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Original Article

Serostatus of echovirus 11, coxsackievirus B3 and enterovirus D68 in cord blood: The implication of severe newborn enterovirus infection

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Abstract *Background:* Maternal transplacental antibody is an important origins of passive immunity against neonatal enterovirus infection. Echovirus 11 (E11) and coxsackievirus B3 (CVB3) are important types causing neonatal infections. There were few investigations of enterovirus D68 (EVD68) infection in neonates. We aimed to investigate the serostatus of cord blood for these three enteroviruses and evaluate the factors associated with seropositivity.

Methods: We enrolled 222 parturient (gestational age 34—42 weeks) women aged 20—46 years old between January and October 2021. All participants underwent questionnaire investigation and we collected the cord blood to measure the neutralization antibodies against E11, CVB3 and EVD68.

Results: The cord blood seropositive rates were 18% (41/222), 60% (134/232) and 95% (211/222) for E11, CVB3 and EVD68, respectively ($p < 0.001$). Geometric mean titers were 3.3 (95% CI 2.9—3.8) for E11, 15.9 (95% CI 12.5—20.3) for CVB3 and 109.9 (95% CI 92.4—131.6) for

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EVD68. Younger parturient age (33.8 ± 3.6 versus 35.2 ± 4.4, p = 0.04) was related to E11 seropositivity. Neonatal sex, gestational age and birth body weight were not significantly different between the seropositive group and the seronegative group.
Conclusion: Cord blood seropositive rate and geometric mean titer of E11 were very low, so a large proportion of newborns are susceptible to E11. The circulation of E11 was low after 2019 in Taiwan. A large cohort of immune naïve newborns existed currently due to lack of protective maternal antibodies. It is imminent to monitor the epidemiology of neonates with enterovirus infections and strengthen the relevant preventive policies.

Introduction

Infection with nonpolio enteroviruses (NPEVs) is very common in children worldwide and may induce severe diseases, especially in young children. Enterovirus A71 (EV A71), enterovirus D68 (EVD68), coxsackievirus B1—5, echovirus 6 and 11 are clinically important NPEVs.[1] However, only the EV A71 vaccine is currently available in some Asian countries.[2]

Enterovirus infection remains a major threat to neonates because of the high case fatality rate and difficulty in early diagnosis. Unlike older children, newborns and young infants have different clinical presentations and susceptible types of enterovirus infection. CVB and echovirus, especially CVB3 and E11, are the most important types related to newborn enterovirus infections which could lead to hepatic necrosis, coagulopathy and central nervous infection.[3—8]

Pregnant women having enterovirus infection, especially coxsackievirus B (CVB), is a risk factor for miscarriage.[9,10] Moreover, one investigation of placental specimens and abortive tissues in Korea found a significantly higher coxsackievirus B3 (CVB3) prevalence in abortion cases.[11] CVB 1—5 are the leading type that causes neonatal myocarditis, with a 30—50% mortality rate.[3] According to Taiwan Centers of Disease Control virology laboratory surveillance data, CVB3 was among the top three circulating types in 2005 and 2012, but had low circulation after 2013.[12]

Echoviruses are another predominant enterovirus type among neonates. One study disclosed that echovirus 11 (E11) was the most common type circulating in children younger than five years of age in Northern Italy.[13] E11 seldom plays the leading role in enterovirus outbreaks. It accounted for 10% and 16% of the predominant types in Taiwan only in 2003 and 2018, respectively.[12]

On the other hand, there have been few investigations of EVD68 infection in neonates. However, one neonatal mouse model provided evidence that EVD68 infection caused pulmonary and neurological damage in neonatal mice.[14] In addition, those neonatal mice having maternal transplacental antibodies against infection totally survived after EVD68 challenge.[14] A total of 92 confirmed EVD68 cases were recorded during 2007 and 2016 in Taiwan.[15] However, there was low circulation of EVD68 after 2019.[12] In this study, we aimed to investigate the cord blood serostatus and associated factors of protective maternal antibodies to improve the understanding and prevention of E11, CVB3 and EVD68.

