The transfer and decay of maternal antibodies against enterovirus A71, and dynamics of antibodies due to later natural infections in Chinese infants: a longitudinal, paired mother–neonate cohort study

Xianglin Wei, Juan Yang*, Lidong Gao*, Lili Wang*, Qiaohong Liao*, Qi Qiu, Kaiwei Luo, Shuanbao Yu, Yonghong Zhou, Fengfeng Liu, Qi Chen, Juanjuan Zhang, Bingbing Dai, Hao Yang, Jiaxin Zhou, Weijia Xing, Xinhua Chen, Min He, Lingshuang Ren, Jinxin Guo, Li Luo, Peng Wu, Zhiyong Chen, H Rogier van Doorn, Simon Cauchemez, Benjamin J Cowling†, Hongjie Yu†*

Summary

Background Since 1997, epidemics of hand, foot, and mouth disease associated with enterovirus A71 (EV-A71) have affected children younger than 5 years in the Asia-Pacific region, including mainland China. EV-A71 vaccines have been licensed for use in children aged 6–71 months in China, but not for infants younger than 6 months. We aimed to assess the dynamics of maternal EV-A71 antibodies to inform choice of potential vaccination strategies to protect infants younger than 6 months, because they have a substantial burden of disease.

Methods We did a longitudinal cohort study with mother–neonate pairs in local hospitals in southern China during 2013–18. We collected cord blood from neonates and venous blood from mothers at delivery. We followed up and collected blood samples from the children at ages 2, 4, 6, 12, 24, and 36 months and tested for the presence of neutralising antibodies against EV-A71 with virus neutralisation assays. Seropositivity, or protective titre, was defined as a neutralisation antibody titre of 16 or higher. We estimated the seroprevalence, geometric mean titre (GMT), and transfer ratio of maternal antibodies. We used a binomial distribution to derive the 95% CIs of seroprevalence. Seropositivity between mothers and neonates was compared by use of an agreement (κ), while GMTs were compared by use of paired Student's *t* tests.

Findings Between Sept 20, 2013, and Oct 14, 2015, 1054 mothers with 1066 neonates were enrolled. The EV-A71 GMT was similar among pairs of neonates (22·7, 95% CI 20·8–24·9) and mothers (22·1, 95% CI 20·2–24·1; p=0·20). The mean transfer ratio of maternal antibodies was 1·03 (95% CI 0·98–1·08). Although 705 (66%) of 1066 neonates acquired protective concentrations of EV-A71 antibodies from mothers, these declined rapidly, with a half-life of 42 days (95% CI 40–44). The time to loss of protective immunity was extended to 5 months in neonates with mothers who had titres of 128 or higher. By age 30 months, 28% of children had become seropositive because of natural infection.

Interpretation EV-A71 maternal antibodies were efficiently transferred to neonates, but declined quickly to below the protective threshold, particularly among those whose mothers had low antibody titres. Our findings suggest that maternal vaccination could be explored to provide neonatal protection against EV-A71 through maternal antibodies. Catch-up vaccination between ages 6 months to 5 years could provide protection to the approximately 30–90% of children that have not had natural EV-A71 infection by that age.

Funding National Science Fund for Distinguished Young Scholars, National Natural Science Foundation of China.

Introduction

Since 1997, large epidemics of hand, foot, and mouth disease (HFMD) associated with enterovirus A71 (EV-A71) among children younger than 5 years have been reported throughout the Asia-Pacific region, including mainland China.[1–4] The estimated case-severity risk is 1·74% and case-fatality risk is 0·055% in mainland China. Younger age and living in rural areas were associated with greater risk of fatal outcomes.[2–4] In the past 5 years, increased circulation of EV-A71 and outbreaks of HFMD have been described outside of Asia, which poses a growing global public health concern.[5–7]

In China, HFMD poses a substantial burden to health, with more than 1 million cases per year, with EV-A71 associated with the most severe and fatal outcomes.[3,4] The case-fatality risk is highest among children younger than 1 year, especially among those younger than 6 months (about 0·17% in children younger than 6 months *vs* 0·11% in those aged 6–11 months).[3] No specific antiviral treatment is available for HFMD. Three licensed inactivated EV-A71 vaccines are available

Lancet Infect Dis 2020

Published **Online** October 5, 2020 https://doi.org/10.1016/S1473-3099(20)30480-1

See **Online/Comment** https://doi.org/10.1016/S1473-3099(20)30452-7

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

*Contributed equally

†Contributed equally

School of Public Health, Fudan University, Key Laboratory of Public Health Safety, Ministry of Education, Shanghai, China (X Wei MD, J Yang PhD, L Wang MSc, Q Liao MSc, Q Qiu MPH, Y Zhou MSc, J Zhang PhD, J Zhou BS, X Chen MD, L Ren MD, J Guo MD, Prof H Yu PhD); **Hunan Provincial Center for Disease Control and Prevention, Changsha, China** (L Gao MSc, K Luo MPH, H Yang MPH); **Institut Pasteur of Shanghai, Chinese Academy of Sciences, Shanghai, China** (L Wang); **Key Laboratory of Surveillance and Early Warning on Infectious Disease, Division of Infectious Disease, Chinese Center for Disease Control and Prevention, Beijing, China** (Q Liao, F Liu MD, S Yu PhD, Q Chen MPH, W Xing PhD, L Luo MSc, Prof H Yu); **Hubei Provincial Center for Disease Control and Prevention, Wuhan, China** (Q Chen); **Anhua County Center for Disease Control and Prevention, Yiyang, China** (B Dai MD, M He MD, Z Chen MD); **School of Public Health, Shandong First Medical University and Shandong Academy of Medical Sciences, Tai'an, China** (W Xing); **WHO Collaborating Centre for Infectious Disease Epidemiology and Control,**

School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China (P Wu PhD, Prof B J Cowling PhD)**; Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam** (H R van Doorn PhD)**; Nuffield Department of Medicine, University of Oxford, Oxford, UK** (H R van Doorn)**; and Mathematical Modelling of Infectious Diseases Unit, Institut Pasteur, UMR2000, Centre National de la Recherche Scientifique, Paris, France** (Prof S Cauchemez PhD)

Correspondence to: Prof Hongjie Yu, School of Public Health, Fudan University, Key Laboratory of Public Health Safety, Ministry of Education, Shanghai 200032, China
yhj@fudan.edu.cn

