# Safety and immunogenicity of 2009 pandemic influenza A H1N1 vaccines in China: a multicentre, double-blind, randomised, placebo-controlled trial

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See Comment pages 6 and 9

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## Summary

**Background** The current influenza pandemic calls for a safe and effective vaccine. We assessed the safety and immunogenicity of eight formulations of 2009 pandemic influenza A H1N1 vaccine produced by ten Chinese manufacturers.

**Methods** In this multicentre, double-blind, randomised trial, 12 691 people aged 3 years or older were recruited in ten centres in China. In each centre, participants were stratified by age and randomly assigned by a random number table to receive one of several vaccine formulations or placebo. The study assessed eight formulations: split-virion formulation containing 7·5 μg, 15 μg, or 30 μg haemagglutinin per dose, with or without aluminium hydroxide adjuvant, and whole-virion formulation containing 5 μg or 10 μg haemagglutinin per dose, with adjuvant. All formulations were produced from the reassortant strain X-179A (A/California/07/2009-A/PR/8/34). We analysed the safety (adverse events), immunogenicity (geometric mean titre [GMT] of haemagglutination inhibition antibody), and seroprotection (GMT ≥1:40) of the formulations. Analysis was by per protocol. Two sites registered their trial with ClinicalTrials.gov, numbers NCT00956111 and NCT00975572. The other eight studies were registered with the State Food and Drug Administration of China.

**Findings** 12 691 participants received the first dose on day 0, and 12 348 participants received the second dose on day 21. The seroprotection rate 21 days after the first dose of vaccine ranged from 69·5% (95% CI 65·9–72·8) for the 7·5 μg adjuvant split-virion formulation to 92·8% (91·9–93·6) for the 30 μg non-adjuvant split-virion formulation. The seroprotection rate was 86·5% (796 of 920; 84·1–88·7) in recipients of one dose of the 7·5 μg non-adjuvant split-virion vaccine compared with 9·8% (140 of 1432; 8·3–11·4) in recipients of placebo (p<0·0001). One dose of the 7·5 μg non-adjuvant split-virion vaccine induced seroprotection in 178 of 232 children (aged 3 years to <12 years; 76·7%, 70·7–82·0), 211 of 218 adolescents (12 years to <18 years; 96·8%, 93·5–98·7), 289 of 323 adults (18–60 years; 89·5%, 85·6–92·6), and 118 of 147 adults older than 60 years (80·3%, 72·9–86·4), meeting the European Union's licensure criteria for seroprotection in all age-groups. In children, a second dose of the 7·5 μg formulation increased the seroprotection rate to 97·7% (215 of 220, 94·8–99·3). Adverse reactions were mostly mild or moderate, and self-limited. Severe adverse effects occurred in 69 (0·6%, 0·5–0·8) recipients of vaccine compared with one recipient (0·1%, 0–0·2) of placebo. The most common severe adverse reaction was fever, which occurred in 25 (0·22%; 0·14–0·33) recipients of vaccine after the first dose and four (0·04%; 0·01–0·09) recipients of vaccine after the second dose compared with no recipients of placebo after either dose.

**Interpretation** One dose of non-adjuvant split-virion vaccine containing 7·5 μg haemagglutinin could be promoted as the formulation of choice against 2009 pandemic influenza A H1N1 for people aged 12 years or older. In children (aged <12 years), two 7·5 μg doses might be needed.

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## Introduction

Since the novel swine-origin influenza A H1N1 virus was identified in Mexico and the USA in April, 2009, it has spread throughout the world, prompting WHO to raise the pandemic alert level to phase 6 on June 11, 2009.[1–3] Vaccination is the most effective measure to control the spread of the virus and to reduce associated morbidity and mortality. Existing evidence shows that the currently used trivalent seasonal influenza vaccines are unlikely to provide protection against the new virus.[4] The development of new vaccines is therefore urgent, especially in China, where a sixth of the world's population lives.

Clinical trials of vaccines against the 2009 pandemic H1N1 virus are in progress in China, Australia, the

USA, and the European Union (EU). These trials recruited fairly small numbers of participants. Data from the USA,[5] the UK,[6] Australia,[7] and China[8] suggest that a segment of the population has pre-existing immunity to the pandemic virus, either as a result of past infections or cross-reaction. Therefore, in clinical trials of vaccines for this pandemic, a placebo group is necessary to distinguish between natural and acquired immunity.

We undertook a multicentre, double-blind, randomised, placebo-controlled trial to assess the safety and immunogenicity of eight formulations of 2009 pandemic influenza A H1N1 vaccine produced by ten Chinese manufacturers.

## Methods

### Participants

This multicentre trial was undertaken between July 22, and Sept 18, 2009, in ten centres in China: Beijing, Wuzhou, and Lipu (Guangxi Autonomous Region); Taizhou and Taixing (Jiangsu Province); Changge and Changshe (Henan Province); Chifeng (Inner Mongolia Autonomous Region); Hengdong (Hunan Province); and Hengshui (Hebei Province). Healthy people aged 3 years or older were recruited for the study. Exclusion criteria included fever (axillary temperature >37·0°C); previous infection with 2009 pandemic H1N1 virus since May, 2009, as shown by medical or laboratory records; previous quarantine imposed by the health authority for being in close contact with a patient with 2009 pandemic H1N1 influenza; severe acute or chronic diseases; history of allergy to vaccines or eggs; immunodeficient disorders; receipt of cytotoxic or immunosuppressive drugs within the past 6 months; or receipt of blood, blood products, or any other vaccines within the past 3 months. Women who were pregnant or lactating, or planning to get pregnant within 2 months were also excluded.

The trial was independently reviewed and approved by the ethics review committee in each centre. Written informed consent was obtained for all participants: those under 12 years of age had consent signed by their parents or guardians; those aged between 12 years and 18 years had consent signed by themselves and by their parents or guardians.

The Chinese Center for Disease Control and Prevention provided basic guidelines for the protocols used in the ten centres, requiring at least 100 participants per group and at least three blood samples from each participant on day 0, 21, 35, or 42 after the first dose. The guidelines also required the trial in each study site to be double-blind, randomised, placebo-controlled, and undertaken in all four of the following age-groups: 3 years to less than 12 years (children), 12 years to less than 18 years (adolescents), 18–60 years (adults), and more than 60 years (older adults). Beyond these basic requirements, each centre could increase the number of participants, as well as the number of blood samples obtained from each participant.

### Vaccine

The seed virus was reassortant strain X-179A (A/California/07/2009-A/PR/8/34), prepared by New York Medical College (Westchester County, NY, USA) by use of classic reassortment technology. The strain was recommended by WHO for the development of 2009 pandemic H1N1 vaccines, and distributed by the US Centers for Disease Control and Prevention and other institutions.[9,10]

The study vaccines and placebo used in the ten centres were provided by ten manufacturers in China. We studied eight vaccine formulations: split-virion formulation containing 7·5 μg, 15 μg, or 30 μg haemagglutinin per dose, with or without adjuvant, and whole-virion formulation containing 5 μg or 10 μg haemagglutinin per dose, with adjuvant. Adjuvant formulations were prepared with aluminium hydroxide, resulting in a final concentration of aluminium of 0·5 mg/mL or 1·2 mg/mL. The study vaccines used in the Beijing centre contained no thiomersal; the vaccines used in the other nine centres contained thiomersal as a preservative. The vaccines were manufactured in embryonated chicken eggs by a process similar to that for seasonal influenza vaccines without the use of antibiotics. The vaccines were prepared in vials or prefilled syringes and formulated in single or multiple doses. Placebo was sterile water for injection in one centre (Chifeng), and phosphate buffered saline in the other nine centres. All formulations of the vaccine and placebo were qualified and quantified by the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP) of China in Beijing.