Materials and methods

Case enrolment

We enrolled parturient women (gestational age 30—42 weeks) aged 20—46 years old between January and October 2021. After written informed consent was obtained, we conducted a questionnaire investigation to collect demographic data, education level, medical history, the number of children in the household, drug history and knowledge of infection prevention. The questionnaire is listed in the supplementary file. Cord blood samples were collected during delivery to measure the neutralization antibodies against E11, CVB3 and EVD68. This study was approved by the Institutional Review Board of National Taiwan University Hospital (202006068RIND).

Neutralizing antibodies against echovirus 11, coxsackievirus B3 and enterovirus D68

The neutralizing antibody test followed the standard protocol of a neutralization test.[16] Serum samples were heat-treated for 30 min at 56 °C, serially diluted twofold (1:4 to 1:256), mixed with 100 50% tissue culture-infective doses (TCID50) of E11 (isolated in 2018), CVB3 (isolated in 2016) and EVD68 (isolated in 2017, clade B3) and incubated for 2 h at 37 °C (E11 and CVB3) or 33 °C (EVD68). Thereafter, rhabdomyosarcoma cells were added to each reaction well and incubated at 37 °C or 33 °C in a 5% CO_2 incubator. Each plate included a cell control, serum control, and virus back-titration. The cytopathic effect was monitored from 4 to 6 days after incubation, and the serotiter was determined when the cytopathic effect was observed in one TCID50 of the virus back-titration. Neutralization titers lower than four were assigned as two, and those higher than 1:256 were assigned as 1:512. The antibody titer of an individual sample was first taken as the logarithmic value, and then the exponent was taken after averaging, which was the geometric mean titer (GMT). Seropositivity was defined as a serotiter ≥1:8.

Statistical analysis

We used independent t tests and Mann—Whitney tests to examine continuous variables. We analysed categorical

variables by the chi-square test and Fisher's exact test. All statistical analyses were performed via PASW version 18.0, and $p < 0.05$ was considered statistically significant.

Results

Demographic data of parturient women and their newborns

We enrolled 222 parturient women with gestational age of 34—42 weeks. The age of the parturient women ranged from 20 to 46 years. The median age was 31.2 years, with 22 (9.9%) women 20—29 years of age, 171 (77%) women 30—39 years of age and 29 (13.1%) women over 40 years of age. Twenty-four (10.8%) participants had an underlying medical history, including 4 (1.8%) with an autoimmune disease, 3 (1.4%) with congenital heart disease, 3 (1.4%) hepatitis B carriers and 3 (1.4%) with hyperthyroidism. Other underlying diseases were valvular heart disease, hypertension, thyroid cancer, hypothyroidism and asthma. Eleven parturient women had used medication during pregnancy. Seven (3.2%) of them used steroids, and 8 (3.6%) women received hydroxychloroquine and/or azathioprine. The other main characteristics of 222 parturient women were summarized in Table 1.

During delivery, cord blood samples were collected from 231 newborns, including 9 twins. Girls accounted for 51.9% (120/231), and 12 newborns had low birth weight (less than 2500 g). The mean gestational age was 38.5 weeks, and 8 newborns were premature (born before 37 weeks of pregnancy).

The seropositive rates of echovirus 11, coxsackievirus B3 and enterovirus D68 in cord blood