Research in context

Evidence before this study

Enterovirus A71 (EV-A71) is responsible for a substantial disease burden of hand, foot, and mouth disease (HFMD) in young children in the Asia-Pacific region, particularly in mainland China. An increased circulation of EV-A71 was described in European and North American countries, leading to outbreaks of severe neurological illness, which poses a growing global public health concern. A 2015 systematic review showed that 30% of children in China were seronegative against EV-A71 and thus remained susceptible at age 5 years. Available licensed EV-A71 vaccines in China cannot be given to infants younger than 6 months, among whom a substantial burden of severe disease occurs. Necessity and timing of vaccination in early life is highly related with maternal-derived antibody levels and their persistence. We searched PubMed, Web of Science, China National Knowledge Infrastructure, and Wanfang up to Feb 10, 2020, using the following search terms: (enterovirus 71 OR EV-A71 OR EV-71 OR EV71) AND (seroepidemiology OR seroepidemiologic OR seroepidemiological OR serosurvey OR seroprevalence OR seroprevalent OR seronegative OR seropositive OR serologic OR serological OR seroincidence OR seroconversion OR seroconvert OR GMT) AND (maternal OR maternally OR mother OR transplacental OR placental OR infant OR newborn OR neonate OR neonatal OR child OR children). Only four cross-sectional studies and one longitudinal cohort study reported the correlation between EV-A71 maternal and neonate antibodies in mother–neonate pairs, with one study presenting the transfer ratio. Of these studies, the cohort study alone characterised the half-life of EV-A71 maternal antibodies, but obtained only two blood specimens from neonates within 6 months of age (ie, the cord blood and venous blood at age 6 months). Another cohort study found that 33% of infants aged 3 months and 7% of infants aged 6 months were positive for EV-A71 antibodies. One study reported the seropositivity and antibody titres in neonates aged 2 and 7 months, but could not estimate the dynamics of maternal antibodies because the blood samples were not collected for neonates at birth. No studies were found that addressed the duration of protection conferred by maternal-transferred antibodies.

Added value of this study

In this study, we assessed the transplacental transfer ratio of EV-A71 antibodies, calculated the half-life of maternal-derived EV-A71 antibody titres, and the time to loss of protective immunity in neonates (with a cutoff titre of 16 or higher for protective titres). To our knowledge, this was the largest population-based study of mother–neonate pairs followed up from birth to age 36 months that quantified the transplacental transfer efficiency of EV-A71 antibodies, and measured the dynamics of EV-A71 antibodies in neonates. Our findings show that the mean transplacental transfer ratio was 1·03 (95%CI 0·98–1·08). Although the majority of neonates acquired protective concentrations of EV-A71 antibodies from their mothers, this protection declined rapidly. After the disappearance of maternal antibodies, antibody titres rose because of natural infection, with about a third of children infected by the age of 2·5 years.

Implications of all the available evidence

Our study showed that protective titres of EV-A71 antibodies could be efficiently transferred to neonates from mothers through the placenta. However, maternal antibodies declined rapidly. The findings provide helpful information to guide EV-A71 vaccination programmes and suggest that maternal vaccination could be explored to provide protection to neonates and infants younger than 6 months against EV-A71 through maternal antibodies. Catch-up vaccination between ages 6 months to 5 years could provide protection to the approximately 30–90% of children that have not had natural EV-A71 infection by that age.

for use in children aged 6–71 months in China.[8,9] Infants younger than 6 months remain susceptible before they receive the first dose of EV-A71 vaccination.

EV-A71 vaccines are used in the private sector in mainland China. To promote their introduction into the National Immunisation Programme, optimal timing and value of catch-up vaccination should be assessed. Information is needed on the presence and persistence of maternal antibodies and dynamics of antibodies from natural infection in early life. Several cross-sectional studies reported correlation of EV-A71 antibody levels in mother–neonate pairs.[10–13] However, only one longitudinal cohort study presented the half-life (42 days) from blood samples collected at birth and at age 6 months.[14] Another study found 33% seropositivity in babies aged 3 months and 7% in those aged 6 months, but had high drop-out rates of 68% at age 3 months and 90% at age 6 months.[15] Mao and colleagues[16] reported seropositivity and antibody titre in babies aged 2 and 7 months, but could not estimate the dynamics of maternal antibodies because the blood samples were not collected for neonates at birth.

In our study, we quantified antibody concentrations for EV-A71 in paired maternal and cord serum samples from a large cohort of mothers and neonates, assessed the transplacental transfer efficiency of maternal antibodies, and analysed the antibody kinetics from birth to age 3 years, including the decline in maternal antibodies and subsequent increases due to natural infection.

Methods

Study design and participants

We established a longitudinal, paired mother–neonate cohort in three townships (Tianzhuang, Jiangnan, and Qingtang) in Anhua county, Hunan Province, southern China from September, 2013, to August, 2018 (appendix 2, p 3). Neonates were eligible for inclusion if

See Online for appendix 2

they were born after Sept 20, 2013, and their mothers had resided in the study sites in the preceding 3 months or longer. Well trained nurses enrolled mother–neonate pairs in six local hospitals. 89% of pregnant women from these three townships give birth in these hospitals.

The 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), and Fudan University (2019–05–0756). We obtained written informed consent from all enrolled mothers for themselves and their neonates.

Procedures

We collected a peripartum venous blood sample (2 mL) from each woman around the time of delivery and a cord blood sample (2 mL) from each neonate (baseline). Mothers were interviewed within 1 week of delivery to obtain information including demographics, gestational weeks, delivery method, and birthweight of neonates. Infant blood samples were taken at 2, 4, 6, 12, 24, and 36 months. The first three follow-ups (at 2, 4, and 6 months) were done allowing 1 week before or after the scheduled age. The last three follow-ups (at 12, 24, and 36 months) were done in February–March or August–November of 2014–18, whichever was closer to the baby's birthday.

At each follow-up visit, we interviewed caregivers face to face about hygiene, vaccination, hospitalisation, and breastfeeding (up to 6 months) of the enrolled children. In February–March, 2017, we did a supplementary investigation to collect information on mothers, including self-reported underlying diseases and health status during pregnancy. Aggregated data on all deliveries in the townships during the study period was retrieved from the Hunan Rural Population Health Information System. EV-A71 activity was identified through enhanced virological surveillance of HFMD in Anhua[17] and national notifiable disease surveillance data in Hunan[3] (appendix 2, pp 3–6).

An EV-A71 vaccine was introduced in 2016, after recruitment and baseline visits were completed. For enrolled children who were vaccinated against EV-A71 during the study period, we excluded from the analyses the antibody titres of blood samples collected after vaccination.

Laboratory procedures

The neutralisation assays are described in appendix 2 (pp 6–8). Briefly, we used the EV-A71 FY573 strain (GenBank accession number HM064456.1), belonging to the same subgenogroup C4 as the circulating strains in Anhua. Serum samples were serially diluted four-fold (1/8 to 1/2048) in duplicate. Neutralising titres were defined as the reciprocal of the highest dilution capable of inhibiting 50% of the cytopathic effect and calculated by use of the Karber method.[18] Neutralisation titres lower than eight were assigned as four, and those higher than 2048 were assigned as 4096.

Statistical analysis

We calculated that a sample size of 900 mother–neonate pairs would allow a 10% annual risk of EV-A71 infection to be estimated to within ±2·5%, with a statistical significance level of 5%, allowing for a drop-out rate of 39%.

