Transplacental transfer efficiency of maternal antibodies against influenza A(H1N1)pdm09 virus and dynamics of naturally acquired antibodies in Chinese children: a longitudinal, paired mother–neonate cohort study

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Summary

Background The 2009 pandemic H1N1 influenza A virus (A(H1N1)pdm09 virus) evolves rapidly and has continued to cause severe infections in children since its emergence in 2009. We aimed to characterise the kinetics of maternally and naturally acquired antibodies against historical A(H1N1)pdm09 strains and to assess the extent to which the response to heterologous strains following infection or vaccination affects observed A(H1N1)pdm09 strain-specific antibody titres in a Chinese paediatric population.

Methods In this retrospective study, we used residual serum samples from 528 mother-neonate pairs from a non-interventional, longitudinal cohort study in southern China conducted from Sept 20, 2013, to Aug 24, 2018, from six local hospitals in Anhua County, Hunan Province, China. Mother-neonate pairs were eligible for inclusion if the neonates were born after Sept 20, 2013, and their mothers had resided in the study sites for at least 3 months. We tested samples with a haemagglutination inhibition (HAI) assay to measure antibody levels against three historical A(H1N1)pdm09 strains that were antigenically similar to the strains that circulated during the 2009 pandemic (A/Hunan-Kaifu/SWL4204/2009 [SWL4204/09 strain], A/Hunan-Daxiang/SWL1277/2016 [SWL1277/16 strain], and A/Hunan-Yanfeng/SWL185/2018 [SWL185/18 strain]). We also determined the seroprevalence, geometric mean titres (GMTs), transfer ratio of maternal antibodies, and the dynamics of maternally and naturally acquired antibodies in children, from birth to 3 years of age.

Findings 1066 mother-neonate pairs were enrolled in the original cohort between Sept 20, 2013, and Oct 14, 2015. Of these, 528 pairs (523 mothers, 528 neonates) were selected for the present study. The median age of the mothers was 25 years (IQR 23 to 29). 291 (55%) of 528 children were boys and 237 (45%) were girls, and most children (452 [86%]) were breastfed before the age of 6 months. The GMTs and the seroprevalence for the SWL4204/09 strain were higher than those for the SWL1277/16 and SWL185/18 strains among mothers (GMTs: 10·4 [95% CI 9·8 to 11·1] *vs* 9·3 [8·7 to 9·8] *vs* 8·0 [7·5 to 8·4], p<0·0001; seroprevalence: 11·1% [95% CI 8·5 to 14·1] *vs* 6·9% [4·9 to 9·4] *vs* 4·6% [3·0 to 6·8], p=0·0003) and among neonates (GMTs: 10·7 [10·0 to 11·5] *vs* 9·4 [8·8 to 10·0] *vs* 8·1 [7·6 to 8·6], p<0·0001; seroprevalence: 13·4% [10·7 to 16·7] *vs* 8·7% [6·5 to 11·5] *vs* 6·1% [4·2 to 8·5], p=0·0002). Regardless of the A(H1N1)pdm09-specific strain, maternal antibodies could be transferred efficiently via the placenta (mean transfer ratios: 1·10 for SWL4204/09 *vs* 1·09 for SWL1277/16 *vs* 1·06 for SWL185/18; p=0·93). The A(H1N1)pdm09 strain-specific antibodies waned below the protective threshold of 1:40 within 2 months after birth. After maternal antibody waning, there were periodic increases and decreases in HAI antibody titres against three A(H1N1)pdm09 strains, and such increases were all significantly associated with a higher immune response to heterologous strains. Vaccination against the SWL4204/09 strain was associated with a poor response to the SWL185/18 strain (β–0·20, 95% CI –0·28 to –0·13; p<0·0001).

Interpretation Our findings suggest low pre-existing immunity against influenza A(H1N1)pdm09 virus among unvaccinated Chinese adult female and paediatric populations. This evidence, together with the rapid decay of maternal antibodies and the observed cross-reactivity among different A(H1N1)pdm09 strains, highlights the importance of accelerating maternal and paediatric influenza vaccination in China.

Funding The Key Program of the National Natural Science Foundation of China.

Introduction

Seasonal influenza causes considerable morbidity and mortality worldwide and is responsible for an estimated 3–5 million cases of severe illness and 290 000–650 000 deaths due to respiratory disease annually.[1] Of the influenza type A viruses (H1N1 and H3N2), which cause most of the widespread seasonal influenza epidemics,[2] the 2009 pandemic H1N1

Lancet Microbe 2023; 4: e893–902

Published Online October 9, 2023 https://doi.org/10.1016/S2666-5247(23)00181-7

For the Chinese translation of the abstract see Online for appendix 1

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Research in context

Evidence before this study

Developing the optimal schedule for paediatric influenza vaccination and facilitating a comprehensive understanding of paediatric risk of 2009 pandemic influenza A virus (A(H1N1)pdm09 virus) infections require a full evaluation of the levels and kinetics of A(H1N1)pdm09-specific antibodies in children. We searched Google Scholar, PubMed, MEDLINE and Web of Science for articles published between Jan 1, 2009, and April 15, 2023, using the search terms "influenza A(H1N1) pdm09 virus" AND "human" AND "sero*", without any language restrictions. Several studies have independently characterised maternal or post-vaccination antibody dynamics in children in placebo groups of clinical trials and in population-based studies since the 2009 pandemic. However, they did not involve any quantification of the long-term kinetics of antibodies specific to the A(H1N1)pdm09 strain in the paediatric population from birth through early childhood.

