virus. While the majority of these vaccines are well tolerated, in Australia, an increased risk of febrile convulsions following immunization with an inactivated, split-virion, unadjuvanted vaccine, Fluvax, led to the suspension of seasonal influenza immunization of healthy children in April 2010. The cause of this association remains unknown, and an investigation is underway. A similar vaccine was utilized in the UK, until the Department of Health advised against its use in younger children and enhanced surveillance for febrile convulsions. Other vaccine products were, however, not associated with an increased risk of febrile convulsions [53].

Effectiveness

Robust immunogenicity does not always result in greater vaccine effectiveness (VE) [54]. This may be, in part, because the 1:40 HAI titer used to measure immunogenicity is extrapolated to all influenza viruses. It is, therefore, important to estimate the effectiveness of vaccines at the population level. Published effectiveness data from 2009–2010 is available from studies conducted in Europe, UK, Canada, China and Korea (Table 2), with VEs ranging from 66.0% to 93.0% [17,54–57]. These studies demonstrate that the monovalent 2009 H1N1 vaccines were effective in preventing infection with 2009 H1N1 virus, in contrast to the 2009–2010 trivalent vaccines, which did not contain the pandemic H1N1 vaccine antigen and showed no evidence of effectiveness against 2009 H1N1 infection, with an adjusted VE of only 9.9% (95% CI, –65.2–50.9%) ([17,54–57]).

Vaccine efficacy studies for the 2010/2011 trivalent influenza vaccines based on mid-season data analysis (Table 3) show evidence of some protection against confirmed 2009 H1N1, however the VEs are lower than the monovalent vaccines administered in 2009/10 (44.1 to

52%) [58–60]. One study also reported that if individuals were vaccinated with monovalent vaccine in the 2009/2010 season, but not with seasonal vaccine in 2010/2011, the VE against current circulating H1N1 viruses was 34%, compared to 46% if individuals were only vaccinated in the 2010/11 season, and 63% if individuals were vaccinated with both monovalent vaccine in the 2009/2010 and the trivalent vaccine in 2010/11 [59]. This suggests that the longevity of protection is poor and that repeat vaccination boosted protective immune responses, reinforcing the recommendation for annual re-immunization.

Concluding remarks

The two major challenges to vaccination during the 2009 H1N1 pandemic were timing and availability of vaccine. The vaccines were well tolerated and surprisingly immunogenic, with inactivated vaccines containing 15µg of HA generally inducing antibody titers $\geq 1:40$ in adults within 2 weeks of the administration of a single dose. This suggests that exposure to H1N1 viruses ‘primed’ the majority of the population, such that only one dose of 2009 H1N1 vaccine was needed for protection. Moreover, the use of an oil-in-water adjuvant permitted dose-reduction, with vaccines containing as little as 3.75 or 7.5µg HA being immunogenic. These factors enabled more vaccine doses to be distributed than was first expected. In future pandemics, we may not be so lucky, and priorities have been set for accelerating vaccine production, including a shift towards cell-based vaccines (Reviewed by [16]). Additionally, the use of adjuvanted vaccines may become more widespread in the future. Case-control studies demonstrated that monovalent 2009 H1N1 vaccines were effective in preventing infection with the 2009 H1N1 virus, but preliminary data suggests that it is important for individuals to be re-immunized annually.

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Highlights

- We summarize lessons regarding 2009 H1N1 influenza vaccines
- 1 dose of inactivated vaccine (15µg HA) was well tolerated & immunogenic in adults
- Oil-in-water adjuvanted vaccines licensed in Europe permitted dose- sparing
- 2009 H1N1 monovalent vaccines were effective in preventing 2009 H1N1 virus infection
- Vaccination with 2009 H1N1 and 2010/11 vaccines boosted protective immunity

Figure 1.