We estimated the seroprevalence, geometric mean titre (GMT), and transfer ratio of maternal antibodies. The ratio of neonate-to-mother titre was log-transformed to obtain normal distribution. The geometric mean of the ratio is a frequently used metric to assess the transfer efficiency of maternal antibodies.[19,20] In this study, the mean transfer ratio of maternal antibodies was defined as the geometric mean of the ratio of neonate-to-mother titre.

The protective immunity of EV-A71 antibodies has not been well characterised. A phase 3 clinical trial of EV-A71 vaccines has shown that a titre of 16 could be considered as a possible serological marker for protection against EV-A71-related HFMD or herpangina.[21] Therefore, we defined seropositivity, or protective concentration, as a titre of 16 or higher in the baseline analysis. Sensitivity analyses were done with use of a cutoff of eight (minimum detectable antibody level in neutralisation assays) and 32. We used a binomial distribution to derive the 95% CIs of seroprevalence (R package binom). Seropositivity between mothers and neonates was compared by use of an agreement (κ), while GMTs were compared by use of paired Student's *t* tests.

We used statistical models to identify factors associated with the level of neutralising antibodies against EV-A71 in neonates and with the transfer ratio, to identify factors associated with seropositivity in neonates, to identify dynamic patterns of antibody titres, to describe dynamic patterns of seroprevalence in all infants, to quantify the half-life of maternal-derived EV-A71 antibody titres and the mean time to loss of protective immunity, and to identify the association between maternal antibody levels and time to loss of immunity of neonates. New infections were defined as seroconversion (an individual whose titres moved from lower to the seropositivity cutoff or higher). We used the Kaplan-Meier estimator to estimate the median time to seroconversion.

In the analysis of EV-A71 antibody dynamics in neonates, records with missing titres due to loss to follow-up were excluded. We also analysed and compared data from participants with complete follow-up with those of all participants.

All analyses were done in R, version 3.6.1, and SAS, version 9.4.

Role of the funding source

The funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

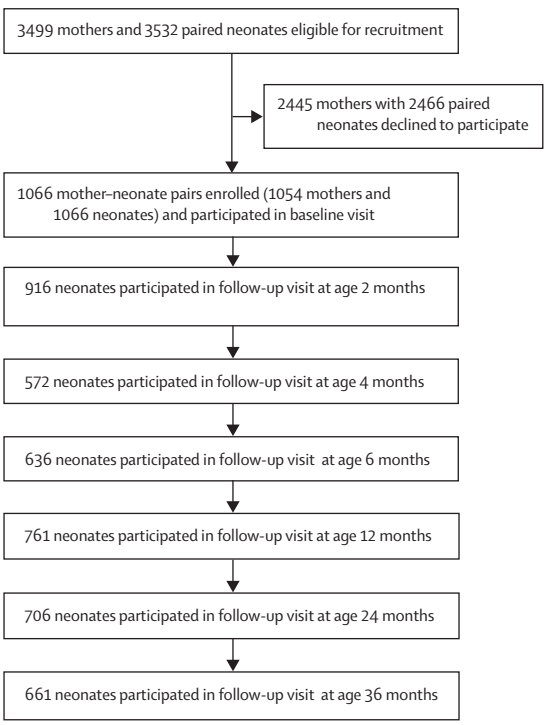

Figure 1: **Recruitment and follow-up visit rates for all participants**

	Number of participants
Mothers	
Age at delivery (years; n=1052)	
16–19	40 (4%)
20–29	768 (73%)
30–48	244 (23%)
Education (n=1020)	
Middle school or lower	711 (70%)
High school	273 (27%)
University or higher	36 (4%)
Township (n=1054)	
Qingtang (2·7 people per 10^4 m^2)	486 (46%)
Jiangnan (2·1 people per 10^4 m^2)	382 (36%)
Tianzhuang (1·5 people per 10^4 m^2)	186 (18%)
Gravidity (n=1048)	
1	321 (31%)
2	431 (41%)
≥3	296 (28%)
Parity (n=1048)	
1	498 (48%)
2	518 (49%)
≥3	32 (3%)
Neonates (n=1066)	
Sex	
Boys	576 (54%)
Girls	490 (46%)
Gestational age at birth (weeks)	
<37	27 (3%)
37–42	998 (94%)
>42	41 (4%)
Birthweight (grams)	
<2500	27 (3%)
2500 to <4000	963 (90%)
≥4000	76 (7%)
Has twin siblings	25 (2%)*

Data are n(%). *12 pairs of twins were included in our study; one more neonate had a twin sibling but his twin sibling did not participate in our study.

Table 1: **Participant characteristics**

Results

Between Sept 20, 2013, and Oct 14, 2015, 3499 pregnant women were approached, 1054 (30%) of whom were enrolled and gave birth to 1066 paired neonates included in the study. For 958 (91%) of 1054 women, blood collection was done on the same day as cord blood collection for paired neonates (appendix 2, p 11). The follow-up rate was over 50% at each visit (figure 1), and 238 (22%) of 1066 neonates completed six follow-ups (full follow-up subgroup). Seven enrolled children were vaccinated against EV-A71 after age 6 months during the study period, and thus we excluded their antibody titres after vaccination. Among them, the first dose was administered at age 10 months for one child in 2016, and at age 21–33 months for six other children in 2017. Local contemporaneous EV-A71 activity is presented in appendix 2 (pp 3–6). A clear peak in EV-A71 cases was detected in March–June each year in 2014–16, with lower circulation detected in 2017 and 2018.

The median age of mothers was 25 years (IQR 23–29); and 576 (54%) of 1066 neonates were boys (table 1). Follow-up time ranged from 1 to 41 months (median 35 months, IQR 18–37). We compared the characteristics of enrolled mothers and neonates with those from the same region who did not participate (appendix 2, pp 11, 14). Mothers were similar in terms of age, gestational age at delivery, parity, and gravidity, whereas women with higher education and those from Qingtang township were relatively overrepresented in our sample. Among neonates, sex and birthweight were similar.