Added value of this study

In this study, we assessed the efficiency of transplacental transfer of maternal antibodies against historical A(H1N1)pdm09 strains, and we determined the kinetics of A(H1N1)pdm09-specific antibodies resulting from placental transfer and later infection in Chinese children, from birth to 3 years of age. To our knowledge, this study is the first to quantitively assess the long-term kinetics of antibodies against historical A(H1N1)pdm09 strains in the Chinese paediatric population in this age group. We found that, regardless of strain, maternal antibodies could be transferred efficiently via the placenta; but titres rapidly declined below the positive threshold value within 2 months after birth. Following the disappearance of maternal antibodies, antibody titres against different strains of influenza A(H1N1)pdm09 virus subsequently increased because of natural infection or vaccination, and then decreased and increased periodically thereafter, consistent with the annual epidemics of the virus. In addition, a dependency between children's SWL185/18 strain-specific antibody titres and their age, previous vaccination against the SWL4204/09-like strain, and the time they had been alive since the SWL185/18 strain epidemic was observed.

Implications of all the available evidence

The findings of this study call for the acceleration of maternal and paediatric influenza vaccination in China to effectively protect both pregnant women and their children. This study also contributes evidence regarding the cross-reactivity between different strains of influenza A(H1N1)pdm09 virus after natural infection or vaccination, which might help relevant stakeholders in planning and implementing paediatric influenza vaccination schedules in China.

influenza A virus (A(H1N1)pdm09 virus) accounts for a substantial burden worldwide, including in China. It is estimated that the mean excess respiratory mortality rate for influenza A(H1N1)pdm09 virus was 1·6 deaths per 100 000 person-seasons in all age groups in China during 2010–15.[3] However, quantification of the burden related to A(H1N1)pdm09 in Chinese children younger than 3 years is lacking. Studies outside China have consistently identified a significantly increased risk of A(H1N1)pdm09-related hospitalisation and admission to intensive care unit among children aged 0–4 years,[4–6] which implies that influenza epidemic control must place a high priority on reducing A(H1N1)pdm09-related morbidity and mortality burden among young children. Prevention of influenza A(H1N1)pdm09 infections in the paediatric population is thus an important health priority in China.

Influenza vaccination is the most effective way to prevent influenza A(H1N1)pdm09 infections, including in young children aged 6 months to 5 years.[7] The influenza vaccine is not included in the national immunisation programme (NIP) in China. Although some local governments offer free influenza vaccines for schoolchildren (6–18 years) and older people (≥60 years),[8] other priority populations for influenza vaccination in China, such as pregnant women, infants, toddlers, and preschool children aged between 3–5 years, are still required to pay for the vaccine. Moreover, no licensed influenza vaccines are available for children younger than 6 months. To promote the introduction of influenza vaccines into the NIP, we need to identify the optimal influenza immunisation schedule for the Chinese paediatric population, which requires a good understanding of the kinetics of their maternally and naturally acquired A(H1N1)pdm09 antibodies. To date, although evidence exists on maternal or post-vaccination antibody dynamics in children shown in placebo groups of clinical trials and in population-based studies,[9–11] it does not involve any quantification of the long-term kinetics of antibodies specific to the A(H1N1)pdm09 strain in the paediatric population from birth through early childhood.

We assessed the efficiency of transplacental transfer of maternal antibodies against historical A(H1N1)pdm09 strains using paired maternal and cord serum samples from a cohort of mother–neonate pairs in southern China. We also determined the kinetics of antibodies specific to the A(H1N1)pdm09 strain resulting from placental transfer and natural infection in Chinese children from birth to age 3 years.

Methods

Study design and participants

We used a subset of archived serum samples from a longitudinal cohort of mother–neonate pairs that was originally established to investigate the seroepidemiological characteristics of paediatric enterovirus A71 infections in Anhua County, Hunan Province, China.

Details of the cohort profile have been provided elsewhere.[12] In brief, mother–neonate pairs were enrolled by well trained nurses in six local hospitals. Mother–neonate pairs were eligible for inclusion if the neonates were born after Sept 20, 2013, and their mothers had resided in the study sites for at least 3 months. A peripartum venous blood sample (2 mL) was collected from each mother (ie, adult female participants) around the time of delivery; blood samples were collected from neonates at birth (baseline, Sept 20, 2013–Oct 14, 2015; 2 mL cord blood sample) and at months 2, 4, 6, 12, 24, and 36 (2 mL venous blood samples). Follow-up visits at 2-months to 6-months were performed within 2 weeks of the scheduled age, while 12-month to 36-month follow-up visits were investigated intensively during either February–March or August–November of 2014–2018, choosing whichever period was closest to a particular child's birthday. This schedule made some follow-up visits take place at an age that was greater than the theoretical maximum age at follow-up (36 months). Participants' epidemiological information was collected using a structured questionnaire (appendix 2, p 11). Influenza vaccination history was collected from vaccination cards, which serve as official and legal documents to show that vaccination schedules are complete,[13] by trained investigators during the final three follow-up visits in September 2017, March 2018, and August 2018.

We calculated a minimum number of participants necessary to conduct this study. We found that a sample size of 450 mother-neonate pairs would allow a 12% annual risk of influenza infection to be estimated within ±3·0%, with a significance level of 5%. All participants in the cohort had stored baseline serum samples available for analysis, but child participant serum samples from later timepoints did not all have sufficient volume for analysis. We therefore selected half of the original cohort participants at baseline (n=528) who had enough serum samples at baseline and subsequent follow-up visits. We categorised child participants at each timepoint into two groups based on seasonal influenza vaccination status (ie, vaccinated and unvaccinated children) if their vaccination cards were available.

This study was approved by the Institutional Review Board from WHO Western Pacific Regional Office (2013.10.CHN.2.ESR), the Chinese Centre for Disease Control and Prevention (201224), Fudan University (2019–05–0756), and the London School of Hygiene & Tropical Medicine (15698). All enrolled mothers provided written informed consent for themselves and their neonates before participation and before psychological, clinical, and laboratory evaluations.

