Immunogenicity and safety of pandemic influenza A (H1N1) 2009 vaccine: systematic review and meta-analysis

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The emergence of the 2009 H1N1 pandemic has highlighted the need to have immunogenicity and safety data on the new pandemic vaccines. There is already considerable heterogeneity in the types of vaccine available and of study performed around the world. A systematic review and meta-analysis is needed to assess the immunogenicity and safety of pandemic influenza A (H1N1) 2009 vaccines. We searched Medline, EMBASE, the Cochrane Library and other online databases up to 1st October 2010 for studies in any language comparing different pandemic H1N1 vaccines, with or without placebo, in healthy populations aged at least 6 months. The primary outcome was seroprotection according to haemagglutination inhibition (HI). Safety outcomes were adverse events. Meta-analysis was performed for the primary outcome. We identified 18 articles, 1 only on safety and 17 on immunogenicity, although 1 was a duplicate. We included 16 articles in the meta-analysis, covering 17 921 subjects. Adequate seroprotection ($\geq$70%) was almost invariably achieved in all age groups, and even after one dose and at low antigen content (except in children under 3 years receiving one dose of non-adjuvanted vaccine). Non-adjuvanted vaccine from international companies and adjuvanted vaccines containing oil in water emulsion (e.g. AS03, MF59), rather than aluminium, performed better. Two serious vaccination-associated adverse events were reported, both of which resolved fully. No death or case of Guillain–Barré syndrome was reported. The pandemic influenza (H1N1) 2009 vaccine, with or without adjuvant, appears generally to be seroprotective after just one dose and safe among healthy populations aged $\geq$36 months; very young children (6–35 months) may need to receive two doses of non-adjuvanted vaccine or one dose of $AS03_{A/B}$-adjuvanted product to achieve seroprotection.

Keywords Immunogenicity, meta-analysis, pandemic influenza, systematic review, vaccine safety, vaccines.

Introduction

In June 2009, the WHO declared the first influenza pandemic of the 21st century.[1] To combat this, pandemic vaccines have been developed and first became commercially available in September 2009. Data on the effectiveness of these vaccines are very limited; however, several studies have evaluated immunogenicity (especially seroprotection) and safety of the vaccine.

The immunogenicity and safety of various formulations of novel H1N1 vaccine have been assessed in various populations and age groups comparing different dosages (1·875–30 μg antigen per dose; single or two doses) with or without adjuvants (e.g. $AS03_A$, $AS03_B$, MF59, alum). Now that over a dozen trials have been reported, there is scope for a meaningful systemic review and meta-analysis to assess the immunogenicity (in particular, seroprotection) and safety of the 2009 influenza A (H1N1) vaccine formulations. This will inform decision-making about the most appropriate dose and formulation of pandemic vaccines. Given possible restrictions on antigen availability, the best compromise between the lowest possible dose and protective immunity is an important consideration.

Methods

Literature search and selection criteria
We searched MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials (CENTRAL) and other clinical trial registries (ClinicalTrial.gov, WHO, and international

Standard Randomised Controlled Trial Number [ISRCTN] registry) using the following search terms: 'influenza', 'flu', 'vaccine* or vaccination', 'immunisation', 'pandemic H1N1', 'H1N1 2009 influenza' and 'swine flu' in all fields with no language restriction for studies published between 1 June 2009 and 1 October 2010. We also checked the references of all relevant articles, including reviews, to identify additional studies. Furthermore, we did a hand-search among leading journals, including New England Journal of Medicine (NEJM), The Lancet, The Lancet Infectious Disease, The Journal of the American Medical Association (JAMA), British Medical Journal (BMJ) and Vaccine.

We included clinical trials measuring serological response to, or adverse events from, pandemic (H1N1) 2009 vaccine at different doses, with or without adjuvant and with or without a control arm, given to healthy people from different age groups.