EV-A71 antibody titres in neonates were positively correlated with those in mothers (ρ=0·88, 95% CI 0·87–0·89), and 82% of paired titres were the same or within a two-fold difference (figure 2A). We observed no difference at baseline in overall GMT between paired mother (22·1, 95% CI 20·2–24·1) and neonate (22·7, 20·8–24·9; p=0·20; figure 2B). With a cutoff of 16 for seropositivity, the EV-A71 seroprevalence among neonates (66%, 95% CI 63–69) was the same as among mothers (66%, 63–68), with κ agreement of 0·75 (0·70–0·79; figure 2C). The choice of antibody titre threshold for seropositivity had little effect on the consistency of seroprevalence in mothers and neonates (appendix 2, p 17). The mean transfer ratio of maternal

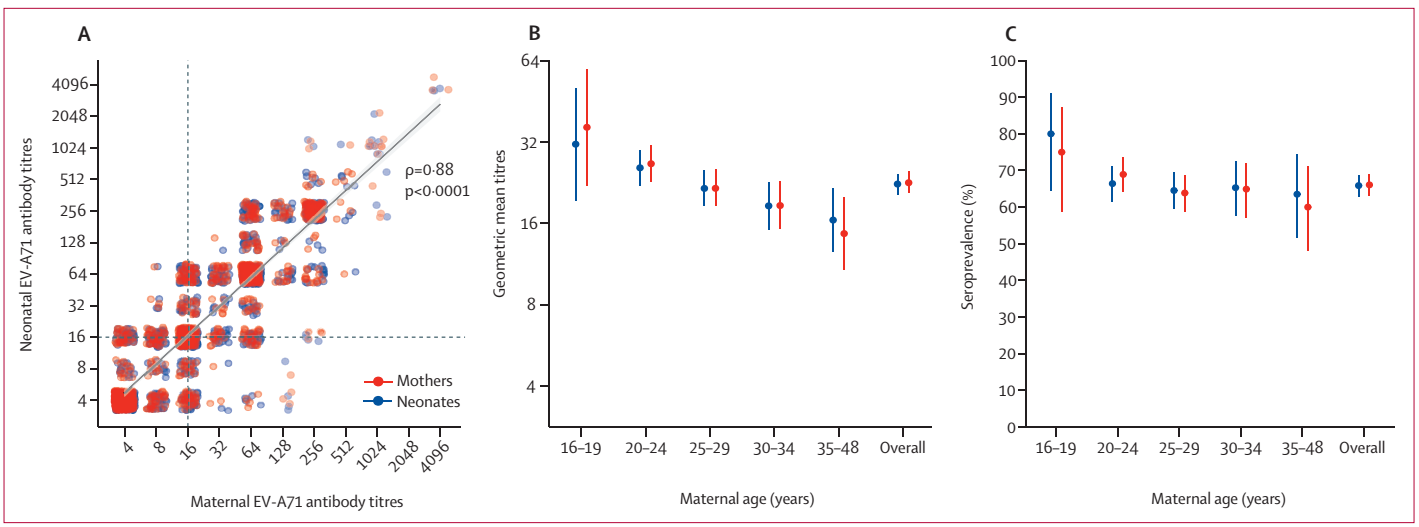

Figure 2: **EV-A71 antibodies in mother–neonate pairs**
(A) Correlation between neonatal and maternal titres (with use of Jitter function in R to avoid overlapping datapoints). (B) Maternal and neonatal geometric mean titres by maternal age. (C) Maternal and neonatal seroprevalence (titre ≥16) by maternal age. EV-A71=enterovirus A71.

antibodies was 1·03 (95% CI 0·98–1·08), ranging from 1/32 to 8 (appendix 2, p 18)—that is, antibody titres in cord blood were 1·03-times higher than those in mothers on average.

The multivariable analysis using a generalised linear mixed model showed that antibody titres in neonates were highly associated with maternal titres, followed by maternal age and gestational age. For every two-fold increase in maternal antibody titres, titres in neonates increased by 87·6% (95% CI 83·8–91·5), and neonates were 4·90-times (4·06–5·91) more likely to acquire protective antibody levels (neutralising titre cutoff of 16). The transfer ratio was higher among younger mothers and in infants with greater gestational age (table 2). However, the transfer ratio decreased by 6·2% (95% CI 4·2–8·1) for every two-fold increase of maternal antibody levels.

Maternal antibody levels in neonates diminished rapidly, followed by titre increases due to natural infection, as was apparent in both GMT and seroprevalence stratified by age groups (figure 3A, B). The generalised linear mixed model fitted the original titres in all neonates well (appendix 2, p 25) and showed that maternal EV-A71 antibody titres declined rapidly to below the protective titre of 16 in 16 days (95% CI 12–20; figure 3C). The proportion of neonates with protective antibody titres decreased rapidly from 66% at birth to lower than 10% at age 5–6 months. Subsequently, median EV-A71 antibody titres gradually increased to the protective threshold and higher by age 30 months (95% CI 27–36) due to natural infection, with a seroprevalence of 28% (figure 3C, D). We observed the same kinetics of EV-A71 antibodies for the full follow-up subgroup (appendix 2, p 25). We further split neonates into two cohorts according to their time of birth. We observed that, compared with the other cohort, the cohort that experienced stronger EV-A71 epidemics had higher antibody titres and seroprevalence due to natural infections after the disappearance of maternal EV-A71 antibodies (appendix 2, pp 26–27).

	β (95% CI)	2^{β} (95% CI)	p value
Factors associated with log-transformed titre of EV-A71 antibodies in neonates			
Maternal age (years)	–0·017 (–0·030 to –0·004)	0·988 (0·979 to 0·997)	0·010
Gestational age (weeks)	0·046 (0·003 to 0·089)	1·033 (1·002 to 1·064)	0·035
Log (maternal titre)	0·908 (0·878 to 0·938)	1·876 (1·838 to 1·915)	<0·0001
Factors associated with seropositivity in neonates (cutoff of 16)			
Log (maternal titre)	1·589 (1·400 to 1·777)	4·897* (4·055 to 5·914)	<0·0001
Factors associated with transfer ratio of EV-A71 antibodies			
Maternal age (years)	–0·017 (–0·030 to –0·004)	0·988 (0·979 to 0·997)	0·010
Gestational age (weeks)	0·046 (0·003 to 0·089)	1·033 (1·002 to 1·064)	0·035
Log (maternal titre)	–0·092 (–0·122 to –0·062)	0·938 (0·919 to 0·958)	<0·0001

Because the antibody titres (including 4, 8, 16, 32, 64, and so on, up to 4096) are exponentiated to the power of 2, we used ln(2)-transformation instead of ln(e)-transformation. In the logistic regression model, we used ln(e)-transformation for seropositivity in neonates to explore the risk factors. β higher than 0 indicates that the predictors were associated with increases of EV-A71 antibodies by (2^{β}–1) folds; β lower than 0 indicates the predictors were associated with decreases of EV-A71 antibodies by (1–2^{β}) folds. EV-A71=enterovirus A71. *For every two-fold increase in maternal antibody titres, neonates were 4·90-times (95% CI 4·06–5·91) more likely to acquire protective antibody levels.