Procedures

Appendix 2 (pp 4–11) describes the annual influenza activity in our study regions, the procedures of haemagglutination inhibition (HAI) assay and its validation using a microneutralisation assay, and the selection of representative influenza A(H1N1)pdm09 strains used in the HAI assay. Briefly, serum samples were tested against three historical A(H1N1)pdm09 strains that were circulating in the study location in 2009–18 and were antigenically similar to the strains that circulated during the 2009 pandemic (A/Hunan-Kaifu/SWL4204/2009 [SWL4204/09 strain], A/Hunan-Daxiang/SWL1277/2016 [SWL1277/16 strain], and A/Hunan-Yanfeng/SWL185/2018 [SWL185/18 strain]; appendix 2 pp 5, 7). HAI antibody titres were determined by testing serial 2-fold dilutions from 1:10 to 1:1280 in duplicate using a standard protocol.[14] A summary of the suppliers for all reagents, kits, and equipment used in this study is provided in appendix 2 (p 10). The HAI antibody titres are expressed as the reciprocal of the highest dilution of serum from complete HAI wells. Samples with undetectable titres (<1:10) were assumed to have a titre of 1:5.

Statistical analysis

We estimated the point estimates of geometric mean titres (GMTs) and seroprevalence of strain-specific maternal antibodies among all mother–neonate pairs, along with the 95% CIs. The 95% CIs of seroprevalence were calculated using the Clopper-Pearson (binomial) method. We also calculated the mean transfer ratio of strain-specific maternal antibodies, which was defined as the geometric mean of the ratio of the neonate-to-mother titre. The threshold of protection against influenza infection in the paediatric population has not been established. Therefore, we defined seropositivity (or protective immunity) as an HAI titre of 1:40 (a generally accepted threshold[15]) or higher, while seronegativity referred to an HAI titre below 1:40 in the main analysis. We also did a sensitivity analysis using an additional protective threshold of 1:110; rationale for this choice is provided in appendix 2 (p 11).[16] For comparison of the study groups, we assessed group means with a *t*-test and proportions with the χ^2 test or Fisher's exact test; all tests were two-sided with a significance threshold of 0·05.

See Online for appendix 2

In the main analysis, we characterised the natural dynamics of A(H1N1)pdm09 strain-specific antibodies by using titre data from children with complete baseline characteristics and without any record of influenza immunisation in their vaccination cards. The baseline characteristics included sex, gestational age, mode of delivery, birthweight, and breastfeeding status. We did a prespecified subgroup analysis to determine the differences between unvaccinated and vaccinated children in the dynamics of antibodies against the SWL4204/09 strain (antigenically similar to the WHO-recommended vaccine strain in 2009–17, A/California/07/2009). Because children are not obliged to provide their vaccination cards at the time of influenza vaccination, there was a possibility that their influenza immunisation record was not completely reported in their vaccination cards. Therefore, in a post-hoc sensitivity analysis, the dynamics of

SWL4204/09 strain-specific antibodies in vaccinated and unvaccinated children were refitted after accounting for the uncertainties in individual vaccination records. In this analysis, 9·4% (the influenza vaccination coverage in our study location[17]) of children who did not have influenza immunisation records (including both children without a vaccination card and those without any influenza immunisation records in their vaccination cards) were assumed to be vaccinated and then included in the vaccinated group; the remaining 90·6% of children without any flu immunisation record were considered unvaccinated, and all children with influenza immunisation records in their vaccination cards were considered vaccinated. In these analyses, we used generalised additive mixed models with spline functions as smooth terms and random effects for child participants to fit the A(H1N1)pdm09 strain-specific antibody dynamics across ages, incorporating participants' baseline characteristics.

To explore potential factors associated with levels of A(H1N1)pdm09 strain-specific antibodies, we first calculated the natural logarithm of A(H1N1)pdm09 strain-specific titres. As the distribution of the log-transformed titres was positively skewed, we used generalised linear mixed models (GLMMs) with a Gaussian distribution and identity link function and predictive variables as fixed effects to assess factors that affect levels of A(H1N1)pdm09 strain-specific antibodies. In the GLMM models, the maternal factors were the mother's age, gravidity, parity, mode of delivery, underlying conditions, socioeconomic status, influenza vaccination history before pregnancy, A(H1N1)pdm09 strain-specific titres, and the length of time they had been alive since the strain epidemic. The neonatal factors included the child's age, baseline characteristics, influenza vaccination status, titres against heterologous A(H1N1)pdm09 strains, having been alive during the strain epidemic and the length of time they had been alive since the strain epidemic. In these models, we also considered each child as a random effect due to repeated measurements for the same individual over time. We used the Kaplan-Meier estimator to determine the age-specific probability of seropositivity among children who had above-threshold titres against the SWL4204/09, SWL1277/16, or SWL185/18 strains at birth. We also determined age-specific probability of seroconversion (ie, a change from seronegativity to seropositivity) in children who had below-threshold titres against the SWL1277/16 or SWL185/18 strain at birth and were not vaccinated during follow-up visits. All analyses were performed in R (version 4.1.1).

Role of the funding source

The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Results

In the original cohort, 1066 mother–neonate pairs were enrolled between Sept 20, 2013, and Oct 14, 2015. Of these, 528 pairs (from 523 mothers, including five pairs of twins) were selected for the present study (figure 1). The maternal and neonatal characteristics were similar between selected and non-selected mother–neonate pairs at baseline, except for their geographical distribution (appendix 2 pp 11–12). Among the selected mother–neonate pairs, all 523 women were included in this study before the SWL185/18 strain epidemic; all child