Outcome measurement

Immunogenicity
We assessed the immunogenicity of pandemic (H1N1) 2009 vaccine using seroprotection (proportion of individuals with post-vaccination HI titre ≥1:40) measured by HI assay. Regulatory agencies require at least 70% of the population aged <65 years to achieve this for a vaccine to be approved.[2–4] The existence of a strong relationship between the HI titre and clinical protection against influenza is recognised, so we assumed that HI titre can be used as a proxy for the protection of inactivated influenza vaccines before vaccine effectiveness data become available from clinical studies.[5,6] We reported the pre-vaccination antibody level for each age group. We do not report microneutralisation data here; few studies[7–9] included it and the results do not materially affect our conclusions. We also compared the seroprotection of non-adjuvanted with adjuvanted vaccines (specifically by comparing vaccines produced by the same company) and expressed the results as risk ratios for the proportion achieving seroprotection in those given non-adjuvanted versus adjuvanted vaccine; this means that a risk ratio significantly >1 indicates that the non-adjuvanted vaccine is superior.

Adverse events
The common adverse events of influenza vaccination can be classified into systemic events, including fever, headache, muscle aches, malaise and local events (injection site), which may include pain, redness and tenderness.

As different studies may use different definitions for adverse events, we adopted easy to understand or commonly used definitions. We also looked for serious adverse events, such as death, hospitalisation, Guillain–Barre syndrome, disability and life-threatening events.

Validity assessment and data extraction

For each study, data extraction and validity assessment were performed by two reviewers (authors: JY, GK) independently. Data extracted included study design, study location, age group of participants, vaccine type, whether adjuvants were used or not, types of adjuvant, dose administered, immunogenicity and adverse events. Any disagreement was solved by discussion or by consulting with the review supervisor (RB). We used the Jadad scoring system to examine the methodology (randomisation, blinding and withdrawals).[10]

Statistical analysis

We used Meta-Analyst 3·13 (Tufts Medical Centre) for meta-analysis.[11] For the point estimate of immunogenicity and 95% confidence intervals (CI), we chose binary analysis with Random (D/L) as the model type and Der-Simonian Laird as the random method. We also used risk ratios to compare the immunogenicity of related (same company) adjuvanted and non-adjuvanted vaccines. Heterogeneity between studies, defined as variation among the results of individual studies beyond that expected from chance, was tested with I^2 statistic for each overall estimate using the same software. Heterogeneity is considered to be mild if $I^2 < 30\%$ and moderate when $I^2 = 30–50\%$; while notable heterogeneity may account for >50% of the variability in point estimates.[12,13] We did heterogeneity tests for each overall estimate of forest plot, which include (i) pre-vaccination antibody level, (ii) seroprotection after 1st and 2nd dose of non-adjuvanted and (alum/AS03$_A$/AS03$_B$) adjuvanted vaccine and (iii) risk ratio of head-to-head comparison (non-adjuvanted versus aluminium hydroxide-adjuvanted vaccine at the same dose). Each overall estimate generated from forest plot was reported in a format of 'weighted estimated, 95% CI, I^2, P value of heterogeneity test'.

Results

Of the 322 papers initially retrieved (Figure S1), 16 studies[7–9,14–26] assessed both immunogenicity and safety, while one other[27] was a review of safety reports from Vaccine Adverse Event Reporting System (VAERS) and Vaccine Safety Datalink (VSD), and another study[28] just reported immune response without safety data. We contacted the authors and found that all the data from one Chinese trial[26] published in NEJM were also reported as part of another larger Chinese study[17] published in The Lancet. Therefore, we included the data only once in our analysis.

A total of 17 921 subjects were involved. Fourteen of 17 studies included (Table S1) were randomised trials. Four studies used placebo as a control.[17,21,25,26] One study compared pandemic vaccine with pandemic and seasonal

influenza vaccine receipt, so we extracted data only from the arm using pandemic vaccine[24]; no others had a control vaccine. One trial[7] only used seed virus grown in cell culture, 15 used egg-based vaccines and the other one[9] used both. They all determined immune responses 21 days after the first and/or second doses, respectively. Seven of these 17 studies examined both adjuvanted and non-adjuvanted vaccines, three assessed only an adjuvanted vaccine and the other seven evaluated only non-adjuvanted vaccines.[19,21] Three studies each were from mainland China and Taiwan, and two studies each were from Australia and the UK, with one each from the USA, Hungary, Costa Rica, Republic of Korea, Spain, Belgium and Germany. Details of study design and methodological quality are in Table S1. Serological responses were most commonly described as the proportion of participants with antibody titres ≥1:40 measured by haemagglutination inhibition (HI) assay (i.e. seroprotection). One study[9] used 1:32 and we included it as if 1:40 in our analysis. Sixteen studies[7–9,14–23,25,26,28] reported pre-vaccination antibody level, and baseline forest plots are given in Figure S2.