The serotiters of cord blood were similar without a fourfold difference between twins, so the serotiter from only one twin was included in the analysis. The overall seropositive rates in cord blood were 18% (41/222, 95% CI 14—24), 60% (134/222, 95% CI 54—67) and 95% (211/222, 95% CI 91—98) for E11, CVB3 and EVD68, respectively ($p < 0.0001$). The cord blood seropositive rates of parturient women among three different maternal age groups, 20—29 years, 30—39 years and over 40 years, are shown in Fig. 1. There was no significant difference of serostatus among different maternal age groups ($p = 0.14$ for E11, $p = 0.52$ for CVB3 and $p = 0.38$ for EVD68, respectively). The GMT were 3.3 (95% CI 2.9—3.8) for E11, 15.9 (95% CI 12.5—20.3) for CVB3 and 109.9 (95% CI 92.4—131.6) for EVD68 (see Fig. 2). None of the cord blood from the 8 preterm babies had E11 antibodies, but the cord blood from 41 (18.9%) term babies was seropositive for E11 ($p = 0.28$). Two (40%) of 5 premature babies were CVB3 seropositive, whereas 132 (60.8%) term newborns had CVB3 antibodies ($p = 0.35$). The seropositive rates of EVD68 were 80% (4/5) and 95.4% (207/217) in the cord blood from premature babies and term babies, respectively ($p = 0.12$).

Table 1 The main characteristics of 222 parturient women.

	Number (%)
Delivery method	
Normal spontaneous delivery	185 (83.3)
Cesarean section	37 (16.7)
Primipara	136 (61.3)
The number of household child	
None	113 (50.9)
Only one	75 (33.8)
More than three	13 (5.9)
Work as a teacher or doctor	50 (22.5)
Contact with children diagnosed with EV infection during pregnancy	12 (5.4)

Abbreviation: EV enterovirus.

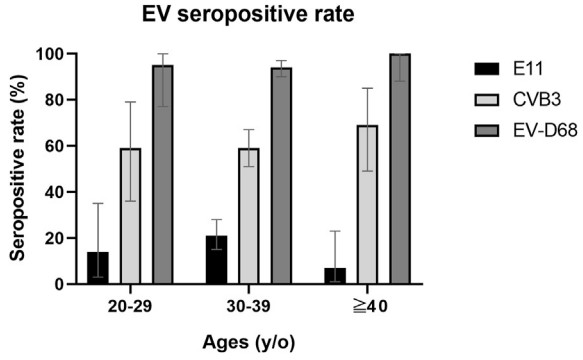

Figure 1. Cord blood seropositive rates of echovirus 11, coxsackievirus B3 and enterovirus D68 among different age groups Cord blood seropositive rates of E11 were 14%, 21% and 7% in the age groups of 20—29 years, 30—39 years and ≥40 years, respectively ($p = 0.14$). The cord blood seropositive rates of CVB3 and EVD68 increased with age ($p = 0.53$ for CVB3 and $p = 0.38$ for EVD68, respectively). Overall, the cord blood seropositive rate was significantly lower for E11 than for CVB3 and EVD68 ($p < 0.0001$).

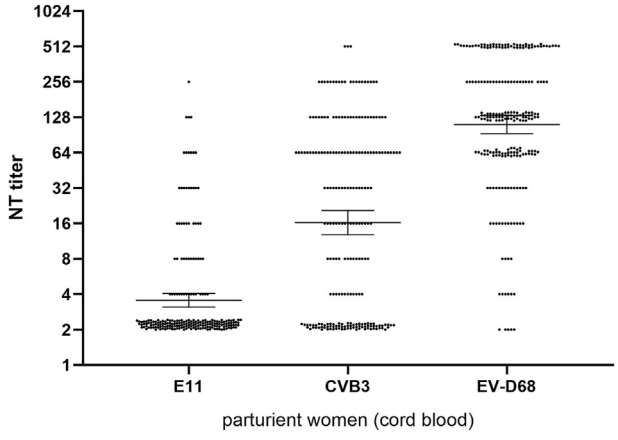

Figure 2. The geometric mean titer (GMT) of echovirus 11, coxsackievirus B3 and enterovirus D68 in cord blood The GMT of E11 (3.3, 95% CI 2.9—3.8) was markedly lower than coxsackievirus B3 (15.9, 95% CI 12.5—20.3) and enterovirus D68 (109.9, 95% CI 92.4—131.6).