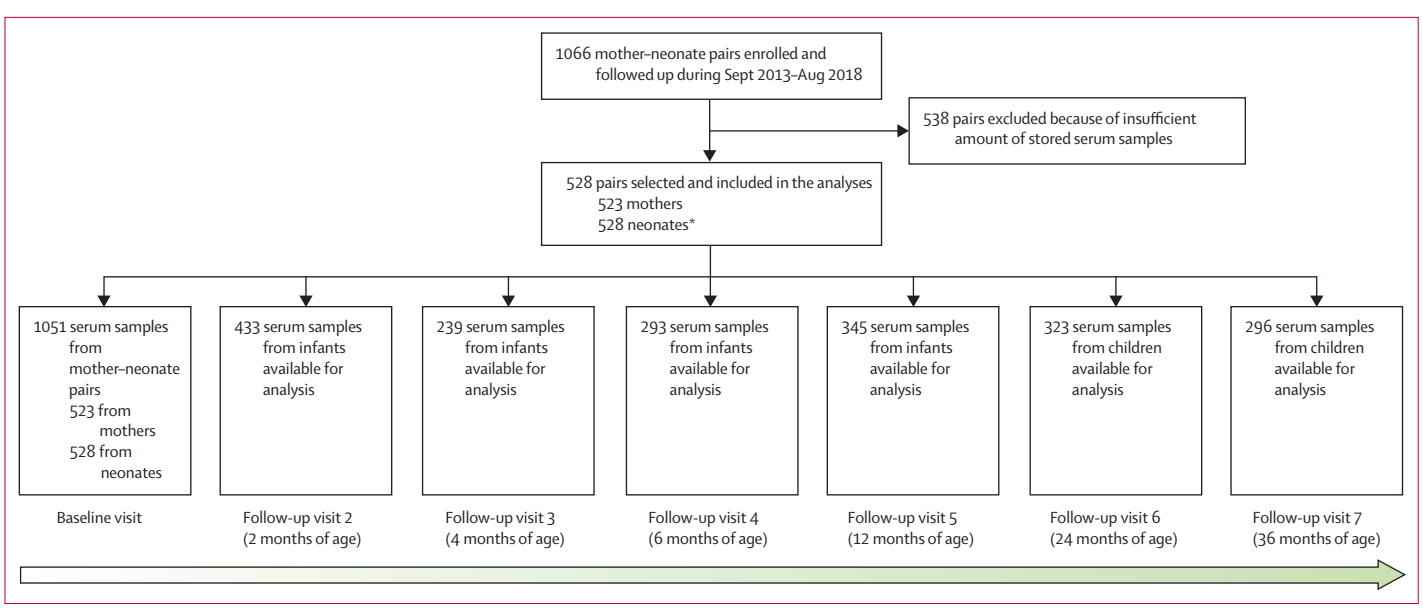

Figure 1: **Selection flowchart of the mother–neonate pairs**
*Includes five pairs of twins.

participants were born and enrolled after the SWL1277/16 strain epidemic, and none of them were born and enrolled during the SWL185/18 strain epidemic, although a minority (n=96, 18%) were followed up during the SWL185/18 strain epidemic (appendix 2 p 13).

The median age of the selected mothers was 25 years (IQR 23–29), and the median gestational age was 40·0 weeks (39·0–40·7; table 1). 291 (55%) of 528 children were boys and 237 (45%) were girls, and most children were breastfed before the age of 6 months (452 [86%]). The follow-up time for child participants ranged from 2 to 42 months (median 35, IQR 7–38). Of the participants with available immunisation records, no adult female participants and 57 (16%) of 351 child participants had received an influenza vaccine (table 1). Among 57 vaccinated children, the median age at vaccination was 14·0 months (IQR 10·0–23·3).

Regardless of maternal age, the GMTs and the seroprevalence for the SWL4204/09 strain were higher than those for the SWL1277/16 and SWL185/18 strains among mothers (GMTs: 10·4 [95% CI 9·8–11·1] *vs* 9·3 [8·7–9·8] *vs* 8·0 [7·5–8·4], p<0·0001; seroprevalence: 11·1% [95% CI 8·5–14·1] *vs* 6·9% [4·9–9·4] *vs* 4·6% [3·0–6·8], p=0·0003) and among neonates (GMTs: 10·7 [10·0–11·5] *vs* 9·4 [8·8–10·0] *vs* 8·1 [7·6–8·6], p<0·0001; seroprevalence: 13·4% [10·7–16·7] *vs* 8·7% [6·5–11·5] *vs* 6·1% [4·2–8·5], p=0·0002; figure 2). A positive correlation of maternal and neonatal titres against the SWL4204/09, SWL1277/16, and SWL185/18 strains was observed (p<0·0001), yet the geometric mean of the ratio of neonate-to-mother titres did not show strain-specific differences (1·10 for SWL4204/09 *vs* 1·09 for SWL1277/16 *vs* 1·06 for SWL185/18; p=0·93; appendix 2 p 13). We also observed significantly higher GMTs among the adult participants younger than 30 years than among those older than 30 years for the SWL4204/09 (p=0·0057) and SWL1277/16 (p=0·036) strains, whereas there was no age-specific difference in their SWL185/18 strain-specific titres (p=0·051; figure 2).

Very few child participants had protective immunity against the three A(H1N1)pdm09 strains at birth and during subsequent follow-up visits (figure 3A; appendix 2 p 14). Maternal antibody titres declined rapidly and reached their lowest mean value at 6·9 months, regardless of the strain (figure 3B). With age, strain-specific antibody titres periodically increased and decreased. Notably, the peak values of mean antibody titres against these strains increased yearly (figure 3B), which was consistent with the annual periodicity of the A(H1N1)pdm09 virus epidemic (appendix 2 p 4). Additionally, age-specific patterns of the antibody titres specific to the SWL4204/09 strain did not significantly differ between vaccinated and unvaccinated children (peak mean titre at 32·8 months: 10·6 *vs* 11·3, p=0·072; appendix 2 p 14), even after adjusting for uncertainties in individual vaccination records (appendix 2 p 15).