All the studies included in this review were clinical trials and published in peer review journals with high impact factors. We have calculated the JADAD score for individual studies and all were three or higher, except for two studies[14,18] (Table S1).

Immunogenicity

Children aged 6–35 months

Five studies[15,18–21] provided pre-vaccination serological data on 1001 children; the percentage with antibody ≥1:40 was 5·8% (95% CI: 4·0–8·4%, $I^2 = 30\cdot9\%$; Figure S2). Nine hundred and sixty-seven children were vaccinated and followed; 135, 170 and 131 of them, respectively received a 7·5, 15 or 30 μg dose of non-adjuvanted vaccine. A total of 324 children received oil in water emulsion vaccines (48, 175 and 101 children, respectively were given 3·75 μg $AS03_A$-, 1·875 μg $AS03_B$- and 1·9 μg $AS03_B$-adjuvanted vaccine).

After 1st/one dose of non-adjuvanted vaccine, the overall weighted proportion with a HI titre ≥1:40 was 56·9% (33·0–78·0%, $I^2 = 48\cdot0\%$); those who were given two doses of non-adjuvanted vaccine had higher seroprotection, 83·6% (64·9–93·4%, $I^2 = 46\cdot1\%$; Table S2 and Figure S3). Participants receiving $AS03_A$ or $AS03_B$ vaccine achieved a proportion of 99·3% (95·2–99·9%, $I^2 = 0\cdot000$) after one dose and 99·4% (97·5–99·8%, $I^2 = 0\cdot000$) after two doses of $AS03_A$ or $AS03_B$ vaccine. The poorest responses were to non-adjuvanted vaccine produced by local manufactures in Republic of Korea and Taiwan.[18,20]

As the study by Oh[20] had low Jadad score, we performed a sensitivity analysis without it; higher seroprotection proportions were shown, 67·7% (44·3–84·7%, $I^2 = 48\cdot0\%$) after 1st dose of non-adjuvanted vaccine and 91·5% (71·2–97·9%, $I^2 = 46\cdot8\%$) after the 2nd dose.

Children aged 3–8 years

There were five reports[14,18–21] and these included pre- and post-vaccination data, respectively from 751 and 746 children. The pre-vaccination proportion was 14·0% (8·1–23·2%, $I^2 = 46\cdot6\%$; Figure S2). Both the Arguedas[14] and Nolan[19] studies reported high antibody percentage before vaccination (up to 33·3% seroprotected). The overall estimate for seroprotection after 1st/one dose of non-adjuvanted vaccine in this age group was 74·8% (60·0–85·4%, $I^2 = 48\cdot0\%$; Table S2 and Figure S3) with poorer responses (as before) after the locally produced vaccines from Republic of Korea and Taiwan.[18,20] The Nolan study (non-adjuvanted product) stood out for showing the highest immune response to vaccination. The adjuvanted vaccine (with MF59) had 7·5 μg haemagglutinin (HA) and induced a higher antibody level (seroprotection = 92·6%, 81·9–97·2%), compared with the non-adjuvanted vaccines containing 15 and 30 μg, although it was only significantly so with the 15 μg of vaccine. Among children receiving two doses, there was even higher seroprotection (Table S2 and Figure S3).

As there was limited information on methodological quality in two reports,[14,20] we performed a sensitivity analysis without them; the overall estimate of seroprotection after one dose (non-adjuvanted vaccine) was actually higher at 81·2% (64·7–91·1%, $I^2 = 47\cdot8\%$). After the 2nd dose, the separate analysis without the data from Oh only was also higher, 97·9% (76·6–99·9%, $I^2 = 44\cdot4\%$).