Factors associated with seropositivity for echovirus 11, coxsackievirus B3 and enterovirus D68

Overall, 98.6% of the enrolled parturient women understood that frequent hand washing and wearing a mask could prevent enterovirus infection, and 73.4% of the participants always or often followed the correct steps of hand washing in their daily life. While 92.8% of parturient women knew that adults were able to get enterovirus infection, 47.7% of women were not sure whether newborns would be infected via vertical transmission. Moreover, 38.3% of participants misunderstood that 75% alcohol had the power to kill enterovirus.

Maternal factors, including age, infectious symptoms before delivery, contact and cluster history, maternal drug history, and neonatal conditions were evaluated for cord blood seropositivity and are summarized in Table 2. Analysis of the questionnaire revealed that the factors related to E11 seropositivity in cord blood were younger parturient women (33.8 ± 3.6 versus 35.2 ± 4.4, $p = 0.04$). One the other hand, the parturient women with cord blood CVB3 seropositivity were slightly older than those who were seronegative (35.4 ± 4.3 versus 34.1 ± 4.2, $p = 0.03$). No significant difference in age was found between EVD68 seropositive and seronegative group ($p = 0.33$). The use of immunosuppressants including steroid during pregnancy was related to a lower cord blood seropositive rate of EVD68 ($p = 0.04$), but not for E11 ($p = 0.12$) and CVB3 ($p = 0.69$). However, the sex, gestational age and birth body weight of the newborns were not significantly different between the seropositive group and the seronegative group for E11, CVB3 and EVD68. Cord blood seropositivity was not related to the delivery method, occupation, education level and the number of parity or children in the household.

Discussion

Cord blood seropositive rate and GMT of E11 were significantly lower than CBV3 and EVD68. As such it is hypothesized that transplacental maternal antibodies for neonatal immunity against E11 infection is also low. Due to the low neutralizing antibody titers, the protective effect is also hypothesized to be low. Conversely, seropositive rates of CVB3 and EVD68 in cord blood were 60% and 95%, respectively. There was no previous seroprevalence data of cord blood for CVB3 so far, which made our results unique. One EVD68 seroepidemiology study in China also found that the seroprevalence rates of EVD68 neutralizing antibodies were 100% in both prenatal women and their neonates.[17] In addition, prenatal women with high EVD68 antibody levels could transfer more antibodies to their neonates and had longer protection. This might be the reason why neonates seldom have EVD68 infection owing to adequate protection from transplacental antibodies.

Transplacentally acquired maternal antibodies effectively prevent neonatal and young infants from specific enterovirus type infections. One EV A71 seroepidemiology study proved that 99% of infants younger than 6 months had adequate maternal antibodies against EV A71.[18] However, infants 6–11 months of age had a higher risk of severe EV A71 infection, possibly due to the waning of maternal antibodies and immature immunity. Besides, we found that premature infants had low seropositive rates in this study. Preterm birth was considered to be a risk factor for severe neonatal enterovirus infection.[3,4] Premature babies, especially those born at or before a gestational age of 32 weeks, received fewer transplacental antibodies, had lower geometric mean titers of antibodies and had a shorter duration of protection from maternal

Table 2 Maternal and neonatal factors associated with cord blood serostatus for echovirus 11, coxsackievirus B3 and enterovirus D68.