	Mothers (n=523)	Children (n=528)*
Age at delivery		
Median age, years	25 (23–29)	..
16–19 years	22 (4%)	..
20–29 years	377 (72%)	..
30–48 years	122 (23%)	..
Missing	2 (<1%)	..
Primiparous	250 (48%)	..
Caesarean section	188 (36%)	..
Sex		
Female	..	237 (45%)
Male	..	291 (55%)
Gestational age		
Median, weeks	..	40·0 (39·0–40·7)
Preterm birth	..	41 (8%)
Full-term birth	..	458 (87%)
Post-term birth	..	29 (5%)
Birthweight		
Median, g	..	3300 (3000–3600)
<2500 g	..	17 (3%)
2500 g to <4000 g	..	471 (89%)
≥4000 g	..	40 (8%)
Breastfeeding		
Yes	..	452 (86%)
No	..	62 (12%)
Missing	..	14 (3%)
Underlying conditions		
Any underlying conditions†	79 (15%)	..
Chronic liver disease	37 (7%)	..
Hypertension	10 (2%)	..
Diabetes	3 (1%)	..
Other diseases‡	36 (7%)	..
Socioeconomic status		
Low	121 (23%)	..
Middle	103 (20%)	..
High	148 (28%)	..
Missing	151 (29%)	..
Vaccination against influenza§		
Yes	0	57 (11%)
No	334 (64%)	294 (56%)
Unknown	189 (36%)	177 (34%)

Data are expressed as n (%) or median (IQ) unless otherwise specified. *Including five pairs of twins. †The numbers presented in each subgroup may not total 79 because one individual had multiple underlying diseases. ‡This category refers to chronic respiratory disease (n=1), cardiovascular disease (n=1), metabolic diseases (n=1), chronic kidney disease (n=2), and other diseases (n=31). Of these, other diseases included cancer, HIV/AIDS, disabilities, chronic haematopathy (eg, sickle-cell disorders), systemic autoimmune diseases (eg, lupus erythaematosus), chromosomal disorders (eg, Down syndrome), and others. §Information on influenza vaccination before and during pregnancy was collected; this information was collected for children during the final three follow-up visits.

Table 1: **Baseline characteristics of mother–neonate pairs**

Strain-specific antibody titres in children were found to be highly associated with their maternal antibody levels and response to heterologous A(H1N1)pdm09 strains