Table 2: **Factors associated with EV-A71 antibodies in mother–neonate pairs**

705 (66%) of 1066 neonates acquired protective antibodies against EV-A71 (titre 16 or higher) from their mothers. Among them, the half-life of EV-A71 antibodies was 42 days (95% CI 40–44), and the time to loss of protective immunity was 58 days (53–62). The time to loss of protective immunity was extended to 5 months in neonates born from 159 (15·1%) of 1054 mothers with titres of 128 or higher (appendix 2, p 28). The choice of protective threshold had little effect on antibody half-life (appendix 2, p 28). The proportion of neonates having protective antibody titres was reduced to 50% at age

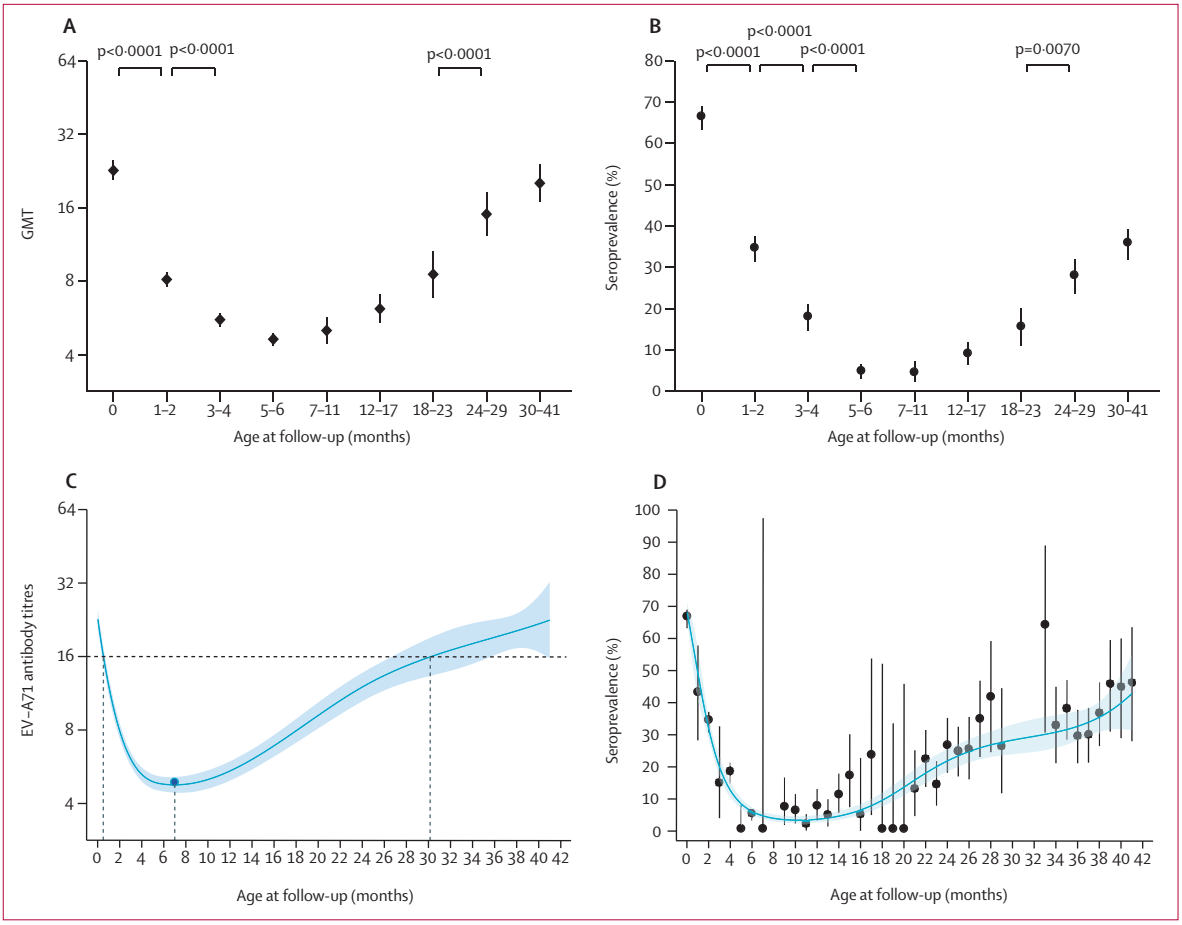

Figure 3: **Dynamic pattern of EV-A71 antibody titres and seroprevalence by age in all neonates**
(A) Observed GMTs stratified by age groups. (B) Observed seroprevalence (with a cutoff of 16 for seropositivity) stratified by age groups. (C) Fitted EV-A71 antibody titre dynamics by use of a generalised linear mixed model. (D) Observed and fitted seroprevalence by use of a generalised linear mixed model (with a cutoff of 16 for seropositivity). EV-A71=enterovirus A71. GMT=geometric mean titre.

2 months, and decreased to 15% at age 4 months (figure 4A). For every two-fold increase of antibody titres in mothers at delivery, the duration of protection was extended by 1 month (figure 4B). The cumulative probability of seroconversion in neonates was 5% at age 12 months, 20% at age 24 months, and 32% at age 36 months (figure 4C).

Additionally, we analysed the effect of breastfeeding duration before age 6 months. We observed no significant association between breastfeeding duration before age 6 months and the risk of seroconversion within age 6 months (hazard ratio 1·086, 95% CI 0·717–1·646, p=0·70) or the risk of seroconversion within age 3 years (0·989, 0·930–1·052, p=0·73; appendix 2, p 28).

Discussion

In this large population-based study of mother–neonate pairs followed up from birth to age 36 months, we quantified the transfer efficiency of maternal EV-A71 antibodies, and we measured the dynamics of maternal antibodies and those in neonates due to natural infections. Our study revealed that the average EV-A71 antibody titres and seroprevalence (titres of 16 or higher) were similar in maternal and cord blood, with a mean transfer ratio of maternal antibodies of 1·03 (95% CI 0·98–1·08). Although 66% of neonates acquired protective concentrations of EV-A71 antibodies from mothers, these declined rapidly. After the disappearance of maternal antibodies, antibody titres increased because of natural infections, with about a third of children seroconverted at age 30 months.

Maternal antibodies can provide protection for neonates in the first months of life against diseases such as measles, rubella, diphtheria, and tetanus.[19,22–25] The protective effects and duration depend on antibody concentrations in mothers, maternal antibody transfer, and persistence in neonates. The transfer of EV-A71 antibodies from mother to infant was efficient. However, on average, maternal antibodies in neonates declined quickly to below a protective level at about age 2 weeks, with a steep drop in proportions of neonates having protective antibody within age 6 months, as reported by

Fu and colleagues.[15] These findings correspond to the clinical observation that infants are at high risk of EV-A71-related HFMD before age 1 year[4] and are most vulnerable to developing severe HFMD before age 6 months,[3] a period when maternal antibodies are waning and the infant's immune response might be incapable of generating a robust neutralising antibody response to infection.

Three inactivated monovalent EV-A71 vaccines have been licensed in mainland China for children aged 6–71 months.[9] Our study showed that administering the first doses at age 6 months might be too late to protect infants younger than that. Therefore, it is important to further investigate the optimal timing of vaccination, which could be administered as early as age 1 month if introduced into the National Immunisation Programme. An inactivated EV-A71 vaccine developed in Taiwan elicits a strong immune response and is tolerable in participants aged 2–6 months.[26] A phase 3 clinical trial is being done to assess its safety and efficacy.[8] We recommend doing clinical studies with EV-A71 vaccines currently used in mainland China, to further assess the doses, safety, and effectiveness in children younger than 6 months.