The number of cases in the 2009 H1N1 pandemic in Canada with respect to availability of vaccine (adapted from Skowronski *et al.*, 2011)

The number of cases in the 2009 H1N1 pandemic peaked at week 43, coinciding with the first vaccine availability

Immunogenicity of monovalent 2009 H1N1 Vaccines

Table 1

Type of Vaccine	Country	Dose of HA (µg)	Subject age	n	Following 1 dose			Following 2 doses			Reference
					% with HAI antibody titers ≥1:40 (95% CI)	% sero-conversion (95% CI)	GMT ^a ratio (95% CI)	% with HAI antibody titers ≥1:40 (95% CI)	% sero-conversion (95% CI)	GMT ratio (95% CI)	
Inactivated, split-virus, unadjuvanted	Australia	15	18–64 years	240	95.0 (89.4–98.1)	74.2 (65.4–81.7)	11.8 (8.9–15.7)	98.3 (94.0–99.8)	82.1 (73.9–88.5)	16.0 (12.4–20.7)	Greenberg <i>et al.</i> , 2009
Inactivated, split-virus, non-adjuvanted	Australia	15	6 months–9 years	370	92.5 (87.6–95.6)	86.8 (80.9–91.0)	13.6 (11.8–15.6)	100 (97.7–100)	97.5 (93.7–99.0)	44.6 (36.3–54.8)	Nolan <i>et al.</i> , 2010
Inactivated, split-virus, unadjuvanted	USA	7.5	18–64 years	141	95 (90–98)	92 (86–96)	38.9 (30.4–49.7)	No data	No data	No data	Plennevaux <i>et al.</i> , 2010
		15	18–64 years	145	98 (94–100)	96 (91–99)	64.3 (50.9–81.2)	No data	No data	No data	
	USA	7.5	6–35 months	92	47 (36–57)	46 (35–56)	3.33 (2.54–4.35)	91 (84–96)	90 (82–95)	32.2 (24.1–43.1)	Plennevaux <i>et al.</i> , 2011
			3–9 years	98	65 (55–75)	63 (53–73)	6.82 (5.09–9.15)	97 (91–99)	96 (90–99)	40.3 (30.5–53.2)	
Inactivated, split-virus, unadjuvanted	USA	15	6–35 months	87	48 (40–61)	48 (37–59)	4.45 (3.42–5.81)	99 (94–100)	99 (94–100)	54.6 (42.3–70.4)	
			3–9 years	91	75 (65–83)	75 (65–83)	9.86 (7.46–13.0)	99 (94–100)	99 94–100)	55.0 (42.7–70.7)	Zhu <i>et al.</i> , 2009
			3–11 years	110	74.5 (65.1–82.5)	No data	64.1/5.8	82.7 (74.0–89.4)	No data	11.4 [†]	
Inactivated, split-virus, unadjuvanted	China	15	12–17 years	110	97.1 (91.9–99.4)	No data	430.7/7.3	97.0 (91.6–99.4)	No data	93.2 [‡]	
			18–60 years	110	97.1 (91.9–99.4)	No data	237.8/6.9	92.6 (85.9–96.8)	No data	53.8 [‡]	
			61+ years	110	79.1 (70.0–86.4)	No data	122.1/6.3	84.1 (75.8–90.5)	No data	28.3 [‡]	Liang <i>et al.</i> , 2010
Inactivated, split-virus, unadjuvanted	China	7.5	3–<12 years	232	76.7 (70.7–82.0)	No data	No data	97.7 (94.8–99.3)	No data	No data	
			12–<18 years	218	96.8 (93.5–98.7)	No data	No data	100 (98.1–100)	No data	No data	
			18–<60 years	323	89.5 (85.6–92.6)	No data	No data	98.4 (96.3–99.5)	No data	No data	
			60+ years	147	80.3 (72.9–86.4)	No data	No data	88.3 (81.7–93.2)	No data	No data	
		15	3–12 years	113	85.1 (78.5–92.,3)	No data	No data	98.4 (97.3–99.1)	No data	No data	
			12–18 years	1091	97.3 (96.1–98.1)	No data	No data	100 (99.6–100)	No data	No data	
			18–60 years	1280	94.3 (92.9–95.5)	No data	No data	97.2 (96.1–98.1)	No data	No data	
			60+ years	842	84.4 (81.8–86.8)	No data	No data	96.6 (95.0–97.8)	No data	No data	
Inactivated, split-virus, MF59-adjuvanted (Novartis)	Italy	7.5	6–23 months (pre-terms ranging from <32 to 42 weeks)	105	94.1–100	94.1–100	9–39.7	100	100	13.6–88.9	Esposito <i>et al.</i> , 2011
Inactivated, surface antigen, MF59- adjuvanted	UK	3.75	20–48 years	25	92 (74–99)	88 (69–98)	29.7 (14.4–61.2)	100 (86–100)	92 (74–99)	45.6 (26.9–77.5)	Clark <i>et al.</i> , 2009