Adolescents aged 9–17 years

Four studies[14,17,18,20] provided data about pre-vaccination seroprotection in 3120 subjects: 19·3% (8·3–38·8%, $I^2 = 49\cdot1\%$; Figure S2) and post-injection seroprotection data from 3036 subjects, 2684 of whom came from a Chinese trial.[17] The summary-weighted estimate for those receiving non-adjuvanted vaccine was higher at 95·7% (90·9–98·0%, $I^2 = 47\cdot5\%$) after the 1st/one dose than after aluminium hydroxide-adjuvanted vaccine (1st dose: 88·1%, 80·2–93·2%, $I^2 = 42\cdot7\%$; Table S2 and Figure S3). MF59-adjuvanted split vaccine was administrated to 52 subjects and produced a better immune response (98·1%, 87·6–99·7%) than other adjuvanted (aluminium hydroxide) and non-adjuvanted vaccines (Table S2 and Figure S3). The analysis without data from Arguedas or Oh showed the overall estimate after one dose of non-adjuvanted vaccine was not materially affected [96·8% (95·2–97·9%, $I^2 = 35\cdot8\%$)].

Two doses of non-adjuvanted vaccine lead to a proportion with seroprotection rate of 99·3% (93·5–99·9%, $I^2 = 46\cdot8\%$), which was again higher than the immune

response to aluminium hydroxide-adjuvanted vaccine (95·2%, 90·6–97·8%, $I^2 = 32·1$%). The sensitivity analysis without the study by Oh gave a slightly higher proportion after 2nd dose of non-adjuvanted vaccine, 99·7% (98·0–99·9%, $I^2 = 37·6$%).

Adults aged 18–60 years
Eight trials had data on seroprotection proportion estimate of 4458 participants[7,8,16,17,22–25] and reported an estimate of 10·2% (6·0–16·8%, $I^2 = 48·1$%; in two studies, there were higher proportions[7,8]; Figure S2).

Seroprotection data showed overall estimates of 92·9% (89·0–95·5%, $I^2 = 45·5$%; Table S2 and Figure S3) after 1st/one dose of non-adjuvanted vaccine, 82·1% (76·7–86·5%, $I^2 = 42·8$%) after alum-adjuvanted vaccine and 93·8% (79·1–98·4%, $I^2 = 43·8$%) after MF59- or AS03$_A$-adjuvanted vaccine. Further improvements were detected for all vaccine types after 2nd dose (non-adjuvanted: 95·9%, 92·5–97·8%, $I^2 = 44·8$%; alum-adjuvanted: 90·7%, 83·9–94·8%, $I^2 = 45·6$% and MF59/AS03$_A$: 97·8%, 91·7–99·5%, $I^2 = 0·000$).

The elderly (aged >60 years)
The pre-injection seroprotection proportion was estimated as 9·6% (4·3–20·1%, $I^2 = 48·8$%) based on the data of 2778 subjects (Figure S2). The seroresponse results were obtained for 2692 participants from six trials.[16,17,21,23,24,28] After 1st/one dose of non-adjuvanted vaccine, the overall seroprotection estimate was 87·3% (82·3–91·0%, $I^2 = 45·4$%; Table S2 and Figure S3); a lower response was shown in those that received aluminium hydroxide-adjuvanted vaccine, 68·1% (57·6–77·0%, $I^2 = 43·6$%). With a low antigen dose (3·75 μg) of AS03$_A$-adjuvanted vaccine, a high proportion, 87·4% (80·1–92·3%), achieved seroprotection. After 2nd dose, all types of vaccine reported better immune responses (non-adjuvanted: 91·2%, 79·7–96·5%, $I^2 = 48·4$%; aluminium hydroxide-adjuvanted: 91·5%, 85·5–95·1%, $I^2 = 33·4$%; AS03$_A$-adjuvanted: 97·0%, 88·8–99·3%).