The available licensed EV-A71 vaccines in China use aluminium adjuvants to improve immunogenicity, which are likely to lead to higher reactogenicity in very young infants.[27] Given the potentially reduced tolerability or safety of vaccinating young infants, another option is the vaccination of mothers during pregnancy to protect infants in the early months of life, which has proven efficacious for influenza and pertussis.[28,29] We estimate that the time to loss of protective immunity can be extended to 5 months in mothers with titres of 128 or higher. However, only 159 (15·1%) of 1054 mothers had antibody titres of 128 or higher. The maternal antibodies in our study reflected the immune response of natural infection rather than vaccination. Previous studies showed that EV-A71 vaccination in children elicited a substantial increase in neutralising antibodies that was persistent, with a GMT of 141 5 years after vaccination.[21,30] These findings emphasise the fact that maternal vaccination might be an alternative effective means of decreasing EV-A71-related HFMD in infants in the first months of life. The antibody response and duration of immunity produced by maternal EV-A71 vaccination, as well as the efficacy or effectiveness of maternal EV-A71 vaccination merits additional research.

With the waning of maternal antibodies in neonates, the probability of seroconversion due to natural infection increased with age. However, about two thirds of children remained susceptible to EV-A71 infection at nearly 3 years of age. Takahashi and colleagues reported that an EV-A71 vaccination coverage level higher than 96% is necessary to achieve population-level immunity because of its very high R_0.[31] To eliminate transmission of EV-A71, in addition to implementing a routine EV-A71 vaccination programme for very young infants (younger than 6 months) of each birth cohort, a one-time catch-up vaccination could protect children aged 6 months to 5 years who have higher risk of infection because of susceptibility and increasing frequent contact with other children.

Our study found that higher maternal antibody levels resulted in a lower placental transfer ratio, which

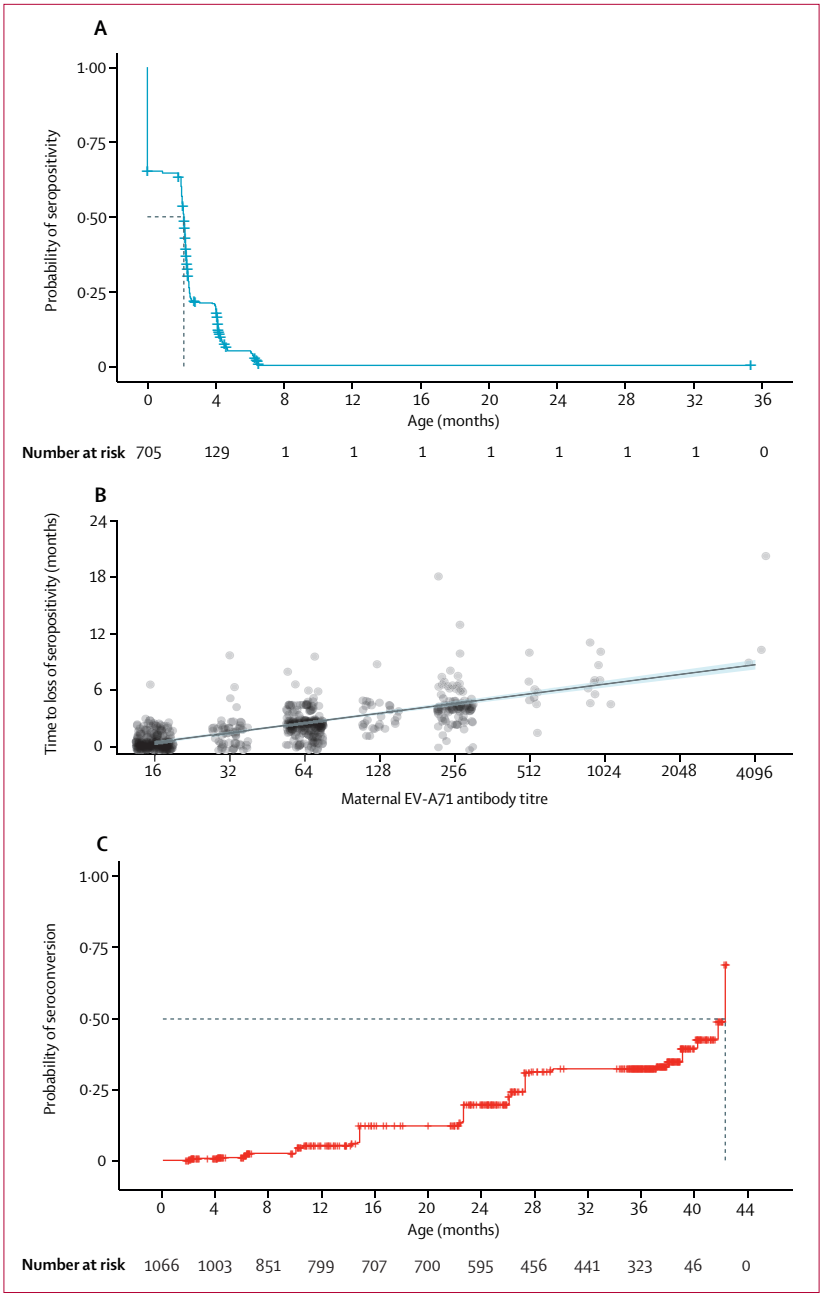

Figure 4: **Loss of protective immunity acquired from mothers and seroconversion in neonates followed up from birth to about age 3 years**
(A) Kaplan-Meier plot of probability of seropositivity (titre of 16 or higher) in neonates who were seropositive at birth. (B) Relationship between time to loss of seropositivity (titre of 16 or higher) and maternal antibody titres. (C) Cumulative probability of seroconversion in all neonates.

decreased by 6·2% (95% CI 4·2–8·1) for every two-fold increase of maternal antibody levels. This occurrence has been shown in other pathogens such as measles.[32] This is explained by saturation of Fc receptors in the placenta, which binds the antibodies transferred from mothers to neonates.[22]

A substantial drop in local EV-A71 prevalence was found after 2016. We did additional analyses stratified by the time of birth of neonates to assess the potential effect of EV-A71 activity in our findings. The cohort experiencing stronger EV-A71 epidemics showed higher antibody titres and seroprevalence due to natural infections than those of the other cohort.

No significant association was found between breastfeeding and the risk of seroconversion. Breastfeeding information was only obtained for the first 6 months of life. This might have limited our exploration of the effect of breastfeeding on seroconversion because natural infections predominantly occurred after age 6 months because of decay of maternal antibodies and increased contact with other children.