Type of Vaccine	Country	Dose of HA (µg)	Subject age	n	Following 1 dose			Following 2 doses			Reference
					% with HAI antibody titers ≥1:40 (95% CI)	% sero- conversion (95% CI)	GMT** ratio (95% CI)	% with HAI antibody titers ≥1:40 (95% CI)	% sero- conversion (95% CI)	GMT ratio (95% CI)	
Inactivated, surface antigen, non-adjuvanted		7.5	18–50 years	26	77 (56–91)	73 (52–88)	25.9 (11.5–58.9)	92 (73–99)	92 (73–99)	53.0 (29.5–95.5)	
		7.5	23–49 years	25	72 (51–88)	72 (51–88)	18.9 (8.8–40.4)	79 (58–93)	79 (58–93)	22.9 (12.7–41.2)	
		15	24–49 years	25	63 (41–81)	52 (31–73)	13.5 (5.6–32.7)	74 (52–90)	74 (52–90)	27.4 (12.2–61.9)	
Inactivated, split-virus, AS03 _B [#] -adjuvanted	UK	1.875	6 months–13 years	435	No data	No data	No data	99.3 [*] (97.9–99.8)	98.7 [*] (97.1–99.6)	89.5 (81.9–97.8)	Waddington <i>et al.</i> , 2010
Whole virion, inactivated, non-adjuvanted		7.5	6 months–13 years	456	No data	No data	No data	88.5 (85.1–91.3)	88.4 (73.8–85.5)	15.0 (13.2–17.2)	
Inactivated, split-virus, AS03 _A [#] -adjuvanted		3.75	18–44 years	70	94 (85–98)	88 (78–95)	60.1 (39.6–91.3)	100 (94–100)	95 (87–99)	94.1 (66.3–133.5)	Nicholson <i>et al.</i> , 2011
			45–64 years	68	77 (65–87)	74 (62–84)	20.2 (13.3–30.5)	91 (81–97)	88 (77–95)	32.3 (22.8–45.8)	
			≥65 years	37	78 (71–84)	75 (68–91)	25.3 (19.1–33.6)	91 (86–95)	87 (81–92)	36.2 (28.5–46.0)	
Whole virion, inactivated, unadjuvanted	UK	7.5	18–44 years	70	51 (43–59)	46 (38–53)	8.3 (6.3–11.0)	54 (46–62)	49 (42–57)	8.2 (6.2–10.8)	
			45–64 years	68	71 (59–82)	36 (25–49)	4.6 (2.9–7.3)	44 (32–57)	41 (29–54)	5.9 (3.9–9.0)	
			≥65 years	34	32 (17–51)	29 (15–48)	4.1 (2.5–6.7)	36 (20–55)	33 (18–52)	3.6 (2.2–6.0)	
Inactivated, split-virus, AS03 _A [#] -adjuvanted	Belgium	3.75	18–60 years	116	96 (86.3–995)	94 (83.5–98.7)	45.3 (30.3–67.7)	94 (83.5–98.7)	92.0 (80.8–97.8)	38.6 (26.0–57.5)	Roman <i>et al.</i> , 2010
			60+ years	116	85.7 (72.8–94.1)	77.6 (63.4–88.2)	13.3 (9.0–19.6)	98.5 (92.0–100)	94.0 (85.4–98.3)	33.6 (24.9–45.3)	
Inactivated, split virus, AS03 _A [#] adjuvanted	Spain	3.75	6–35 months	53	100 (92.6–100)	97.9 (88.7–99.9)	46.3 (35.4–60.5)	100 (92.9–100)	100 (92.6–100)	323 (231–449)	Carmona <i>et al.</i> , 2010
Inactivated, split virus, AS03 _B [#] adjuvanted		1.9	6–35 months	104	100 (96.4–100)	99.0 (94.6–100)	54.5 (46.4–64.0)	100 (96.3–100)	100 (96.3–100)	347 (288–418)	
Inactivated, whole-virion, alum-adjuvanted	Hungary	6	18–60 years	103	No data	74.3 (64.6–82.4)	9.1 (7.7–10.7)	No data	No data	No data	Vajo <i>et al.</i> , 2010
			60+ years	75	No data	61.3 (49.4–72.4)	6.3 (5.2–7.6)	No data	No data	No data	
Live attenuated 2009 H1N1, non-adjuvanted	USA	10 ^{7.5} FFU of live virus per 0.5ml	2–17 years	261	No data	11.1	3.53/2.81	No data (26.4% had antibody titers ≥1:32)	32.0	2.71 [†]	Mallory <i>et al.</i> , 2010
			18–49 years	240	No data	6.1	3.44/3.00	No data (13.5% had antibody titers ≥1:32)	14.9	1.62 [†]	