Comparisons of adjuvanted vaccines versus non-adjuvanted vaccines produced by the same company
Based on the data from two studies[17,25] (one study[17] had a third one[26] as a subset), use of aluminium hydroxide as an adjuvant did not improve the immune response compared with non-adjuvanted vaccine, and in fact, it was significantly decreased after one dose in three age groups, 9–17, 18–60 and >60 years compared to a plain split vaccine (Figure S4). One study[7] showed a higher seroprotection proportion in participants vaccinated with MF59-adjuvanted vaccine (77–96% after 1st dose and 92–100% after 2nd dose) than those who were given non-adjuvanted vaccine (63–72% after 1st dose and 67–76% after 2nd dose).

Adverse events

Seventeen[7–9,14–27] of the 18 studies provide safety data, 4[17,21,25,26] of which compared pandemic vaccine with placebo. Fourteen studies[7–9,15–20,22–26] used a structured diary card to record adverse events after vaccination, 10[7–9,16–23] of which also collected unsolicited adverse events. One study[14] did not report the collection method of adverse events. The common local and systematic adverse events were defined and collected in each study; however, some studies reported adverse events by combining vaccine doses, with/without adjuvants and age groups, and also different grading scales are observed among these studies. Therefore, we are not able to synthesise these data by meta-analysis.

The pandemic influenza A (H1N1) vaccines were associated with two serious adverse events: one 8-year-old child developed an episode of 4-day high fever (39·7°C) within 1 day of the first vaccination (no adjuvant, 30 μg).[19] This child made a full recovery. One adult with multiple allergies and mastocytosis had allergic symptoms 1 hour after receiving the first dose of non-adjuvanted (30 μg HA) vaccine and this event also revolved.[22] No death or case of Guillain–Barré syndrome (GBS) was reported. The severities of local and systematic adverse events were relatively mild to moderate, although high proportions of some adverse events were reported (pain: 88·9%; muscle aches: 54·0%; Table S3). Pain at the injection site after vaccination was the most frequently reported local adverse event in 14 of the 16 studies, followed by swelling and redness. Fever and headache were reported to be the most common systematic adverse events with malaise and muscle aches also quite common. A proportion of 10·9% (4·9–16·1%) of children under 3 years developed fever (>37·5°C) after the vaccination (Table S5). There was one febrile convulsion reported among 967 children aged 6 months to 3 years and none in 746 aged 3–8 years; it was stated to be associated with concurrent pneumonia.[19] Mild systematic adverse events (nausea, vomiting, diarrhoea, fever and irritability) were frequently found among children aged 6–35 months; the proportions of fever (>37·5°C) were 10·9% and 4·4%, respectively, in children aged 6–35 months and 3–8 years (Tables S4 and S5).

There were only three studies[7,17,25] where comparisons were made between adjuvanted and non-adjuvanted vaccines at the same dosage. Two Chinese studies[17,25] involving 13 397 participants given influenza vaccine with or without aluminium hydroxide adjuvant showed in general that local and systemic reactions were more common with the alum-adjuvanted product. In the large study (in The Lancet) by Liang *et al.*,[17] aluminium hydroxide-adjuvanted vaccine was associated with a significantly higher proportion of local reactions; however, the smaller study by Wu *et al.*[25] did not report any significant differences; the

third study[7] involving 176 adults compared recipients of an MF59-adjuvanted vaccine with those given non-adjuvanted vaccine. Pain was the most frequent local reaction and was more common with MF59-adjuvanted vaccine (65% versus 39%, $P = 0.003$). Participants receiving two doses of MF59-adjuvanted vaccine had a higher proportion of muscle ache ($P = 0.02$) than those who were given one dose only, whereas no significant difference of systemic reactions, in frequency or severity, was detected between MF59-adjuvanted and non-adjuvanted vaccines.