Originally, the primary objective of our wider study was to estimate the infection rate among neonates. Therefore, the sample size calculation was based on the annual risk of EV-A71 infection. However, the main purpose of this part of the study was to estimate the transfer and dynamics of maternal antibodies on the basis of this cohort. The sample size was found to be sufficient to estimate $\log_{(GMTs)}$ to within ±1 and seroprevalence to within ±2·5%.

Compared with previous studies,[10–15] our study was strengthened by the cohort's sequential longitudinal design and a large sample size, with frequent follow-up visits and high follow-up rates. Substantial differences were observed in factors such as mothers' education, township of residence, parity, and birthweight of neonates between the infants who did and did not participate in the study, as well as follow-up visits. However, the disparity would not have biased our findings because these factors were not associated with the transfer ratio of maternal antibodies, antibody titres, and seropositivity in neonates (appendix 2, pp 11, 14–16).

The maternal age at delivery in our study (median 25 years, IQR 23–29) was similar to that of women across China (28·5 years).[33] The GMT of EV-A71 antibodies in mothers in our study (22·1) was consistent with those of other studies across China (24·0–26·6).[10,11,13] Accordingly, the transfer characteristics of maternal antibodies in our study are likely to represent those of China. However, the seasonality and activity of EV-A71 varied between geographical regions. Two peaks in spring and autumn were observed in southern China, whereas only one peak in summer was observed in northern China.[3] Additionally, EV-A71 has been circulating in the Asia-Pacific region since the 1990s, whereas EV-A71 activity remained at a low level in Europe and the USA for decades, where an increasing number of EV-A71-associated outbreaks occurred in the past 5 years.[5–7,34,35] Accordingly, the generalisability of our results on natural infections to northern China and other countries should be done with caution because of variations in EV-A71 exposure history.

We found that the transfer ratio of maternal EV-A71 antibodies was 1·03, which is similar to that from a study done in Guangzhou, China.[10] However, the transfer ratio of EV-A71 antibodies was lower than that of measles (1·65), mumps (1·53), and rubella (1·62).[20] Many factors might have affected the transfer capability, such as IgG subclasses, maternal nutrition, maternal HIV infection, diabetes, and hypergammaglobulinaemia.[22] In this study, we did not explore these factors, and this could be a topic for additional research.

Our study had some limitations. First, we were unable to determine whether maternally derived EV-A71 antibodies protected against clinical illness because this would have required frequent and regular active surveillance of each participant for episodes of HFMD throughout follow-up. Second, the last three follow-up visits were done in the spring and autumn of each year, which did not necessarily correspond to the exact ages of 12, 24, and 36 months of neonates. This discrepancy led to variations in the exact age of infants at follow-up visits and small numbers in some age groups, which could influence the precision of seroprevalence estimates. As shown in figure 3D, only 11 children were included in the age 33 months group, which had a wide 95% CI.

Protective titres of EV-A71 antibodies were efficiently transferred to neonates from mothers through the placenta. However, titres declined rapidly to below protective levels up to the time of the first dose of EV-A71 vaccination at age 6 months. Our study was a longitudinal cohort study with mother–neonate pairs that followed up children from birth up to age 36 months with serial serology. Studies such as ours are essential to obtain robust estimates of maternal antibodies against EV-A71, better understand their dynamics, and inform policy making on EV-A71 vaccination. It is important to assess the potential benefits of vaccinating children younger than 6 months and pregnant women, as well as the benefits of one-time catch-up vaccination for children aged between 6 months and 5 years.

Contributors

HYu conceived, designed and supervised the study. XW, JY, LG, QL, FL, KL, SY, QC, HYa, JZho, BD, WX, MH, LL, PW, and ZC coordinated and participated in data collection. LW, SY, QQ, YZ, XC, LR, and JG did the laboratory tests. XW, JY, QL, JZha, and JZho analysed the data. XW and JY prepared the figures and the first draft of the manuscript. HRvD, SC, BJC, and HYu commented on the data and its interpretation and revised the content critically. All authors contributed to the review and revision, approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Declaration of interests

HYu has received research funding from Sanofi Pasteur, GlaxoSmithKline, Yichang HEC Changjiang, and Shanghai Roche Pharmaceutical Company, outside this study. HRvD has received grants

from Pfizer and Sanofi, outside this study, and reports grants from Oxford Li Ka Shing Foundation during the conduct of the study. BJC has received honoraria from Roche and Sanofi, outside this study. All other authors declare no competing interests.

Acknowledgments

We acknowledge that this work was a continuous research project conducted by HYu, who was a former employee of the Chinese Center for Disease Control and Prevention and moved to Fudan University in May, 2017. We thank staff members of the Anhua County-level, Yiyang Prefecture-level, and Hunan Provincial-level departments of health for providing assistance with administration and data collection; staff members at the Anhua County-level, Yiyang Prefecture-level, and Hunan Provincial-level Centers for Disease Control and Prevention and study hospitals (Anhua People's Hospital, Anhua Maternal and Child Health Hospital, Anhua Chinese Medicine Hospital, Anhua Second People's Hospital, Jiangnan Township Health Center, Qingtang Township Health Center, and Tianzhuang Township Health Center) for providing assistance with field investigation, administration, and data collection. We thank staff from the Institut Pasteur of Shanghai, Chinese Academy of Sciences, for their help with data collection and laboratory assays. HYu acknowledges financial support from the National Science Fund for Distinguished Young Scholars (81525023), the National Natural Science Foundation of China (81473031), the Li Ka Shing Oxford Global Health Programme (LG33), the National Science and Technology Major Project of China (2018ZX10201001–010, 2018ZX10713001–007, and 2017ZX10103009–005), and Emergency Response Mechanism Operation Program, Chinese Center for Disease Control and Prevention (131031001000015001). SC acknowledges financial support from the Investissement d'Avenir programme, the Laboratoire d'Excellence Integrative Biology of Emerging Infectious Diseases programme (ANR-10-LABX-62-IBEID).

References

1 WHO. A Guide to clinical management and public health response for hand, foot and mouth disease (HFMD). 2011. https://iris.wpro.who.int/handle/10665.1/5521 (accessed Dec 26, 2019).

2 Ooi MH, Wong SC, Lewthwaite P, Cardosa MJ, Solomon T. Clinical features, diagnosis, and management of enterovirus 71. *Lancet Neurol* 2010; **9:** 1097–105.

3 Xing W, Liao Q, Viboud C, et al. Hand, foot, and mouth disease in China, 2008–12: an epidemiological study. *Lancet Infect Dis* 2014; **14:** 308–18.

4 Yang B, Liu F, Liao Q, et al. Epidemiology of hand, foot and mouth disease in China, 2008 to 2015 prior to the introduction of EV-A71 vaccine. *Euro Surveill* 2017; **22:** 16–824.

5 Messacar K, Spence-Davizon E, Osborne C, et al. Clinical characteristics of enterovirus A71 neurological disease during an outbreak in children in Colorado, USA, in 2018: an observational cohort study. *Lancet Infect Dis* 2020; **20:** 230–39.