### Randomisation and masking

In each of the ten centres, participants were randomly assigned to treatment within each age-group. Vaccine and placebo used for each age-group were labelled with sequential numbers according to a random number table generated with the block method by a statistician not involved in the rest of the trial. Block size varied by age-group and centre. The same sequential numbers were also assigned to participants according to their sequence of enrolment. Participants received vaccine or placebo according to their sequential numbers. Masking was achieved by covering all labels with aluminium foil.

Although the study sites followed the same general guidelines, the procedures at each site were not identical. In the Beijing centre, older adults (>60 years) received one dose of 10 μg whole-virion formulation; all other participants received two doses of vaccine or placebo 21 days apart. In the Hengdong centre, all age-groups (<12 years, 12 to <18 years, 18–60 years, >60 years) had a placebo group, with approximately a third of participants in each age-group receiving placebo. The remaining

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nine centres had a placebo group in adults only because of concerns about recruitment of children in a culture that can be reluctant to consider randomisation to a placebo; in these nine centres, 110 (5%), 100 (6%), 110 (8%), 110 (9%), 120 (11%), 100 (11%), 120 (11%), 110 (11%), 100 (11%) of participants, respectively, received placebo. The whole-

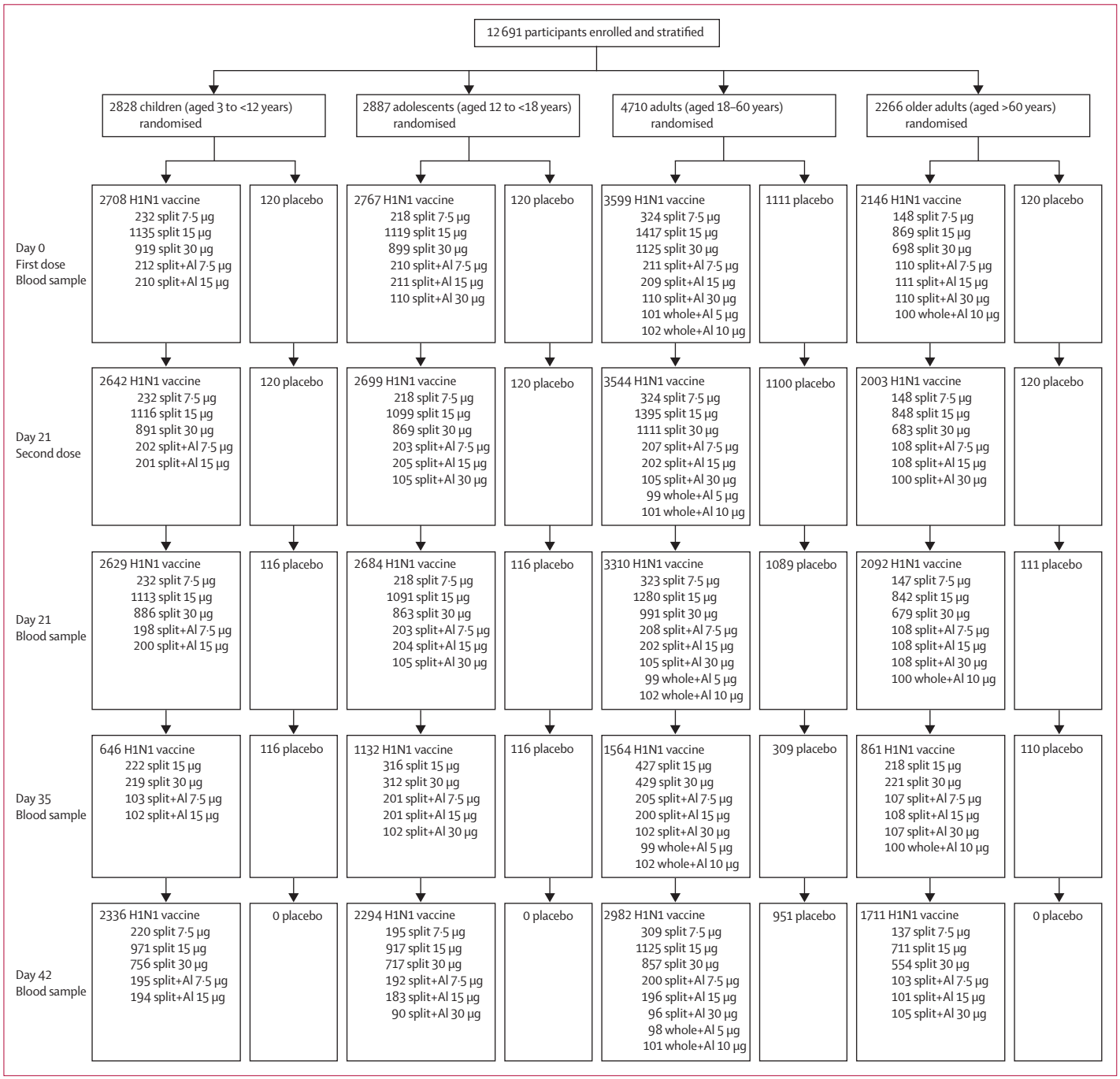


***Figure 1*: Enrolment and follow-up of participants**

253 participants dropped out after receiving the first dose (four for an adverse event associated with vaccination; 35 for adverse events unrelated to vaccination; 91 for unwillingness to continue; 58 for being unable to comply with the study schedule; and 65 for other reasons). 343 participants dropped out after receiving the second dose (one for an adverse event associated with vaccination; four for adverse events unrelated to vaccination; 62 for unwillingness to continue participation; 254 for being unable to comply with the study schedule; and 22 for other reasons). Some participants in the Hengdong centre only received one dose of vaccine or placebo. The number of participants who gave blood samples differed from the number of participants who received vaccine or placebo because, according to the study design, fewer participants were needed to give blood samples than to receive vaccine or placebo. Split=non-adjuvant split-virion formulation. Split+Al=aluminium-adjuvant split-virion formulation. Whole+Al=aluminium-adjuvant whole-virion formulation.