The CDC review of safety data on non-adjuvanted vaccines[27] looked at nearly four thousand reports from the US VAERS and examined more than 430 000 electronic records in VSD. There was no significant difference detected between pandemic H1N1 and seasonal influenza vaccines in terms of serious adverse events, although they found somewhat more adverse events post-vaccination with H1N1 vaccine compared with seasonal influenza vaccine (82 versus 47 reports per 1 million vaccine doses distributed). During December 2009–August 2010, the European Medicines Agency provided regular updates of safety surveillance data on both non-adjuvanted and adjuvanted vaccines. It concluded that the benefit–risk profile of pandemic H1N1 vaccine, with or without adjuvant, continued to be positive, and the majority of post-vaccination adverse events were considered to be non-severe.[29]

Discussion

Pandemic influenza A (H1N1) 2009 vaccine, with or without adjuvant, at most doses, was immunogenic and generally safe in people aged from 36 months to over 60 years. A striking finding is that both healthy young children (in 2 of 5 studies) and the elderly (in 7 of 7 studies) could respond vigorously to just one dose of vaccine and fulfilled the seroprotection criteria of leading regulatory agencies.[2,3] As the existence of a strong relationship between HI titre and clinical effectiveness against influenza has been confirmed recently,[5] we are able to provide timely information for governments, academics and medical practitioners on the new pandemic vaccines; this is not contingent on comparing the data with past meta-analyses of seasonal vaccines.[30,31]

The three studies[18,20,21] in young children (6–35 month) where immunogenicity did not fulfil seroprotection criteria all involved non-adjuvanted vaccine. Two[18,20] of these studies were by manufacturers where vaccines proved generally less immunogenic in all paediatric age bands. One study showed one dose of 15 μg non-adjuvanted vaccine was highly immunogenic[19] comparing favourably with the 70% cut-off. The apparent better immune response to vaccine among young children (6–35 months) in the Nolan (Australian) study[19] and the poorer responses in other

studies[18,20,21] need to be interpreted in the context of assays not all being performed by the one laboratory. The higher pre-vaccination antibody titres in Australian children may indicate some were primed by mild/asymptomatic infections with H1N1 2009 virus; it is noteworthy that date of recruitment in Australia was no later than the other studies (in Republic of Korea,[20] Taiwan[18] and United States[21]), but the Australian study was performed in August in the Southern Hemisphere winter when the disease was peaking (Figure S2). Use of single-dose AS03$_A$- or AS03$_B$-adjuvanted vaccine lead to generally higher immunogenicity despite a low amount of antigen; a 2nd dose of non-adjuvanted vaccine is likely to be required in this age group as only one of four studies showed a strong response to one dose.[19] Two doses were recommended by authorities for young children.[27,32] Even after two doses, there were two studies using non-adjuvanted vaccine at the 7.5 μg dose that did not demonstrate adequate immunogenicity,[9,20] whereas six other comparisons[9,15,18,19] using 15 or 30 μg non-adjuvanted, or lower dose (1.9 or 3.75 μg) AS03$_A$/AS03$_B$-adjuvanted vaccines, resulted in adequate levels of antibody response.

Single-dose vaccine performed well in adults aged 18–60 years (Figure S3). It was clear in this age group that there were better responses to the adjuvanted (MF59 or AS03$_A$) vaccines, despite lower antigen contents. Based on the consideration that, in order to cover large numbers, the lowest dose vaccine may be needed during a pandemic, use of MF59- or AS03$_A$-adjuvanted vaccine would contribute to an increase in vaccine production.

Use of aluminium derivatives as adjuvants did not improve the immune response. Indeed, results were significantly worse compared to a non-adjuvanted split virus vaccine. Notable heterogeneities were detected among five of six overall estimates. These findings were drawn from two studies,[17,25] both of which used batches of vaccines provided by the same pharmaceutical company in China, so caution is required before discounting the value of aluminium hydroxide or alum adjuvants altogether. However, research with H5N1 vaccines has also shown no benefit (and perhaps detriment) from using alum adjuvants.[33]

Meta-analysis of safety outcomes is particularly difficult, because, in the main, of poor uniformity in documenting outcomes. Also, there were only three studies e.g. that compared, head-to-head, adjuvanted vaccine against non-adjuvanted vaccines. There was a suggestion that alum-adjuvanted vaccine was less well tolerated. There was some statistical evidence that pain after injection and muscle ache were more frequent with a MF59-adjuvanted vaccine compared with non-adjuvanted vaccine. In 1976, the vaccine for a swine-origin influenza virus was shown to have significant association with higher risk of GBS among the vaccinated population, the reason for which remains

unknown. The trials in this review included healthy volunteers only and had relatively small size; therefore, although no cases of GBS were reported, surveillance systems at the general population level are required to better assess the safety profile of pandemic H1N1 vaccine in term of rare adverse events.