6 Bubba L, Broberg EK, Jasir A, et al. Circulation of non-polio enteroviruses in 24 EU and EEA countries between 2015 and 2017: a retrospective surveillance study. *Lancet Infect Dis* 2020; **20:** 350–61.

7 Böttcher S, Diedrich S, Keeren K. Increased detection of enterovirus A71 infections, Germany, 2019. *Euro Surveill* 2019; **24:** 1900556.

8 Lin J-Y, Kung Y-A, Shih S-R. Antivirals and vaccines for enterovirus A71. *J Biomed Sci* 2019; **26:** 65.

9 Chinese Center for Disease Control and Prevention. Technical guidelines for the use of inactivated enterovirus 71 vaccines. *Chin J Vaccines Immunization* 2016; **22:** 458–64 (in Chinese).

10 Fu C, Lu L, Wu H, et al. Placental antibody transfer efficiency and maternal levels: specific for measles, coxsackievirus A16, enterovirus 71, poliomyelitis I-III and HIV-1 antibodies. *Sci Rep* 2016; **6:** 38874.

11 Xia W, Li Y, Yin D, et al. Investigation on neutralizing antibody level of enterovirus type 71 and coxsackievirus group A type 16 among mothers and newborns in six counties. *Chin J Vaccines Immunization* 2013; **19:** 164–67 (in Chinese).

12 Ji H, Li L, Liu Y, et al. Seroepidemiology of human enterovirus 71 and coxsackievirusA16 in Jiangsu province, China. *Virol J* 2012; **9:** 248.

13 Li Z, Liu T, Yang G, Xia X. Seroepidemiological study of enterovirus A71 in children in Lipu county of Guangxi province in 2010. *Applied Prev Med* 2013; **19:** 281–83 (in Chinese).

14 Luo ST, Chiang PS, Chao AS, et al. Enterovirus 71 maternal antibodies in infants, Taiwan. *Emerg Infect Dis* 2009; **15:** 581–84.

15 Fu C, Shen J, Lu L, et al. Pre-vaccination evolution of antibodies among infants 0, 3 and 6 months of age: a longitudinal analysis of measles, enterovirus 71 and coxsackievirus 16. *Vaccine* 2017; **35:** 3817–22.

16 Mao QY, Liao XY, Yu X, et al. Dynamic change of mother-source neutralizing antibodies against enterovirus 71 and coxsackievirus A16 in infants. *Chin Med J (Engl)* 2010; **123:** 1679–84.

17 Gao L, Zou G, Liao Q, et al. Spectrum of enterovirus serotypes causing uncomplicated hand, foot, and mouth disease and enteroviral diagnostic yield of different clinical samples. *Clin Infect Dis* 2018; **67:** 1729–35.

18 WHO. Polio laboratory manual, 4th edn. 2004. https://www.who.int/immunization/documents/WHO_IVB_04.10/en/ (accessed June 23, 2016).

19 Scott S, Cumberland P, Shulman CE, et al. Neonatal measles immunity in rural Kenya: the influence of HIV and placental malaria infections on placental transfer of antibodies and levels of antibody in maternal and cord serum samples. *J Infect Dis* 2005; **191:** 1854–60.

20 van den Berg JP, Westerbeek EA, Smits GP, van der Klis FR, Berbers GA, van Elburg RM. Lower transplacental antibody transport for measles, mumps, rubella and varicella zoster in very preterm infants. *PLoS One* 2014; **9:** e94714.

21 Zhu FC, Meng FY, Li JX, et al. Efficacy, safety, and immunology of an inactivated alum-adjuvant enterovirus 71 vaccine in children in China: a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2013; **381:** 2024–32.

22 Wilcox CR, Holder B, Jones CE. Factors affecting the FcRn-mediated transplacental transfer of antibodies and implications for vaccination in pregnancy. *Front Immunol* 2017; **8:** 1294.

23 de Voer RM, van der Klis FR, Nooitgedagt JE, et al. Seroprevalence and placental transportation of maternal antibodies specific for *Neisseria meningitidis* serogroup C, *Haemophilus influenzae* type B, diphtheria, tetanus, and pertussis. *Clin Infect Dis* 2009; **49:** 58–64.

24 Chu HY, Steinhoff MC, Magaret A, et al. Respiratory syncytial virus transplacental antibody transfer and kinetics in mother-infant pairs in Bangladesh. *J Infect Dis* 2014; **210:** 1582–89.

25 Hughes MM, Englund JA, Edwards K, et al. Pertussis seroepidemiology in women and their infants in Sarlahi District, Nepal. *Vaccine* 2017; **35:** 6766–73.

26 Huang LM, Chiu CH, Chiu NC, et al. Immunogenicity, safety, cross-reaction, and immune persistence of an inactivated enterovirus A71 vaccine in children aged from two months to 11 years in Taiwan. *Vaccine* 2019; **37:** 1827–35.

27 Petrovsky N. Comparative safety of vaccine adjuvants: a summary of current evidence and future needs. *Drug Saf* 2015; **38:** 1059–74.

28 WHO. Vaccines against influenza. WHO position paper—November 2012. *Wkly Epidemiol Rec* 2012; **87:** 461–76.

29 Mazzilli S, Tavoschi L, Lopalco PL. Tdap vaccination during pregnancy to protect newborns from pertussis infection. *Ann Ig* 2018; **30:** 346–63.

30 Hu Y, Zeng G, Chu K, et al. Five-year immunity persistence following immunization with inactivated enterovirus 71 type (EV71) vaccine in healthy children: a further observation. *Hum Vaccin Immunother* 2018; **14:** 1517–23.

31 Takahashi S, Liao Q, Van Boeckel TP, et al. Hand, foot, and mouth disease in China: modeling epidemic dynamics of enterovirus serotypes and implications for vaccination. *PLoS Med* 2016; **13:** e1001958.

32 Hartter HK, Oyedele OI, Dietz K, Kreis S, Hoffman JP, Muller CP. Placental transfer and decay of maternally acquired antimeasles antibodies in Nigerian children. *Pediatr Infect Dis J* 2000; **19:** 635–41.

33 Song J, Zhang J. Parity, timing of birth, and fertility: a comparative study on the trends and differential mechanism of the mean age at childbearing in China, Japan, and Korea. *Popul Res* 2017; **41:** 3–14 (in Chinese).

34 Puenpa J, Wanlapakorn N, Vongpunsawad S, Poovorawan Y. The history of enterovirus A71 outbreaks and molecular epidemiology in the Asia-Pacific region. *J Biomed Sci* 2019; **26:** 75.

35 Solomon T, Lewthwaite P, Perera D, Cardosa MJ, McMinn P, Ooi MH. Virology, epidemiology, pathogenesis, and control of enterovirus 71. *Lancet Infect Dis* 2010; **10:** 778–90.