| | Non-adjuvant split-virion formulation | | | Adjuvant split-virion formulation | | | Adjuvant whole-virion formulation | | All vaccine formulations | Placebo |
|---|---|---|---|---|---|---|---|---|---|---|
| | 7·5 μg | 15 μg | 30 μg | 7·5 μg | 15 μg | 30 μg | 5 μg | 10 μg | | |
| **Children (3 to <12 years; n=2828)** | | | | | | | | | | |
| Number | 232 | 1135 | 919 | 212 | 210 | .. | .. | .. | 2708 | 120 |
| Age (years) | 7·2 (2·5) | 7·2 (2·5) | 7·1 (2·6) | 7·2 (2·5) | 7·3 (2·7) | .. | .. | .. | 7·2 (2·5) | 7·1 (2·5) |
| Sex (female) | 105 (45%) | 528 (47%) | 454 (49%) | 104 (49%) | 109 (52%) | .. | .. | .. | 1300 (48%) | 60 (50%) |
| **Adolescents (12 to <18 years; n=2887)** | | | | | | | | | | |
| Number | 218 | 1119 | 899 | 210 | 211 | 110 | .. | .. | 2767 | 120 |
| Age (years) | 14·1 (1·7) | 14·0 (1·6) | 13·9 (1·6) | 14·2 (1·6) | 14·3 (1·5) | 14·0 (1·6) | .. | .. | 14·0 (1·6) | 13·3 (1·4) |
| Sex (female) | 103 (47%) | 559 (50%) | 461 (51%) | 104 (50%) | 109 (52%) | 55 (50%) | .. | .. | 1391 (50%) | 60 (50%) |
| **Adults (18–60 years; n=4710)** | | | | | | | | | | |
| Number | 324 | 1417 | 1125 | 211 | 209 | 110 | 101 | 102 | 3599 | 1111 |
| Age (years) | 44·1 (13·4) | 42·8 (12·0) | 43·0 (12·1) | 41·6 (11·3) | 41·2 (11·7) | 40·8 (13·8) | 41·3 (10·0) | 41·1 (9·9) | 42·4 (11·9) | 41·6 (11·3) |
| Sex (female) | 191 (59%) | 807 (57%) | 662 (59%) | 119 (56%) | 114 (55%) | 55 (50%) | 58 (57%) | 62 (61%) | 2068 (57%) | 667 (60%) |
| **Older adults (>60 years; n=2266)** | | | | | | | | | | |
| Number | 148 | 869 | 698 | 110 | 111 | 110 | .. | 100 | 2146 | 120 |
| Age (years) | 66·3 (4·5) | 66·4 (4·7) | 66·3 (4·9) | 65·3 (3·3) | 64·8 (2·9) | 65·4 (3·4) | .. | 66·4 (4·2) | 66·1 (4·6) | 65·0 (4·3) |
| Sex (female) | 79 (53%) | 445 (51%) | 367 (53%) | 55 (50%) | 55 (50%) | 55 (50%) | .. | 44 (44%) | 1100 (51%) | 60 (50%) |
| **Total (n=12 691)** | | | | | | | | | | |
| Number | 922 | 4540 | 3641 | 743 | 741 | 330 | 101 | 202 | 11220 | 1471 |
| Age (years) | 31·3 (23·2) | 31·3 (23·4) | 31·2 (23·5) | 27·6 (21·8) | 27·4 (21·7) | 40·0 (22·6) | 41·3 (10·0) | 53·6(14·8) | 32·3 (22·7) | 38·4 (17·2) |
| Sex (female) | 478 (52%) | 2339 (52%) | 1944 (53%) | 382 (51%) | 387 (52%) | 165 (50%) | 58 (57%) | 106 (52%) | 5859 (52%) | 847 (58%) |

Data are mean (SD) or n (%).

*Table 1:* **Demographic characteristics of participants, by study group and age-group**

virion formulation was not given to children and adolescents because of concerns about adverse reactions. Doses of vaccine and placebo were administered intramuscularly into the deltoid region. Both participants and investigators were masked to treatment assignment.

## Procedures

We obtained blood samples (3–5 mL) from participants on day 0 (immediately before the first dose), day 21 (immediately before the second dose), and day 42 (21 days after the second dose, apart from in one centre, Hengdong). In four centres (Beijing, Taizhou, Taixing, and Hengdong), blood samples were also taken on day 35 (14 days after the second dose). As per the study protocol, the participants gave blood specimens on a "first-come, first-give" basis. Once the required number of blood samples had been reached, no more were taken, but individuals could still receive vaccination.

Participants were observed for immediate adverse events for 30 min after receipt of vaccine or placebo. For the next 3 days, participants recorded solicited adverse events by use of a structured questionnaire in the form of a diary card. Participants were required to return their diary cards, which were reviewed by investigators before data entry. Participants were also asked to report any unsolicited adverse events as they arose throughout the study. Symptoms of Guillain-Barré syndrome were of special interest. A serious adverse event was defined as any untoward medical occurrence that resulted in death, life-threatening medical conditions, persistent or substantial disability or incapacity, admission to hospital, or extended length of an existing hospital admission. Information about serious adverse events and adverse events of special interest was obtained by diary cards, spontaneous reports, and hospital admission records throughout the study. All adverse events were assessed and graded according to standardised scales established by the Division of Microbiology and Infectious Diseases, US National Institutes of Health[11,12] (webappendix pp 1–2). Axillary temperatures of 37·1–37·5°C, 37·6–39·0°C, and higher than 39·0°C were graded as mild, moderate, and severe fever, respectively.

See Online for webappendix

We assayed serum samples by the haemagglutination inhibition method against homologous X-179A strain. These assays were done in accordance with established procedures with the use of turkey erythrocytes, as in previous reports.[13,14] The haemagglutination inhibition assays were undertaken under masked conditions by the NICPBP of China.

## Statistical analysis

With 100 participants per treatment group, the study had a statistical power of at least 80% to detect a serocoversion rate of more than 40% in each group.