In Western Australia (WA), there were reports of increased febrile convulsions after 2010 seasonal influenza vaccination that included pandemic H1N1 strain. However, a definitive explanation has not yet been found.[34] Among the children aged <3 years that received monovalent pandemic H1N1 vaccine, there was just one episode of febrile convulsion reported (an 18-month-old boy, 20 days after 1st dose of 15 μg non-adjuvanted vaccine)[19] and was considered to be non-vaccine associated by the authors.

Mild to 'notable' heterogeneities are detected in most of the overall estimates (28 of 31 estimates, I^2: 30·9–91·0%). As not all studies provided detailed information, we are not able to perform full analysis for the sources of heterogeneity. However, we believe that the heterogeneities may be associated with: (i) substantially higher pre-vaccination antibody level in some groups of vaccinees. Participants in studies by Plennevaux[21], Nolan[19], Arguedas[14], Clark[7] and Greenberg[8] had higher pre-vaccination antibody seroprotection proportions (up to 48·0%) and (ii) use of vaccine provided by local manufactories in several studies: the studies by Oh,[20] Lu,[18] Kung[16] and Kao[28] used vaccines produced locally. Substantially lower immune responses from these vaccines decreased the overall estimates.

This study has some other limitations. Our review included several studies of lower quality (Jadad scores of 1 in 2 studies[14,18] and 3 in 7 studies[7,9,14,15,18,20,23–25]). However, sensitivity analyses removing the two lowest quality studies made no material difference to the estimates. The measurement of immunogenicity has some limitations. First, immunogenicity in our meta-analysis only refers to the antibody against the homologous virus contained in the monovalent vaccine (A/California/07/2009-A/PR/8/34); however, this virus has been reported to have developed some phylogenetic clades and different subclades.[35] Secondly, the degree of association between immunogenicity and clinical efficacy needs to be further evaluated despite recent supportive evidence.[5] Thirdly, there is known to be considerable inter-laboratory variation in antibody measurement and an antibody standard was not routinely applied. Finally, we did not report data on seroconversion or on microneutralisation titres as these were not routinely available.

Authors Contributors

All authors participated in the design, analysis and interpretation of the study. JY, GK and RB were involved in all phases of the study. JY led the statistical analysis. GK assisted JY in data extraction. HR and GK performed the methodological quality assessment. JY, GK, RB, LH and IR wrote the manuscript.

Conflicts of interest

Robert Booy has received funding from CSL, Roche, Sanofi, GlaxoSmithKline (GSK) and Wyeth to attend and present at scientific meetings; any funding received is directed to a research account at the Children's Hospital at Westmead. Leon Heron has performed consultancy work for Novartis for which payment was made to the National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases. He has had travel expenses covered by GSK and has conducted sponsored research and investigator-driven research with funding from GSK, Wyeth, Merck, CSL, Roche and Sanofi Pasteur. The other authors declare that they have no conflict of interest in relation to this work.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Results of literature search and studies analysed (adapted from PRISMA 2009 Flow Diagram[29]).

Figure S2. Forest plots of pre-vaccination antibody concentration.

Figure S3. Forest plots of serological response following pandemic (H1N1) 2009 vaccination, by age group and by doses.

Figure S4. Risk ratio comparing the immunogenicity of non-adjuvanted to adjuvanted of pandemic influenza A (H1N1) 2009 vaccine, by age group and by doses.

Table S1. Characteristics of studies included.

Table S2. Overall estimates of forest plots with heterogeneity test results.

Table S3. The most frequently reported adverse events (if any, proportion) of studies included.

Table S4. Definitions and grades of fever.

Table S5. Proportion of fever in different age groups.

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