Factors	E11			CVB3			EVD68		
	Seropositive	Seronegative	P value	Seropositive	Seronegative	P value	Seropositive	Seronegative	P value
	N = 41	N = 181		N = 134	N = 88		N = 211	N = 11	
Maternal age (mean ± SD)	33.8 ± 3.6	35.2 ± 4.4	0.04	35.4 ± 4.3	34.1 ± 4.2	0.03	35.0 ± 4.4	33.7 ± 3.0	0.33
Had infection symptoms/signs during pregnancy	19 (46.3%)	59 (32.6%)	0.10	48 (35.8%)	30 (34.1%)	0.79	74 (35.1%)	4 (36.4%)	1.0
Household children had enterovirus infection	3 (15.0%)	3 (3.1%)	0.06	5 (7.0%)	1 (2.2%)	0.4	5 (4.5%)	1 (20.0%)	0.24
Immunosuppressants use	4 (9.8%)	7 (3.9%)	0.12	6 (4.5%)	5 (5.7%)	0.69	9 (4.3%)	2 (18.2%)	0.04
Newborn sex (boys/girls)	16/25 (0.64)	90/91 (0.99)	0.22	66/68 (0.97)	40/48 (0.83)	0.58	101/110 (0.92)	5/6 (0.83)	0.88
GA at birth <37 weeks	0 (0%)	5 (2.8%)	0.28	2 (1.5%)	3 (3.4%)	0.35	4 (1.9%)	1 (9.1%)	0.12
BBW <2500 g	1 (2.4%)	8 (4.4%)	0.56	4 (3.0%)	5 (5.7%)	0.32	8 (3.8%)	1 (9.1%)	0.39

Abbreviations: E11 echovirus 11, CVB3 coxsackievirus B3, EVD68 enterovirus D68.

antibodies.[19,20] Maternal antibody protection would even fade away when there was a declining trend of specific enterovirus type circulation.[21] Lacking specific enterovirus maternal antibodies, especially E11, may lead to increased severe case numbers and a higher case fatality rate of neonatal enterovirus infections.

Lower seropositivity of E11 was also found in another study conducted by our team and the study enrolled healthy, community-dwelling 16 to 50-year-olds from the northern (Taipei City), eastern (Hualien County), western (Yunlin County) and southern (Kaohsiung City) regions of Taiwan between May and November 2017, before the latest large E11 circulation in Taiwan in 2018.[12,15,22] We used the previous blood samples of 20 to 40-year-old nonpregnant women of the aforementioned study to test E11 neutralization antibody. The overall seropositive rate of E11 in these nonpregnant women in 2017 was 31.3% (42/134) and the overall GMT of E11 was low, 4.6 (95% CI 3.8—5.5). Seroepidemiology studies of EVD68 and EV A71 in China, the Netherlands, Malaysia and Taiwan all showed that seropositivity and antibody titers increased with age but declined in elderly individuals.[16,17,23—25] Nevertheless, the E11 seropositive rate in cord blood decreased when parturient women were over 40 years of age in our study.

Household contact transmission of enterovirus has been previously proven.[26,27] Only 12 parturient women were confirmed to have contact with children diagnosed with enterovirus infection such as hand-foot-mouth disease and herpangina during pregnancy. The questionnaire analysis didn't reveal that those having household children diagnosed with enterovirus infection had a significantly higher seropositive rate of E11, CVB3 or EVD68 in cord blood. The use of immunosuppressants was found to be a factor associated with a higher seronegative rate of EVD68, but not for E11 or CVB3. Nevertheless, whether immunosuppressants influence seropositivity needs further research due to the small sample size in this study.

In our study, the majority of parturient women understood the correct way of enterovirus infection prevention and practiced in daily life. However, 47.7% of women were not sure whether newborns would be infected via vertical transmission. Moreover, 38.3% of participants misunderstood that 75% alcohol had the power to kill enterovirus. To prevent neonates from having enterovirus infections, we should put more emphasis on surveillance and health education for different clinical manifestations and transmission routes of neonatal enterovirus infection in the future.

There are some limitations in our study. First, we collected cord blood rather than maternal and neonatal samples to measure the neutralizing antibodies of specific enterovirus types. We did not calculate the direct transplacental ratio of maternal antibodies. However, previous investigations showed that maternal serum antibodies had a high correlation with cord blood or neonatal blood samples.[22,28,29] Second, part of the questionnaire analysis was dependent on self-assessment rather than medical records. Therefore, there may be some recall bias. Third, the number of women in different maternal age groups was inconsistent. The statistical analysis may have bias due to unbalanced case numbers.