| | Non-adjuvant split-virion formulation | | | Adjuvant split-virion formulation | | | Adjuvant whole-virion formulation | | All vaccine formulations | Placebo |
|---|---|---|---|---|---|---|---|---|---|---|
| | 7·5 µg | 15 µg | 30 µg | 7·5 µg | 15 µg | 30 µg | 5 µg | 10 µg | | |
| All adverse events | 96/922 (10·3%; 8·3–12·3) | 949/4540 (20·9%; 19·7–22·1) | 968/3641 (26·6%; 25·2–28·0) | 207/743 (27·9%; 24·6–31·1) | 200/741 (27·0%; 23·8–30·2) | 135/330 (40·9%; 35·6–46·2) | 17/101 (16·8%; 9·4–24·3) | 23/202 (11·4%; 7·0–15·8) | 2594/11 220 (23·1%; 22·3–23·9) | 233/1471 (15·8%; 14·0–17·1) |
| Dose | | | | | | | | | | |
| First | 68/922 (7·4%; 5·7–9·1) | 685/4540 (15·1%; 14·0–16·1) | 708/3641 (19·5%; 18·2–20·8) | 115/743 (15·5%; 12·9–18·1) | 123/741 (16·6%; 13·9–19·3) | 78/330 (23·6%; 19·0–28·2) | 14/101 (13·9%; 7·1–20·6) | 22/202 (10·9%; 6·6–15·2) | 1813/11 220 (16·2%; 15·5–16·8) | 145/1471 (9·9%; 8·3–11·4) |
| Second | 35/922 (3·8%; 2·6–5·0) | 387/4458 (8·7%; 7·9–9·5) | 403/3544 (11·3%; 10·3–12·4) | 127/720 (17·6%; 14·9–20·4) | 105/714 (14·7%; 12·1–17·3) | 76/318 (23·9%; 19·2–28·6) | 5/98 (5·1%; 1·6–11·5) | 5/100 (5·0%; 0·7–9·2) | 1143/10 886 (10·5%; 9·9–11·1) | 107/1471 (7·3%; 6·0–8·7) |
| Grade | | | | | | | | | | |
| Mild | 89/922 (9·7%; 7·7–11·6) | 847/4540 (18·7%; 17·5–19·8) | 814/3641 (22·4%; 21·0–23·7) | 178/743 (24·0%; 20·9–27·0) | 183/741 (24·7%; 21·6–27·8) | 114/330 (34·5%; 29·4–39·7) | 15/101 (14·9%; 7·8–21·9) | 21/202 (10·4%; 6·2–14·6) | 2261/11 220 (20·2%; 19·4–20·9) | 208/1471 (14·1%; 12·4–15·9) |
| Moderate | 8/922 (0·9%; 0·3–1·5) | 109/4540 (2·4%; 2·0–2·8) | 160/3641 (4·4%; 3·7–5·1) | 24/743 (3·2%; 2·0–4·5) | 15/741 (2·0%; 1·0–3·0) | 13/330 (3·9%; 1·8–6·0) | 2/101 (2·0%; 0–4·7) | 3/202 (1·5%; 0–3·2) | 334/11 220 (3·0%; 2·7–3·3) | 25/1471 (1·7%; 1·0–2·4) |
| Severe | 0 | 15/4540 (0·3%; 0·2–0·5) | 29/3641 (0·8%; 0·5–1·1) | 6/743 (0·8%; 0·2–1·5) | 6/741 (0·8%; 0·2–1·5) | 12/330 (3·6%; 1·6–5·7) | 1/101 (1·0%; 0–3·0) | 0 | 69/11 220 (0·6%; 0·5–0·8) | 1/1471 (0·1%; 0–0·2) |
| Age-group | | | | | | | | | | |
| Children (3 to <12 years) | 34/232 (14·7%; 10·1–19·2) | 284/1135 (25·0%; 22·5–27·5) | 303/919 (33·0%; 29·9–36·0) | 58/212 (27·4%; 21·3–33·4) | 47/210 (22·4%; 16·7–28·1) | .. | .. | .. | 726/2708 (26·8%; 25·1–28·5) | 15/120 (12·5%; 6·5–18·5) |
| Adolescents (12 to <18 years) | 29/218 (13·3%; 8·8–17·8) | 309/1119 (27·6%; 25·0–30·2) | 299/899 (33·3%; 30·2–36·3) | 56/210 (26·7%; 20·6–32·7) | 56/211 (26·5%; 20·5–32·5) | 49/110 (44·5%; 35·1–54·0) | .. | .. | 798/2767 (28·8%; 27·2–30·5) | 16/120 (13·3%; 7·2–19·5) |
| Adults (18–60 years) | 26/324 (8·0%; 5·1–11·0) | 256/1417 (18·1%; 16·1–20·1) | 2471125 (22·0%; 19·5–24·4) | 64/211 (30·3%; 24·1–36·6) | 63/209 (30·1%; 23·9–36·4) | 44/110 (40·0%; 30·7–49·3) | 17/101 (16·8%; 9·4–24·3) | 20/102 (19·6%; 11·8–27·4) | 737/3599 (20·5%; 19·2–21·8) | 195/1111 (17·6%; 15·3–19·8) |
| Older adults (>60 years) | 6/148 (4·1%; 0·8–7·3) | 100/869 (11·5%; 9·4–13·6) | 119/698 (17·0%; 14·3–19·8) | 29/110 (26·4%; 18·0–34·7) | 34/111 (30·6%; 21·9–39·3) | 42/110 (38·2%; 29·0–47·4) | .. | 3/100 (3·0%; 0–6·4) | 333/2146 (15·5%; 14·0–17·1) | 7/120 (5·8%; 1·6–10·1) |
| Type of event | | | | | | | | | | |
| Local | 1/922 (0·1%; 0–0·3) | 265/4540 (5·8%; 5·2–6·5) | 313/3641 (8·6%; 7·7–9·5) | 136/743 (18·3%; 15·5–21·1) | 126/741 (17·0%; 14·3–19·7) | 100/330 (30·3%; 25·3–35·3) | 9/101 (8·9%; 3·3–14·6) | 10/202 (5·0%; 1·9–8·0) | 960/11 220 (8·6%; 8·0–9·1) | 41/1471 (2·8%; 1·9–3·6) |
| Systemic | 94/922 (10·2%; 8·2–12·2) | 783/4540 (17·2%; 16·1–18·3) | 794/3641 (21·8%; 20·5–23·1) | 103/743 (13·9%; 11·4–16·4) | 103/741 (13·9%; 11·4–16·4) | 50/330 (15·2%; 11·3–19·0) | 12/101 (11·9%; 5·5–18·3) | 14/202 (6·9%; 3·4–10·5) | 1953/11 220 (17·4%; 16·7–18·1) | 199/1471 (13·5%; 11·8–15·3) |

Data are number of participants with adverse event/number of recipients (%; 95% CI). Adverse events up to 3 days after vaccination are reported. Numerator is total number of adverse reactions by participant after both doses (except for adverse events for first dose and second dose). If a participant had adverse reactions after both doses, we counted the participant only once in the numerator. Similarly, if a participant had adverse reactions at different timepoints after one dose, we counted the participant only once in the numerator. Denominator is number of recipients of first dose (except for adverse events for second dose).

***Table 2:*** **Participants with adverse events after vaccine or placebo**

The immunogenicity endpoints were based on the haemagglutination inhibition licensure criteria established by the EU Committee for Medicinal Products for Human Use.[15,16] The primary immunogenicity endpoints were geometric mean titre (GMT) and seroprotection rate. In this report, haemagglutination inhibition titre of 1:40 or greater was deemed seroprotective against 2009 pandemic H1N1, on the basis of criteria established for seasonal influenza.[15] A seroprotection rate of 70% was deemed to be protective against the virus.

Because all ten study centres followed the same basic set of guidelines, we regarded these centres as part of a multicentre clinical trial. We first pooled compatible data from different centres to assess the safety and immunogenicity of the vaccine by use of descriptive statistics (eg, GMT of haemagglutination inhibition antibody, seroprotection rate, adverse reaction rate). Before data analysis, GMTs were transformed into $\log_{10}$ titres to account for skewed distributions. Analysis was by per protocol.

To account for within-centre correlation, we used a mixed model approach to analyse GMTs and cumulative adverse events at day 21 after the first dose. Study centres were judged as a random effect, whereas age-group and