** GMT: geometric mean titer

* antibody titer determined by microneutralization

[#] AS03_A contains 11.86mg tocopherol oil-in-water adjuvant; AS03_B contains 5.93mg tocopherol

[†] calculated using figures from original reference

Table 2
Effectiveness of monovalent 2009 H1N1 vaccines against confirmed 2009 H1N1 virus infection

Type of Vaccine	Country	Time of analysis	Number of ILI patients	Adjusted Vaccine Effectiveness (95% CI)	Reference
Inactivated, unadjuvanted, 15µg HA; Inactivated, MF59-adjuvanted, 3.75µg HA	Korea	December 2009–March 2010	416	73.4 (49.1–86.1)	Song <i>et al.</i> , 2011
Inactivated, split virion AS03 adjuvanted, 3.75µg HA; whole virion, non-adjuvanted, 7.5µg HA	UK	November 2009–January 2010	5,808	72.0 (21–90)	Hardelid <i>et al.</i> , 2011
Inactivated, AS03-adjuvanted, 3.75µg HA	Canada	8 November – 5 December 2009	552	93.0 (69–98)	Skowronski <i>et al.</i> , 2011
Inactivated, split-virion, non-adjuvanted, 15µg HA	China	September 2009–January 2010	95,244	87.3 (75.4–93.4)	Wu <i>et al.</i> , 2010
Unspecified	France, Hungary, Ireland, Italy, Romania, Portugal, Spain	November 2009–February 2010	2,902	66.0 (69.9–93.2)	Valenciano <i>et al.</i> , 2011

Table 3
Effectiveness of 2010/11 trivalent inactivated vaccines against confirmed 2009 H1N1 virus infection

Country	Time of analysis	Number of ILI patients	Adjusted VE in preventing 2009 H1N1 (95% CI)	Reference
UK	September 2010–January 2011	3,480	46 (7–69)	Pebody <i>et al.</i> , 2011
Spain	12 December 2010–12 February 2011	1,061	52 (6–75)	Savulescu <i>et al.</i> , 2011
France, Hungary, Ireland, Italy, Romania, Poland, Portugal, Spain	November 2010–January 2011	1,671	44.1 (14.3–72.7)	Kissling <i>et al.</i> , 2011