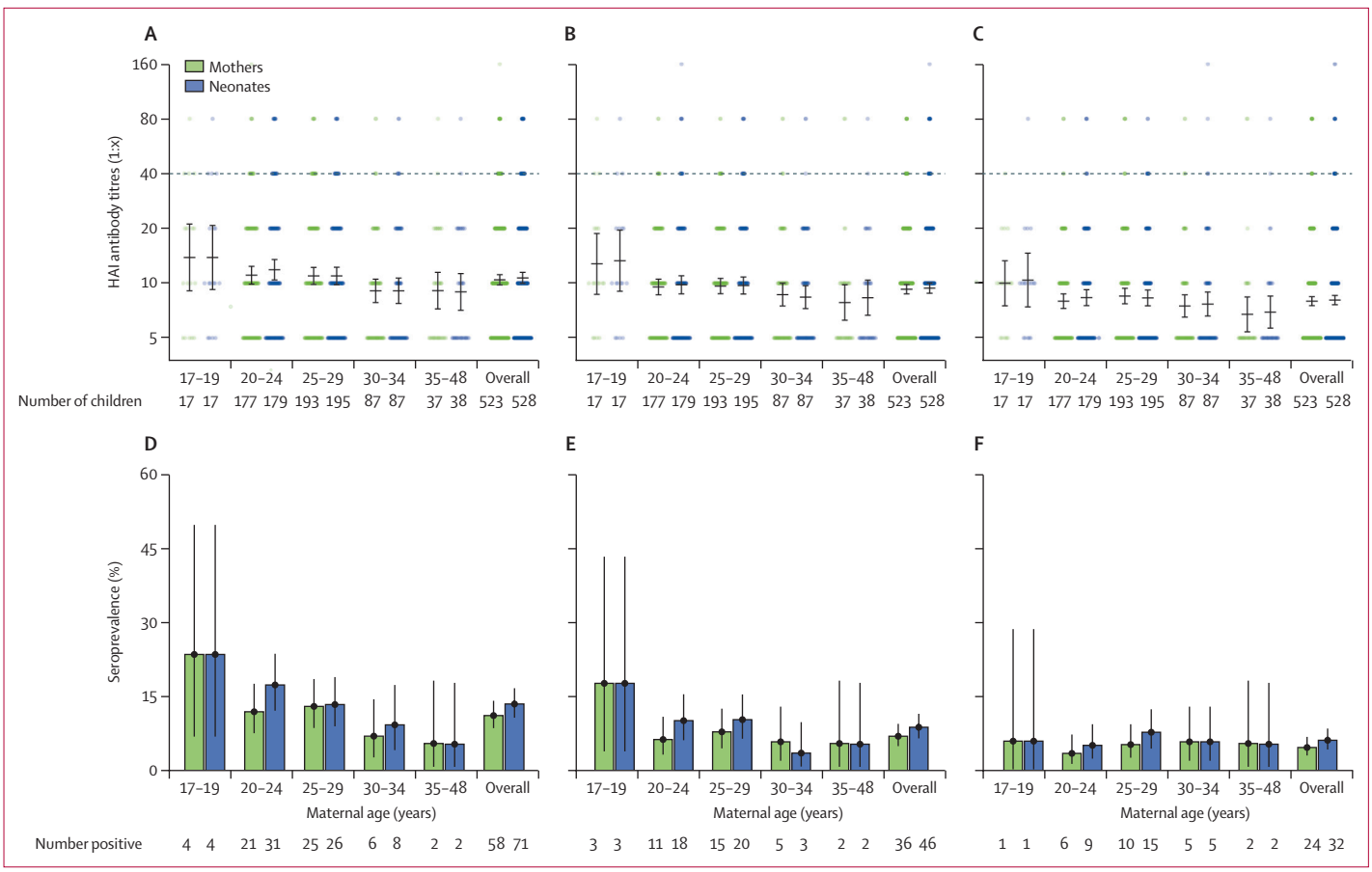

Figure 2: **Antibodies against influenza A(H1N1)pdm09 virus in mother–neonate pairs**
Maternal and neonatal antibody titres against the SWL4204/09 (A), SWL1277/16 (B), and SWL185/18 (C) strains by maternal age; and maternal and neonatal seroprevalence of the SWL4204/09 (D), SWL1277/16 (E), and SWL185/18 (F) strains by maternal age. Note that the numbers below the x-axis in panels A–C refer to the total number of participants and the numbers below the x-axis in panels D–F refer to the number of participants who were seropositive to a given strain in each age group. In panels A–C, the blue and green points show individual antibody titres, and the horizontal solid line with error bars refers to the geometric mean titres and 95% CIs; the bar plus black point with error bars in panels D–F represents the seroprevalence and 95% CIs. The dashed line in (A–C) denotes the protective threshold value of 1:40. HAI=haemagglutination inhibition.

after maternal antibody waning (table 2; appendix 2 pp 15–17). There was a positive correlation between maternal and neonatal titres against homologous strains (p<0·0001), and a negative one between maternal and neonatal titres against heterologous strains (p<0·0001), except for maternal SWL4204/09 and neonatal SWL185/18 titres (p=0·71). In child participants, we observed a positive correlation between naturally acquired antibody titres against heterologous strains (p<0·0001). Older age (p<0·0001) and having been alive during the SWL185/18 strain epidemic (p=0·013) were significantly associated with increases in SWL185/18 strain-specific titres in children. In particular, children's SWL185/18 strain-specific titres increased with the length of time during which they had been alive since the SWL185/18 epidemic (p=0·0069). Vaccinated children were more likely than unvaccinated children to have higher antibody titres against the SWL4204/09 strain (p<0·0001), and to have a poor response to the SWL185/18 strain (p<0·0001).

Kaplan-Meier analyses suggested that the cumulative probability of children having seropositivity specific to the SWL4204/09, SWL1277/16, and SWL185/18 strains was reduced to 0·50 before 2·3 months of age (appendix 2 p 17). The cumulative probability of seroconversion to the SWL1277/16 strain in children was 0·528 (95% CI 0·343–0·661) at 40·1 months, which was similar to the probability of seroconversion to the SWL185/18 strain at 39·8 months (0·390, 0·209–0·530, p=0·090), even when a higher protective threshold was used (p=0·67; appendix 2 p 18).

Discussion

On the basis of a prospective longitudinal, paired mother–neonate cohort study, we quantified the kinetics of maternally and naturally acquired A(H1N1)pdm09-specific antibodies in Chinese children from birth until 3 years of age. We showed that A(H1N1)pdm09-specific antibodies could be efficiently transferred via the placenta regardless of the strain, but that infants rapidly lost their

maternally derived immunity after birth. After waning of maternal antibodies, A(H1N1)pdm09 strain-specific titres subsequently increased either due to natural infection or vaccination, and then fell and increased periodically thereafter. We also demonstrated the association of children's SWL185/18 strain-specific titres with their age and the time they had been alive since the strain epidemic, indicating a possible association between strain-based cumulative exposure and individuals' immune responses. Moreover, we revealed differences in SWL4204/09 and SWL185/18 strain-specific titres between vaccinated and unvaccinated children.

Low levels of SWL4204/09 strain-specific antibodies were observed in adult women (GMT 10·4, seroprevalence 11%). These estimates were consistent with those observed in unvaccinated participants of the same age in Guangdong Province, China, which is close to our study location (GMT 9·1, 95% CI 7·8–10·5).[18] By contrast, these estimates were lower than those observed in regions with higher influenza epidemic intensity,[19] such as Beijing city (GMTs 15·3–16·8).[20] Regardless of the immunological mechanism (eg, boosting from reinfections or cross-reactivity from antigenically similar strains), it is evident that there are strain-specific differences in GMTs and seroprevalence among adult female participants, which can be explained by differences in time elapsed at sampling since the strain epidemic.

We observed that neonatal A(H1N1)pdm09 antibodies declined quickly to below the protective threshold within 2 months after birth. This decline is temporally associated with the sharp increase in the number of influenza A cases among infants aged 2 months or older,[21] which together reveal a need to improve protective immunity in infants through maternal immunisation before they are eligible for vaccination at 6 months of age. Many factors determine the transfer capability and levels of maternal A(H1N1)pdm09 antibodies. Our estimated serum levels of maternal A(H1N1)pdm09 antibodies were unlikely to be attributable to antibodies transferred via breastmilk because breastmilk antibodies only affect infants' mucosal immunity and cannot cross the mucosal barrier (which is already closed at birth) and affect their systemic immunity.[22] IgG subclasses,[23] maternal underlying diseases (eg, hypergammaglobulinaemia), and placental malaria[24] are likely to play a role in the transfer of maternal A(H1N1)pdm09 antibodies; however, we did not explore these factors because of the predefined study topic.

Our results indicated that antibody titres after infection did not persist year-round and had already declined before the next season.[25] In particular, we observed that the sharp increase in the probability of seroconversion to the SWL1277/16 or SWL185/18 strain after 36 months of age corresponded to the age of children starting nursery school, underlining the need for timely influenza vaccination among nursery schoolchildren to reduce their risk of developing an infection. The extent to which A(H1N1)pdm09 strain-specific antibodies after infection or vaccination can protect children against clinical illness is of public health relevance. The present study did not address the evidence gap on this topic because of the lack of active surveillance of influenza-like illness or viral