| | First dose | | | | Second dose | | | |
|---|---|---|---|---|---|---|---|---|
| | All vaccine formulations (n=11 220) | | Placebo (n=1471) | | All vaccine formulations (n=10 886) | | Placebo (n=1471) | |
| | Total | Severe | Total | Severe | Total | Severe | Total | Severe |
| Local reactions | 598 (5·3%; 4·9–5·8) | 29 (0·3%; 0·2–0·4) | 24 (1·6%; 1·1–2·4) | 0 | 460 (4·2%; 3·9–4·6) | 6 (0·1%; 0·1–0·1) | 18 (1·2%; 0·7–1·9) | 0 |
| Pain | 511 (4·6%; 4·2–5·0) | 2 (0·1%; 0–0·1) | 18 (1·2%; 0·7–1·9) | 0 | 415 (3·8%; 3·5–4·2) | 0 | 15 (1·1%; 0·6–1·7) | 0 |
| Induration | 45 (0·4%; 0·3–0·5) | 11 (0·1%; 0·1–0·2) | 0 | 0 | 23 (0·2%; 0·1–0·3) | 1 (0·1%; 0–0·1) | 0 | 0 |
| Redness | 52 (0·5%; 0·4–0·6) | 15 (0·1%; 0·1–0·2) | 0 | 0 | 12 (0·1%; 0·1–0·2) | 0 | 0 | 0 |
| Swelling | 58 (0·5%; 0·4–0·7) | 14 (0·1%; 0·1–0·2) | 0 | 0 | 31 (0·3%; 0·2–0·4) | 5 (0·1%; 0·1–0·1) | 0 | 0 |
| Exanthema | 9 (0·1%; 0·1–0·2) | 0 | 0 | 0 | 2 (0·1%; 0–0·1) | 0 | 0 | 0 |
| Itching | 50 (0·5%; 0·3–0·6) | 0 | 5 (0·3%; 0·1–0·8) | 0 | 20 (0·2%; 0·1–0·3) | 0 | 3 (0·2%; 0·1–0·6) | 0 |
| Other | 20 (0·2%; 0·1–0·3) | 0 | 4 (0·3%; 0·1–0·7) | 0 | 3 (0·1%; 0·1–0·1) | 0 | 0 | 0 |
| Systemic reactions | 1404 (12·5%; 11·9–13·1) | 29 (0·3%; 0·2–0·4) | 127 (8·6%; 7·3–10·2) | 1 (0·1%; 0–0·4) | 765 (7·0%; 6·6–7·5) | 6 (0·1%; 0·1–0·1) | 89 (6·1%; 4·9–7·4) | 0 |
| Fever | 1072 (9·6%; 9·1–10·1) | 25 (0·2%; 0·1–0·3) | 97 (6·6%; 5·4–8·0) | 0 | 597 (5·5%; 5·1–5·9) | 4 (0·1%; 0·1–0·1) | 68 (4·6%; 3·6–5·8) | 0 |
| Allergy | 24 (0·2%; 0·1–0·3) | 2 (0·1%; 0–0·1) | 1 (0·1%; 0–0·4) | 0 | 16 (0·2%; 0·1–0·2) | 1 (0·1%; 0–0·1) | 2 (0·1%; 0·1–0·5) | 0 |
| Headache | 162 (1·4%; 1·2–1·7) | 0 | 17 (1·2%; 0·7–1·8) | 1 (0·1%; 0–0·4) | 49 (0·5%; 0·3–0·6) | 0 | 12 (0·8%; 0·4–1·4) | 0 |
| Fatigue | 160 (1·4%; 1·2–1·7) | 0 | 16 (1·1%; 0·6–1·8) | 1 (0·1%; 0–0·4) | 74 (0·7%; 0·5–0·9) | 0 | 13 (0·9%; 0·5–1·5) | 0 |
| Nausea | 56 (0·5%; 0·4–0·7) | 0 | 5 (0·3%; 0·1–0·8) | 1 (0·1%; 0–0·4) | 21 (0·2%; 0·1–0·3) | 0 | 3 (0·2%; 0·1–0·6) | 0 |
| Diarrhoea | 54 (0·5%; 0·4–0·6) | 1 (0·1%; 0–0·1) | 4 (0·3%; 0·1–0·7) | 0 | 30 (0·3%; 0·2–0·4) | 0 | 2 (0·1%; 0·1–0·5) | 0 |
| Myalgia | 75 (0·7%; 0·5–0·8) | 2 (0·1%; 0–0·1) | 6 (0·4%; 0·2–0·9) | 0 | 16 (0·2%; 0·1–0·2) | 0 | 0 | 0 |
| Cough | 73 (0·7%; 0·5–0·8) | 0 | 6 (0·4%; 0·2–0·9) | 0 | 32 (0·3%; 0·2–0·4) | 0 | 6 (0·4%; 0·2–0·9) | 0 |
| Other | 24 (0·2%; 0·1–0·3) | 1 (0·1%; 0–0·1) | 2 (0·1%; 0·1–0·5) | 0 | 31 (0·3%; 0·1–0·4) | 1 (0·1%; 0–0·2) | 1 (0·1%; 0–0·4) | 0 |
| Total | 1813 (16·2%; 15·5–16·9) | 58 (0·5%; 0·4–0·7) | 145 (9·9%; 8·4–11·5) | 1 (0·1%; 0–0·4) | 1143 (10·5%; 9·9–11·1) | 12 (0·1%; 0·1–0·2) | 107 (7·3%; 6·0–8·7) | 0 |

Data are number of participants with adverse event (%; 95% CI). Adverse events up to 3 days after vaccination are reported.

***Table 3:*** **Participants with adverse reactions by dose**

vaccine formulation were regarded as fixed effects. We used PROC MIXED to model GMT and PROC GLIMMIX to model adverse events (SAS version 9.20).

Hualan Biological and Sinovac Biotech registered their studies with ClinicalTrials.gov, numbers NCT00956111 and NCT00975572, respectively. The other eight studies were registered with the State Food and Drug Administration of China.

### Role of the funding source

The sponsors of the study participated in study design and trial monitoring; they had no role in data collection, laboratory testing, statistical analysis, or writing of the report. The first and corresponding authors (X-FL and YW) had full access to the data in the study. The corresponding author took full responsibility for the decision to submit the report for publication.

## Results

12 691 participants were enrolled in the ten centres (figure 1 and table 1), including 2828 children (aged 3 to <12 years), 2887 adolescents (12 to <18 years), 4710 adults (18–60 years), and 2266 older adults (61–87 years). 596 (4·7%) participants dropped out during the study period (figure 1).

12 691 participants received the first dose on day 0, and 12 348 participants received the second dose on day 21. These participants completed the 3-day safety

| | All vaccine formulations | Placebo |
|---|---|---|
| **Children (3 to< 12 years)** | | |
| Number | 2706 | 120 |
| GMT | 5·3 (5·2–5·4) | 5·1 (5·0–5·3) |
| Titre ≥1:10 | 160 (5·9%; 5·0–6·8) | 3 (2·5%; 0·5–7·1) |
| Titre ≥1:40 | 16 (0·6%; 0·3–1·0) | 1 (0·8%; 0–4·6) |
| **Adolescents (12 to <18 years)** | | |
| Number | 2767 | 120 |
| GMT | 7·0 (6·8–7·2) | 6·2 (5·6–6·8) |
| Titre ≥1:10 | 703 (25·4%; 23·8–27·1) | 22 (18·3%; 11·9–26·4) |
| Titre ≥1:40 | 174 (6·3%; 5·4–7·3) | 5 (4·2%; 1·4–9·5) |
| **Adults (18–60 years)** | | |
| Number | 3598 | 1110 |
| GMT | 6·4 (6·3–6·6) | 6·6 (6·3–6·8) |
| Titre ≥1:10 | 741 (20·6%; 19·3–22·0) | 232 (20·9%; 18·5–23·4) |
| Titre ≥1:40 | 136 (3·8%; 3·2–4·5) | 52 (4·7%; 3·5–6·1) |
| **Older adults (>60 years)** | | |
| Number | 2145 | 120 |
| GMT | 6·3 (6·2–6·5) | 6·3 (5·7–7·0) |
| Titre ≥1:10 | 393 (18·3%; 16·7–20·0) | 23 (19·2%; 12·6–27·4) |
| Titre ≥1:40 | 79 (3·7%; 2·9–4·6) | 5 (4·2%; 1·4–9·5) |
| **Total** | | |
| Number | 11 216 | 1470 |
| GMT | 6·3 (6·2–6·3) | 6·4 (6·2–6·6) |
| Titre ≥1:10 | 1997 (17·8%; 17·1–18·5) | 280 (19·0%; 17·1–21·2) |
| Titre ≥1:40 | 405 (3·6%; 3·3–4·0) | 63 (4·3%; 3·3–5·5) |

Data are mean (95% CI) or n (%; 95% CI). GMT=geometric mean titre. Titre=haemagglutination inhibition antibody titre. Although 11 220 participants in the vaccine group and 1471 participants in the placebo group had blood samples taken on day 0, some blood samples were spoiled during transportation and were not suitable for haemagglutination inhibition testing.

***Table 4:*** **Pre-existing antibody against 2009 pandemic influenza A H1N1 in participants, by age-group**

observation and were included in the safety analysis. Blood samples were obtained from 12 686 participants: 12 147 on day 21, 4854 on day 35, and 10 274 on day 42. Haemagglutination inhibition titres were estimated from these blood samples in the immunogenicity analysis.