In conclusion, transplacentally acquired maternal antibodies plays an important role in neonatal immunity against enteroviral infection. Lack of maternal antibodies against E11 leads to severe neonatal enterovirus infection and even death. The circulation of E11 was low after 2019 in Taiwan. A large cohort of immune naïve newborns existed currently due to lack of protective maternal antibodies. On the other hand, the circulation of CVB3 and EVD68 was also low after 2019 in Taiwan. However, the cord blood seropositive rate and GMT of CVB3 and EVD68 were higher than E11. A possible explanation is that we did not detect the circulation in the community due to high asymptomatic infections. Our study calls for warning information to monitor the epidemiology of neonates with enterovirus infections and strengthen the relevant preventive policies.

Declaration of competing interest

All authors have no conflicts to declare.

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References

1. Liu DP, Wang TA, Huang WT, Chang LY, Wang ET, Cheng SH, et al. Disease burden of enterovirus infection in Taiwan: implications for vaccination policy. *Vaccine* 2016;**34**:974—80.
2. Nguyen TT, Chiu CH, Lin CY, Chiu NC, Chen PY, Le TTV, et al. Efficacy, safety, and immunogenicity of an inactivated, adjuvanted enterovirus 71 vaccine in infants and children: a multiregion, double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet* 2022;**399**:1708—17.
3. Chuang YY, Huang YC. Enteroviral infection in neonates. *J Microbiol Immunol Infect* 2019;**52**:851—7.
4. Sandoni M, Ciardo L, Tamburini C, Boncompagni A, Rossi C, Guidotti I, et al. Enteroviral infections in the first three months of life. *Pathogens* 2022;**11**:60.
5. Chen YC, Yang SL, Yang H, Lin TY, Hsieh YC, Huang KA, et al. Clinical characteristics of echovirus 11 and coxsackievirus B5 infections in Taiwanese children requiring hospitalization. *J Microbiol Immunol Infect* 2021;**54**:581—7.
6. Modlin JF, Polk BF, Horton P, Etkind P, Crane E, Spiliotes A. Perinatal echovirus infection: risk of transmission during a community outbreak. *N Engl J Med* 1981;**305**:368—71.
7. Lin TY, Kao HT, Hsieh SH, Huang YC, Chiu CH, Chou YH, et al. Neonatal enterovirus infections: emphasis on risk factors of severe and fatal infections. *Pediatr Infect Dis J* 2003;**22**:889—94.
8. Khetsuriani N, Lamonte A, Oberste MS, Pallansch M. Neonatal enterovirus infections reported to the national enterovirus surveillance system in the United States, 1983-2003. *Pediatr Infect Dis J* 2006;**25**:889—93.
9. Jaïdane H, Halouani A, Jmii H, Elmastour F, Mokni M, Aouni M. Coxsackievirus B4 vertical transmission in a murine model. *Virol J* 2017;**14**:16.