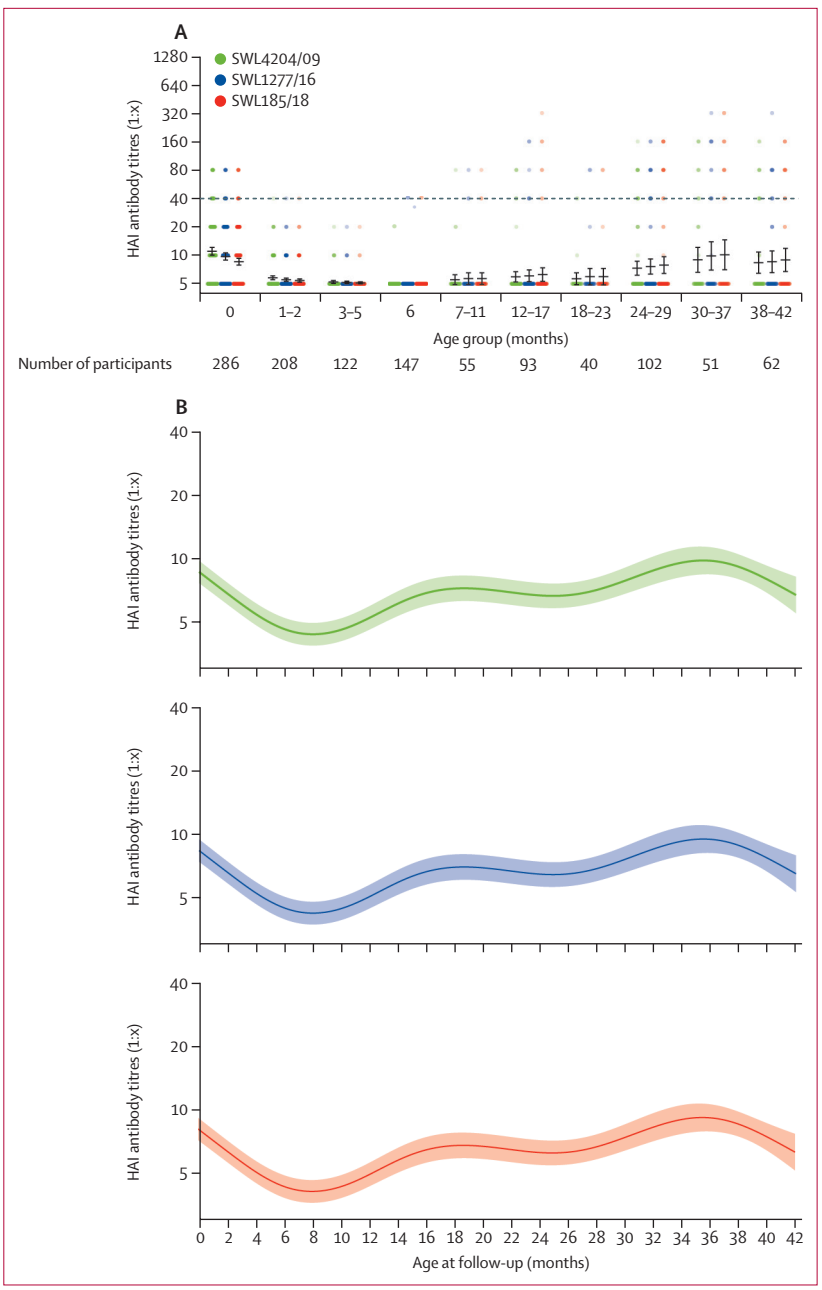

Figure 3: **Dynamic pattern of antibodies against influenza A(H1N1)pdm09 virus**
(A) Observed and (B) predicted antibody titres against the SWL4204/09, SWL1277/16, and SWL185/18 strains by use of generalised additive mixed models. Note that the numbers below the x-axis in (A) refer to the total number of child participants in each age group. In panel A, the blue, green and red points show individual antibody titres, and the horizontal solid line with error bars refers to the geometric mean titres and 95% CIs. The dashed line in (A) denotes the protective threshold value of 1:40. Solid lines and shaded areas in panel B show the predicted mean antibody titre and its 95% CI.

	Number of participants	SWL4204/09 strain		SWL1277/16 strain		SWL185/18 strain	
		b (95% CI)	p value	b (95% CI)	p value	b (95% CI)	p value
Maternal factors							
Age at delivery (continuous variable, years)	515	−0·00 (−0·01 to −0·00)	0·027	0·00 (−0·00 to 0·01)	0·11	−0·00 (−0·00 to 0·00)	0·96
Log(maternal HAI titres)	515	..	..	..	..	..	..
SWL4204/09	515	0·15 (0·12 to 0·17)	<0·0001	−0·10 (−0·12 to −0·07)	<0·0001	0·01 (−0·03 to 0·04)	0·71
SWL1277/16	515	−0·10 (−0·13 to −0·08)	<0·0001	0·10 (0·08 to 0·13)	<0·0001	−0·05 (−0·09 to −0·01)	0·011
Neonatal factors							
Age at follow-up visits (continuous variable, months)	515	−0·01 (−0·01 to −0·01)	<0·0001	−0·01 (−0·55 to 0·53)	0·97	0·01 (0·00 to 0·01)	<0·0001
Vaccination							
No	232	Ref (1)	..	Ref (1)	..	Ref (1)	..
Yes	51	0·12 (0·05 to 0·19)	0·0005	0·05 (−0·01 to 0·10)	0·10	−0·20 (−0·28 to −0·13)	<0·0001
Unknown	283	−0·01 (−0·04 to 0·02)	0·53	−0·01 (−0·03 to 0·02)	0·54	0·03 (−0·01 to 0·07)	0·14
Log(children's HAI titres)							
SWL4204/09	515	..	..	0·58 (0·55 to 0·60)	<0·0001	0·11 (0·06 to 0·15)	<0·0001
SWL1277/16	515	0·86 (0·83 to 0·90)	<0·0001	..	..	0·82 (0·77 to 0·86)	<0·0001
SWL185/18	515	0·08 (0·05 to 0·12)	<0·0001	0·43 (0·41 to 0·45)	<0·0001	..	..
Having been alive during the strain epidemic							
Yes, SWL4204/09 strain	0	..	..	..	..	..	..
Yes, SWL1277/16 strain	515	..	..	..	..	..	..
Yes, SWL185/18 strain	93	..	..	..	..	0·11 (0·02 to 0·19)	0·013
Time for which participants had been alive since the strain epidemic*†							
SWL1277/16	515	..	..	0·01 (−0·53 to 0·55)	0·96	..	..
SWL185/18	93	..	..	..	..	0·02 (0·01 to 0·03)	0·0069

HAI=haemagglutination inhibition. *Defined as the period between the sampling date and the date of the emergence of this strain. †For child participants who were alive during the epidemic of the SWL185/18 strain, an additional GLMM was performed to determine the effects of the cumulative time that participants had been alive since the SWL185/18 epidemic on their antibody titres.