Diary cards from all recipients of the first dose and all recipients of the second dose were returned and analysed. All eight formulations of the vaccine studied in the ten centres were well tolerated. No immediate systemic allergic reactions, serious adverse events, or events suggestive of Guillain-Barré syndrome were reported. Adverse reactions were mostly mild or moderate, and self-limited.

2594 (23·1%, 95% CI 22·3–23·9) participants assigned to vaccine reported solicited adverse reactions compared with 233 (15·8%, 14·0–17·1) assigned to placebo. 1813 (16·2%, 15·5–16·8) participants reported solicited adverse reactions after the first dose of vaccine compared with 1143 (10·5%, 9·9–11·1) after the second dose. The most frequent local reaction was pain at the injection site and the most frequent systemic reaction was fever (table 2; webappendix pp 3–5).

Severe adverse effects occurred in 69 (0·6%, 95% CI 0·5–0·8) recipients of vaccine compared with one recipient (0·1%, 0–0·2) of placebo (table 2). The most common severe adverse reaction was fever, which occurred in 25 (0·22%; 0·14–0·33) recipients of vaccine after the first dose and four (0·04%; 0·01–0·09) recipients of vaccine after the second dose compared with no recipients of placebo after either dose (table 3).

At baseline, detectable haemagglutination inhibition antibody (titre ≥1:10) was seen in 1997 (17·8%) of 11 216 participants in the vaccine group and 280 (19·0%) of 1470 participants in the placebo group (table 4); seroprotective concentrations of the antibody (titre ≥1:40) were seen in 405 (3·6%) participants in the vaccine group and 63 (4·3%) participants in the placebo group. These proportions were lower in children, but did not differ substantially between the other age-groups (table 4). Similar findings were obtained for GMT.

One dose of vaccine induced robust immune responses in all formulation groups (table 5, figure 2, and webappendix p 6). The seroprotection rate 21 days after the first dose of vaccine ranged from 69·5% (95% CI 65·9–72·8) for the adjuvant split-virion formulation containing 7·5 µg haemagglutinin to 92·8% (91·9–93·6) for the non-adjuvant split-virion formulation containing 30 µg antigen (table 5). The rate was 86·5% (796 of 920; 84·1–88·7) in recipients of one dose of the 7·5 µg non-adjuvant split-virion vaccine compared with 9·8% (140 of 1432; 8·3–11·4) in recipients of placebo ($p<0·0001$).

The second dose of vaccine boosted seroprotection rates to more than 80% 21 days after vaccination (table 6). The boosting effect of the second dose was more pronounced in children (3 to <12 years), who developed a lower level of protection after the first dose of vaccine (table 6). In other age-groups, the second dose of vaccination had limited boosting effects. In participants assigned to placebo, the seroprotection rate increased to 10·8% (95% CI 8·9–13·0) after the second dose (table 6, webappendix p 7).

Two centres assessed the 15 µg split-virion formulations with or without aluminium adjuvant (webappendix p 7). GMTs for non-adjuvant formulations (167·8 and 225·0, respectively) were significantly higher than were those for adjuvant formulations (92·2 and 110·6, respectively; $p<0·0001$).

In the mixed model analysis of cumulative adverse events, the covariance parameter estimate for the study centres was 0·325 (SE 0·157), suggesting moderate within-centre correlation. Of the various vaccine formulations, the non-adjuvant split-virion formulation containing 7·5 µg haemagglutinin was associated with the lowest risk of cumulative adverse events during the

| | Non-adjuvant split-virion formulation | | | Adjuvant split-virion formulation | | | Adjuvant whole-virion formulation | | Placebo |
|---|---|---|---|---|---|---|---|---|---|
| | 7·5 μg | 15 μg | 30 μg | 7·5 μg | 15 μg | 30 μg | 5 μg | 10 μg | |
| **Children (3 to <12 years)** | | | | | | | | | |
| Number | 232 | 1113 | 886 | 198 | 200 | .. | .. | .. | 116 |
| GMT | 78·6 (64·2–96·2) | 85·1 (78·5–92·3) | 107·1 (98·1–116·8) | 38·4 (33·3–44·2) | 52·2 (45·2–60·3) | .. | .. | .. | 6·3 (5·4–7·2) |
| Titre ≥1:40 | 178 (76·7%; 70·7–82·0) | 902 (81·0%; 78·5–83·2) | 777 (87·7%; 85·4–89·8) | 111 (56·1%; 48·8–63·1) | 145 (72·5%; 65·8–78·6) | .. | .. | .. | 6 (5·2%; 1·9–10·9) |
| **Adolescents (12 to <18 years)** | | | | | | | | | |
| Number | 218 | 1091 | 863 | 203 | 204 | 105 | .. | .. | 116 |
| GMT | 578·1 (465·1–718·6) | 522·9 (476·6–573·7) | 696·9 (627·9–773·5) | 99·9 (83·7–119·2) | 166·7 (137·5–201·9) | 234·6 (177·3–310·5) | .. | .. | 7·0 (6–8·1) |
| Titre ≥1:40 | 211 (96·8%; 93·5–98·7) | 1061 (97·3%; 96·1–98·1) | 847 (98·1%; 97·0–98·9) | 172 (84·7%; 79·0–89·4) | 186 (91·2%; 86·4–94·7) | 100 (95·2%; 89·2–98·4) | .. | .. | 6 (5·2%; 1·9–10·9) |
| **Adults (18–60 years)** | | | | | | | | | |
| Number | 323 | 1280 | 991 | 208 | 202 | 105 | 99 | 102 | 1089 |
| GMT | 316·6 (260·8–384·3) | 330·4 (302–361·5) | 414·2 (372·2–461·0) | 77·6 (63·1–94·0) | 121·2 (98·1–149·7) | 187·5 (143·6–244·7) | 87·6 (65·4–117·3) | 155·7 (117–207·1) | 8·6 (8–9·2) |
| Titre ≥1:40 | 289 (89·5%; 85·6–92·6) | 1207 (94·3%; 92·9–95·5) | 932 (94·0%; 92·4–95·4) | 153 (73·6%; 67·0–79·4) | 168 (83·2%; 77·3–88·1) | 93 (88·6%; 80·9–94·0) | 76 (76·8%; 67·2–84·7) | 93 (91·2%; 83·9–95·9) | 116 (10·7%; 8·9–12·6) |
| **Older adults (>60 years)** | | | | | | | | | |
| Number | 147 | 842 | 679 | 108 | 108 | 108 | .. | 100 | 111 |
| GMT | 105·7 (80·5–138·6) | 162·8 (144·9–182·9) | 244·2 (215·0–277·3) | 44·3 (34·0–57·8) | 85·3 (64·2–113·4) | 95·7 (71–129·1) | .. | 37·8 (29·3–48·8) | 8·0 (6·7–9·6) |
| Titre ≥1:40 | 118 (80·3%; 72·9–86·4) | 710 (84·4%; 81·8–86·8) | 616 (90·7%; 88·3–92·8) | 62 (57·4%; 47·5–66·9) | 81 (75·0%; 65·7–82·8) | 83 (76·9%; 67·8–84·4) | .. | 54 (54·0%; 43·7–64·0) | 12 (10·8%; 5·7–18·1) |
| **Total** | | | | | | | | | |
| Number | 920 | 4326 | 3419 | 717 | 714 | 318 | 99 | 202 | 1432 |
| GMT | 215·6 (191·4–242·9) | 228 (216·6–240·0) | 299·6 (282·8–317·4) | 63·1 (57·2–69·5) | 99·4 (89·5–110·4) | 160·7 (136·0–189·9) | 87·6 (65·4–117·3) | 77·3 (62·4–95·7) | 8·2 (7·7–8·6) |
| Titre ≥1:40 | 796 (86·5%; 84·1–88·7) | 3880 (89·7%; 88·7–90·6) | 3172 (92·8%; 91·9–93·6) | 498 (69·5%; 65·9–72·8) | 580 (81·2%; 78·2–84·0) | 276 (86·8%; 82·6–90·3) | 76 (76·8%; 67·2–84·7) | 147 (72·8%; 66·1–78·8) | 140 (9·8%; 8·3–11·4) |

Data are mean (95% CI) or n (%; 95% CI). GMT=geometric mean titre. Titre=haemagglutination inhibition antibody titre.