10. Axelsson C, Bondestam K, Frisk G, Bergström S, Diderholm H. Coxsackie B virus infections in women with miscarriage. *J Med Virol* 1993;**39**:282—5.
11. Hwang JH, Kim JW, Hwang JY, Lee KM, Shim HM, Bae YK, et al. Coxsackievirus B infection is highly related with missed abortion in Korea. *Yonsei Med J* 2014;**55**:1562—7.
12. Taiwan Centers of Disease Control. https://www.cdc.gov.tw. [Accessed 10 March 2022].
13. Bubba L, Martinelli M, Pellegrinelli L, Primache V, Tanzi E, Pariani E, et al. A 4-year study on epidemiologic and molecular characteristics of human parechoviruses and enteroviruses circulating in children younger than 5 Years in northern Italy. *Pediatr Infect Dis J* 2017;**36**:13—9.
14. Sun S, Bian L, Gao F, Du R, Hu Y, Fu Y, et al. A neonatal mouse model of Enterovirus D68 infection induces both interstitial pneumonia and acute flaccid myelitis. *Antivir Res* 2019;**161**:108—15.
15. Lee JT, Shih WL, Yen TY, Cheng AL, Lu CY, Chang LY, et al. Enterovirus D68 seroepidemiology in Taiwan, a cross sectional study from 2017. *PLoS One* 2020;**15**:e0230180.
16. Huang WC, Huang LM, Kao CL, Lu CY, Shao PL, Cheng AL, et al. Seroprevalence of enterovirus 71 and no evidence of cross-protection of enterovirus 71 antibody against the other enteroviruses in kindergarten children in Taipei city. *J Microbiol Immunol Infect* 2012;**45**:96—101.
17. Sun S, Gao F, Hu Y, Bian L, Wu X, Su Y, et al. A cross-sectional seroepidemiology study of EV-D68 in China. *Emerg Microb Infect* 2018;**7**:99.
18. Luo ST, Chiang PS, Chao AS, Liou GY, Lin R, Lin TY, et al. Enterovirus 71 maternal antibodies in infants, Taiwan. *Emerg Infect Dis* 2009;**15**:581—4.
19. van den Berg JP, Westerbeek EA, van der Klis FR, Berbers GA, van Elburg RM. Transplacental transport of IgG antibodies to preterm infants: a review of the literature. *Early Hum Dev* 2011;**87**:67—72.
20. Linder N, Taushtein I, Handsher R, Ohel G, Reichman B, Barzilai A, et al. Placental transfer of maternal poliovirus antibodies in full-term and pre-term infants. *Vaccine* 1998;**16**:236—9.
21. Salur L, Oikarinen S, Tauriainen S, Mandel M, Hyöty H, Uibo R. Enterovirus infections in young infants: are children still protected by maternal antibodies? *Hum Vaccine* 2011;**7**:966—71.
22. Lee JT, Yen TY, Shih WL, Lu CY, Liu DP, Huang YC, et al. Enterovirus 71 seroepidemiology in Taiwan in 2017 and comparison of those rates in 1997, 1999 and 2007. *PLoS One* 2019;**14**:e0224110.
23. Xiang Z, Li L, Ren L, Guo L, Xie Z, Liu C, et al. Seroepidemiology of enterovirus D68 infection in China. *Emerg Microb Infect* 2017;**6**:e32.
24. Karelehto E, Koen G, Benschop K, van der Klis F, Pajkrt D, Wolthers K. Enterovirus D68 serosurvey: evidence for endemic circulation in The Netherlands, 2006 to 2016. *Euro Surveill* 2019;**24**:1800671.
25. Chan YF, Sam IC, Nayan E, Tan XH, Yogarajah T. Seroepidemiology of enterovirus D68 infection in kuala lumpur, Malaysia between 2013 and 2015. *J Med Virol* 2022;**94**:2607—12.
26. Hoang CQ, Nguyen HD, Ho NX, Vu THT, Pham TTM, Nguyen KT, et al. Incidence of infection of enterovirus 71 and coxsackieviruses A6 and A16 among household contacts of index cases in dong thap province, southern vietnam. *BioMed Res Int* 2020;**2020**:9850351.
27. Chang LY, Tsao KC, Hsia SH, Shih SR, Huang CG, Chan WK, et al. Transmission and clinical features of enterovirus 71 infections in household contacts in Taiwan. *JAMA* 2004;**291**:222—7.
28. Wei X, Yang J, Gao L, Wang L, Liao Q, Qiu Q, et al. 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. *Lancet Infect Dis* 2021;**21**:418—26.
29. Flannery DD, Gouma S, Dhudasia MB, Mukhopadhyay S, Pfeifer MR, Woodford EC, et al. Assessment of maternal and neonatal cord blood SARS-CoV-2 antibodies and placental transfer ratios. *JAMA Pediatr* 2021;**175**:594—600.