Table 2: **Multivariable analyses of factors associated with HAI antibody titres against three influenza A(H1N1)pdm09 strains in child participants**

surveillance during the study period, but it could be a topic for further studies. We also observed cross-reactivity between different A(H1N1)pdm09 strains. The SWL185/18 strain-specific antibody titres in newborn babies could be attributed to pre-existing SWL4204/09 or SWL1277/16 antibodies, as the SWL185/18 strain was not circulating at their birth. Annual increases in the SWL4204/09 strain-specific titres were probably conferred by cross-reactivity from other heterologous strains, as this strain had been completely replaced by the SWL1277/16 strain when infant participants were born. We also found that previous vaccination was associated with a higher response to the SWL4204/09 strain and a lower response to the SWL185/18 strain. The higher response to the SWL4204/09 strain might be because children had been vaccinated with the SWL4204/09-like strain and, therefore, a greater immune response might have been mounted because of the strong immunogenicity of the vaccine. In comparison, the lower response to the SWL185/18 strain was probably due to partial cross-protection induced by vaccination against heterologous strains of pandemic influenza virus, as was shown in a ferret model.[26]

This study has several limitations. We could not exclude the possibility that children with A(H1N1)pdm09 virus infections were underdetected because some infections could have occurred between follow-up visits and antibodies waned by the time of the visit. This underdetection of infections might have resulted in an underestimated A(H1N1)pdm09 antibody level in some age groups. Additionally, the estimated natural dynamics of A(H1N1)pdm09 antibodies were based on vaccination cards. We attempted to address the impact of uncertainties in the accuracy of individual vaccination records on our estimates by using influenza vaccination coverage to adjust for such uncertainties, but the number of vaccinated children who were misclassified into the unvaccinated group might have been measured incorrectly. Other concerns regard the selection of the protective threshold for A(H1N1)pdm09 infections in paediatric populations and a lack of cross-check results using a second assay. Since the protective threshold has not been established in paediatric populations, the use of two different thresholds resulted in different estimated age-specific A(H1N1)pdm09 immunity in children. Nevertheless, both estimates consistently support low pre-existing A(H1N1)pdm09

immunity in paediatric populations. We were unable to perform another non-HAI assay to validate the current results because of the insufficient amount of stored cohort serum samples. However, the high consistency between the results of the HAI assay and the microneutralisation assay from our validation test (as shown in appendix 2 pp 8–10) and previous studies[27,28] support the robustness of our overall conclusions. Finally, we enrolled more male child participants both in the original cohort and in this study, because of an unbalanced sex ratio among newborn children (male-to-female ratio: 1·14[29]) in our study location. Given gender-specific differences in the immune response to virus infection,[30] as well as region-specific and time-specific variations in virus strain circulation, demography, and vaccination coverage, caution should be taken when generalising our findings to areas outside our study region.

In conclusion, low pre-existing A(H1N1)pdm09 immunity among unvaccinated pregnant women and rapid decay of maternal antibodies in infants underline the importance of accelerating and expanding maternal influenza vaccination in China to effectively protect both pregnant women and infants. Observed low rates of A(H1N1)pdm09 infections in the paediatric population and cross-reactivity between different strains of influenza A(H1N1)pdm09 virus can be used to guide relevant stakeholders in planning and implementing paediatric influenza vaccination schedules in China. These findings highlight the necessity of population-based longitudinal serological studies in accurately determining target populations for influenza vaccination. Mechanistic modelling studies will also be important to improve the understanding of the role of maternal antibody levels in determining individual immune response levels following the first A(H1N1)pdm09 infection or vaccination, and the true levels of A(H1N1)pdm09 strain-specific immunity and inter-strain cross-reactivity in paediatric populations.

Contributors

HY conceived, designed, and supervised the study. ML, ZZhao, ZZhan, CH, XC, JZ, QL, and JY participated in data collection. ML, JC, MX, NL, LR, LY, WZ, HSh, NZ, and WL performed the laboratory tests. WW, ML, JC, XC, ZZhao, and XZ analysed the data. WW, ML, and JC prepared the figures and the first draft of the manuscript. HY, HSa, and MJ commented on the data and their interpretation and critically revised the content. ML and WW verified the data underlying the study. All authors had full access to all the data in the study and accept responsibility for the decision to submit for publication.

Declaration of interests

HY has received research funding from Sanofi Pasteur, GlaxoSmithKline, Yichang HEC Changjiang Pharmaceutical Company, Shanghai Roche Pharmaceutical Company, and SINOVAC Biotech Ltd. All other authors declare no competing interests.

Data sharing

The original database containing confidential individual information cannot be made public. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments

The views expressed in this publication are those of the author(s) and not necessarily those of the National Institute for Health Research (NIHR) or the UK Department of Health and Social Care. HY acknowledges financial support from the Key Program of the National Natural Science Foundation of China (82130093) and the NIHR (16/137/109) using UK aid from the UK Government to support global health research. WW acknowledges financial support from the National Postdoctoral Program for Innovative Talent (BX2021072) and the China Postdoctoral Science Foundation (2022M720753).

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