*Table 5:* **Haemagglutination inhibition antibody response 21 days after first dose of vaccine or placebo**

21 days after the first dose; higher antigen contents were associated with higher risks of adverse events (webappendix p 8). In the mixed model analysis of GMTs, the covariance parameter estimate was 0·097, suggesting little within-centre correlation. When we examined GMTs in relation to various formulations, the non-adjuvant split-virion formulations had the best outcomes; higher antigen content was associated with higher GMTs (webappendix p 9). Adjuvant whole-virion formulations were associated with the lowest GMTs (webappendix p 9). Children had the lowest GMTs, whereas adolescents and adults aged 18–60 years had higher GMTs than did adults older than 60 years (webappendix p 9).

## Discussion

Our study showed that all formulations of the 2009 pandemic H1N1 vaccine produced by ten Chinese manufacturers were safe and immunogenic. In adolescents (12 to <18 years), adults (18–60 years), and older adults (>60 years), one dose of the non-adjuvant split-virion vaccine containing 7·5 μg haemagglutinin induced nearly the same level of immune response as did formulations containing more antigen. This formulation required the lowest amount of antigen, which is important during a pandemic when the vaccine is in high demand and production is limited. It was also associated with the lowest frequency of adverse reactions. Therefore, this formulation seemed to have the best overall outcome. In young children, however, a two-dose schedule of the 7·5 μg non-adjuvant formulation seemed necessary. Compared with the non-adjuvant split-virion formulation, the adjuvant split-virion and whole-virion formulations were associated with lower immunogenicity and higher rates of adverse reactions.

In clinical trials assessing the immunogenicity of influenza vaccine in the context of a pandemic, potential concurrent infection with the pandemic virus could be a powerful confounder. Therefore, a placebo group is essential in such trials. In our study, all ten centres had a placebo group in adults aged 18–60 years; in one centre a

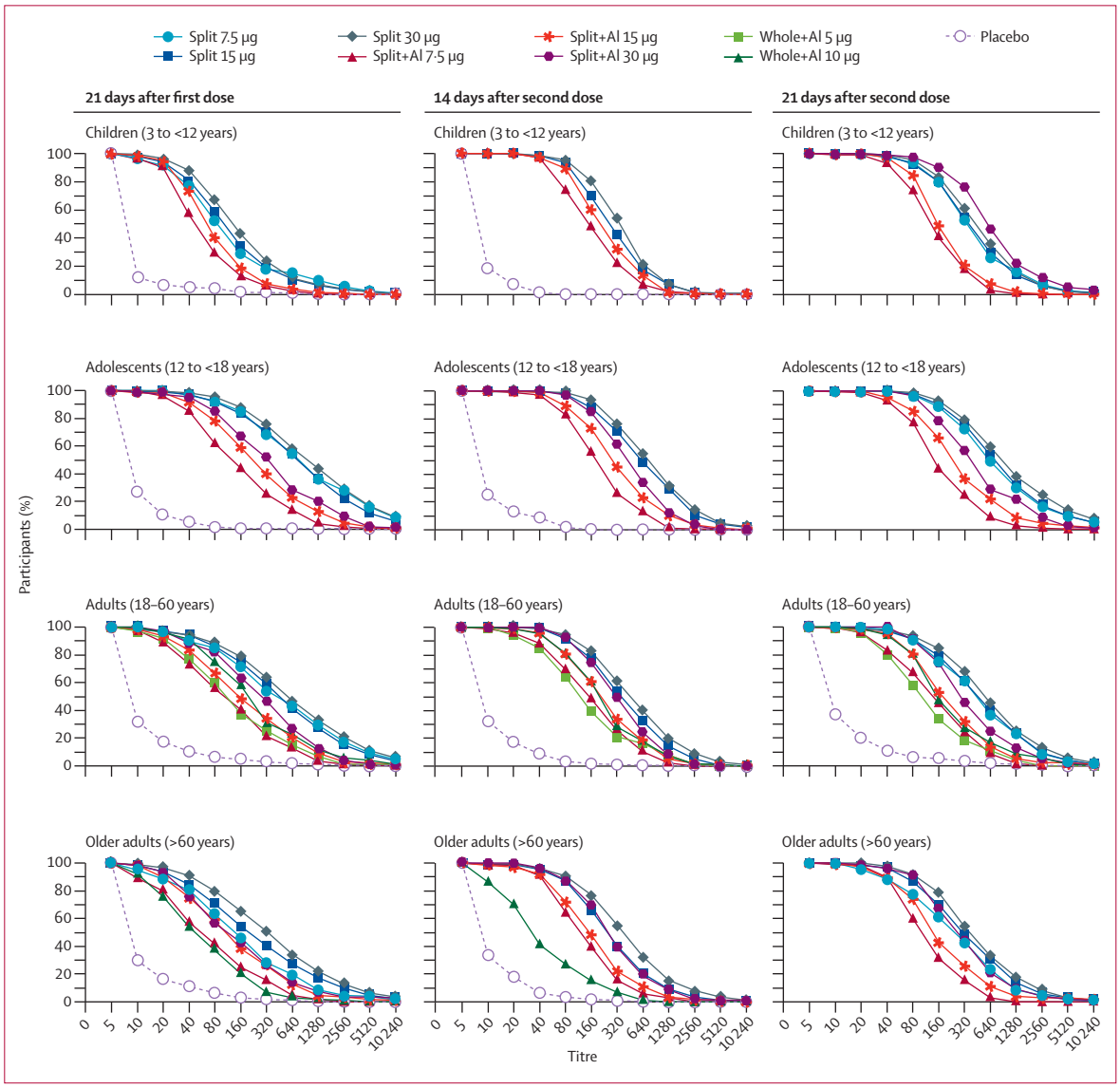


***Figure 2*: Reverse cumulative distribution curves of antibody titres, by follow-up time after receipt of vaccine or placebo**
Split=non-adjuvant split virion formulation. Split+Al=aluminium-adjuvant split-virion formulation. Whole+Al=aluminium-adjuvant whole-virion formulation. Titres are expressed as the reciprocal of the dilution. The limit of detection was a titre of 1:5.

placebo group was included in all age-groups. Our findings showing that the vaccinated groups had excellent antibody responses, whereas the placebo groups had only slight antibody responses, adds weight to the credibility of our results.

At baseline, nearly a fifth of all study participants had detectable antibody against 2009 pandemic H1N1 virus (titre ≥1:10) before vaccination. Additionally, approximately 4% had seroprotective concentrations of antibody, which is higher than the rate of seroprotection reported in China's remote Guangxi Province (1·7%),[8] but lower than that reported in the USA (6–9%).[4] In our study, the baseline seroprotection rate in older adults (>60 years) was similar to that in adults aged 18–60 years and adolescents. This finding contrasts with rates reported in the USA, where a third of adults older than 60 years had seroprotective concentrations of antibody before vaccination.[4] The high rate of seroprotection in the US elderly population has been attributed to previous exposure (through infection or vaccination) to a virus that is genetically and antigenically similar to the 2009 pandemic H1N1 virus.[4] Our findings suggest that, by contrast with older adults in the USA, previous exposures did not affect the Chinese population of similar ages.

Aluminium adjuvant is thought to improve the immune response to vaccine. However, in our study, the adjuvant formulations were less effective than were the

| | Non-adjuvant split-virion formulation | | | Adjuvant split-virion formulation | | | Adjuvant whole-virion formulation | | Placebo |
|---|---|---|---|---|---|---|---|---|---|
| | 7·5 μg | 15 μg | 30 μg | 7·5 μg | 15 μg | 30 μg | 5 μg | 10 μg | |
| **Children (3 to <12 years)** | | | | | | | | | |
| Number | 220 | 971 | 756 | 195 | 194 | .. | .. | .. | .. |
| GMT | 273·4 (233·1–320·5) | 273·7 (254·4–294·4) | 314·6 (290·7–340·4) | 94·9 (83·6–107·7) | 121·1 (107·3–136·7) | .. | .. | .. | .. |
| Titre ≥1:40 | 215 (97·7%; 94·8–99·3) | 955 (98·4%; 97·3–99·1) | 747 (98·9%; 97·9–99·5) | 181 (92·8%; 88·2–96·0) | 188 (96·9%; 93·4–98·9) | .. | .. | .. | .. |
| **Adolescents (12 to <18 years)** | | | | | | | | | |
| Number | 195 | 917 | 717 | 192 | 183 | 90 | .. | .. | .. |
| GMT | 513·4 (425·7–619·2) | 561·5 (516·3–610·7) | 711·1 (644·1–785·1) | 114 (98·0–132·5) | 188·3 (157·4–225·2) | 310·3 (242·1–397·6) | .. | .. | .. |
| Titre ≥1:40 | 195 (100%; 98·1–100·0) | 917 (100%; 99·6–100·0) | 716 (99·9%; 99·2–100·0) | 179 (93·2%; 88·7–96·3) | 175 (95·6%; 91·6–98·1) | 90 (100%; 96–100·0) | .. | .. | .. |
| **Adults (18–60 years)** | | | | | | | | | |
| Number | 309 | 1125 | 857 | 200 | 196 | 96 | 98 | 101 | 951 |
| GMT | 320·7 (277·3–371) | 328·4 (303·4–355·4) | 403 (367·3–442·1) | 95·5 (81·3–112·1) | 141·9 (120·1–167·6) | 236·3 (187·7–297·5) | 81·1 (63·9–103·1) | 140·4 (109·9–179·5) | 9·1 (8·5–9·8) |
| Titre ≥1:40 | 304 (98·4%; 96·3–99·5) | 1094 (97·2%; 96·1–98·1) | 837 (97·7%; 96·4–98·6) | 166 (83·0%; 77·1–87·9) | 187 (95·4%; 91·5–97·9) | 96 (100%; 96·2–100·0) | 79 (80·6%; 71·4–87·9) | 95 (94·1%; 87·5–97·8) | 103 (10·8%; 8·9–13·0) |
| **Older adults (>60 years)** | | | | | | | | | |
| Number | 137 | 711 | 554 | 103 | 101 | 105 | .. | .. | .. |
| GMT | 163·3 (129·2–206·4) | 232·2 (210·6–256) | 288·8 (260–320·8) | 77·9 (64·9–93·5) | 114·3 (88·9–147·0) | 202·9 (161·8–254·4) | .. | .. | .. |
| Titre ≥1:40 | 121 (88·3%; 81·7–93·2) | 687 (96·6%; 95·0–97·8) | 540 (97·5%; 96·0–98·7) | 92 (89·3%; 81·7–94·5) | 90 (89·1%; 81·3–94·4) | 101 (96·2%; 90·5–99·0) | .. | .. | .. |
| **Total** | | | | | | | | | |
| Number | 861 | 3724 | 2884 | 690 | 674 | 291 | 98 | 101 | 951 |
| GMT | 307·6 (281·2–336·5) | 334·5 (320·6–349·0) | 408 (388·7–428·2) | 97·1 (89·9–104·9 | 141·7 (130–154·5) | 243·3 (212·5–278·6) | 81·1 (63·9–103·1) | 140·4 (109·9–179·5) | 9·1 (8·5–9·8) |
| Titre ≥1:40 | 835 (97·0%; 95·6–98) | 3653 (98·1%; 97·6–98·5) | 2840 (98·5%; 98–98·9) | 618 (89·6%; 87–91·7) | 640 (95·0%; 93–96·5) | 287 (98·6%; 96·5–99·6) | 79 (80·6%; 71·4–87·9) | 95 (94·1%; 87·5–97·8) | 103 (10·8%; 8·9–13·0) |

Data are mean (95% CI) or n (%; 95% CI). GMT=geometric mean titre. Titre=haemagglutination inhibition antibody titre.

*Table 6:* **Haemagglutination inhibition antibody response 21 days after second dose of vaccine or placebo**

non-adjuvant formulations for similar antigen contents. Similar results have been reported in trials of the influenza A H5N1 vaccine.[17,18] There have been no satisfactory explanations for this finding; however, one suggestion is the delay in the release of antigens absorbed onto aluminium.

Our study has at least two limitations. First, influenza-like illnesses were not actively reported during the 42-day follow-up period in any of the study centres. However, the national influenza surveillance system showed no circulation of 2009 pandemic H1N1 virus in the study areas (Chinese Center for Disease Control and Prevention, unpublished data) and data for the placebo group also suggested no relevant circulation of the virus in the study areas. Second, we did not obtain detailed information about the 596 participants who dropped out of the trial; hence we were unable to provide a detailed breakdown of the reasons for dropping out of the study.

Previous studies have shown that one dose of non-adjuvant split-virion vaccine containing 15 μg haemagglutinin could induce satisfactory immune responses in people aged 12–64 years.[7,19] A single 7·5 μg dose of MF59-adjuvant vaccine also generated protective antibody responses in adults aged 18–50 years.[6] Our study suggested that one 7·5 μg dose of non-adjuvant split-virion vaccine could induce protective levels of antibody in individuals aged 3 years or older. However, in children (aged 3 to <12 years, two 7·5 μg doses of the formulation might be necessary to induce a satisfactory immune response. Our findings will help to inform the development of an antigen-sparing strategy for mass immunisation during the pandemic, when demand for vaccine far exceeds production capacity.

Thus, we recommend that non-adjuvant split-virion vaccine containing 7·5 μg haemagglutinin is adopted as the vaccine of choice against 2009 pandemic H1N1 in adolescents and adults. A two-dose schedule of this formulation might be needed in children.

**Contributors**

X-FL, H-QW, and YW designed the study. JW, F-CZ, R-CL, S-LX, Y-LZ, F-JL, and S-HY managed the study and served as principle investigators.

J-ZW and H-HF undertook the immunological assays. W-DY, KA, D-JF, X-LC, F-CQ, C-JJ, Y-HZ, Z-JG, P-YC, ZC, and K-MY were responsible for vaccine batch production. All authors contributed to data interpretation and to the writing of the report.

#### Conflicts of interest

W-DY, KA, D-JF, X-LC, F-CQ, C-JJ, Y-HZ, Z-JG, P-YC, ZC, and K-MY are employed by manufactures of the study vaccines. JW, F-CZ, R-CL, S-LX, Y-LZ, F-JL, and S-HY have received research funding from the manufacturers of the study vaccines. All other authors declare that they have no conflicts of interest.

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