Clinical Epidemiology and Global Health 8 (2020) 1302–1305

Contents lists available at ScienceDirect

Clinical Epidemiology and Global Health

journal homepage: www.elsevier.com/locate/cegh

The cytomegalovirus seroprevalence among children in Mostar, Bosnia and Herzegovina: A hospital cross-sectional study

Damir Franjić[a], Helena Karlović[a], Borko Rajič[a,b], Igor Azinović[a], Martina Komšić[c], Vinka Mikulić[a,d], Božo Šušak[a,c], Tamara Miličević[b], Maja Arapović[a], Željka Bilinovac[b], Ivanka Mikulić[a,d], Jurica Arapović[a,c,*]

[a] School of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina
[b] Department of Paediatrics, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina
[c] Department of Infectious Diseases, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina
[d] Department of Laboratory Medicine, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina

ARTICLE INFO

Keywords:
Cytomegalovirus
Children
Seroprevalence
Risk factors

ABSTRACT

Background: Cytomegalovirus (CMV) seroprevalence varies between 60% in developed countries and 100% in developing countries. The aim of this study was to assess CMV seroprevalence in representative sample of children in Bosnia and Herzegovina and to identify possible factors associated with CMV.

Methods: Blood samples and surveys were collected from 253 children at the Department of Paediatrics and Department of Infectious diseases of University Clinical Hospital Mostar. Blood sera were tested for the presence of CMV IgG antibodies by ELISA. CMV seroprevalence was correlated with age, gender, household members, presence of pets and animals in household, attendance of day-care and summer camps and use of already used cutlery.

Results: Overall CMV seroprevalence among children was 56.5% (P = 0.038) and raised with age of children from 44.4% in early preschool children up to 73.9% in adolescents (P = 0.002). There was no significant difference in the CMV seroprevalence between genders (P = 0.732). Significantly higher rate of the CMV seroprevalence was observed among children having pets or domestic animals (P = 0.015), among those who attended summer sport camp or school camp (P = 0.04) and among children using of dirty cutlery and glasses (P = 0.039).

Conclusions: More than half of tested children were CMV seropositive with significantly higher rate in older children. Higher CMV seroprevalence was in correlations with possession of the pets or domestic animals, attendance at the summer sport camp or school camp and use of dirty cutlery and glasses.

1. Introduction

Cytomegalovirus (CMV) belongs to *herpesviridae* family *and betaherpesvirinae* subfamily. Common feature of all herpesviruses is their ability to stay in human body for the whole life in latent form after primary infection.[1,2] Generally, CMV infections are more spread among population of developing countries and those countries with low socioeconomic status (SES). Previous studies showed CMV seroprevalence between 40 and 79% in western European countries,[3,4] while in Asia, Africa and South America seroprevalence is 96–100%.[5,6] In addition, study performed on a group of children between four and 12 years old reported seroprevalence between 40 and 60% in developed European countries, while in Africa and Asia the seroprevalence of almost 100% was reported.[7] Humans are only reservoirs of CMV and natural transmission is made by direct or indirect, close and intimate contact between persons. CMV is mostly found in oropharyngeal secretions, urine, cervical and vaginal secretions, sperm, breast milk, tears, faeces and blood.[2] Because of its nature of transmission, CMV is widespread among all age groups, especially children having poor hygiene habits, attending collectives and kindergartens, sharing cutlery and glasses.[8] Close contact transmission has been previously described in previous studies where family members or children at kindergartens were included, and most probable source of CMV was upper respiratory tract or urine.[9,10] Therefore, young children are source of CMV for their families because they acquire infection from other children at day care centres transmitting virus to their siblings.[10] Moreover, households with larger

* Corresponding author. Department of Infectious Diseases, University Clinical Hospital Mostar, Kralja Tvrtka b.b., 88000, Mostar, Bosnia and Herzegovina.
E-mail address: jurica.arapovic@mef.sum.ba (J. Arapović).

https://doi.org/10.1016/j.cegh.2020.05.001
Received 24 January 2020; Received in revised form 27 April 2020; Accepted 1 May 2020
Available online 19 May 2020

number of members have been associated with higher CMV seroprevalence because of more interpersonal contact.[11] Also higher CMV seroprevalence was found among adolescents that had more sexual partners or that had sexually transmitted diseases.[12] Acute CMV infection in immunocompetent hosts is asymptomatic or it is presented as infective mononucleosis, especially in adolescents.[13,14] CMV IgG is present in sera of children that were either in close contact with virus reservoir or the antibodies were transmitted from mother to baby through placenta.[15,16] There is still no commercially available vaccine against CMV that would prevent infection in childhood, but there are a lot of pre-clinic trials and experimental trails that are promising.[17]

The aim of this study was to determine CMV seroprevalence among paediatric population and to discover risk factors associated with CMV infection in Bosnia and Herzegovina.

2. Subjects and methods

2.1. Study design and subjects

This prospective cross-sectional study included 253 children at the Department of Paediatrics and Department of Infectious Diseases of University Clinical Hospital Mostar (UCHM) in time period from November 2018 to May 2019. The study included children aged from one to 18 years. Infants were excluded from the study because of CMV IgGs crossing placental barrier. The sera of children admitted at UCHM were taken during routine diagnostic procedures at different Paediatric wards. During sampling period 734 children were admitted to the wards. We excluded 167 children because they were infants and those 314 did not either answer to questionnaire or did not sign in agreement for sera collection. The data were collected using questionnaire designed for children. If they were younger than 14 years then questionnaire was filled by their parents. Information on socio-demographic and lifestyle parameters were obtained by standardized self-administered questionnaires.

Subjects were divided in four groups by age: young pre-school children (two and three years old), pre-school children (four to six years old), school children (seven to 12 years old) and adolescents (13 to 18 years old). Age groups were chosen according to different habits and exposures to risk factors. Place of residence was categorised as urban or rural. Number of household members was divided in three groups: less than four, four to five, more than five. Subjects answered to questions (Yes or No) about presence of children younger than six years in their household, everyday contact with animals, attending kindergarten and attending summer school camp or sport camp. Children's habits using dirty cutlery and glasses were examined by Likert scale question: never, sometimes, often and very often. Sexual habits of adolescents were also in questionnaire but they were excluded from analysis due to lack of data.

Along with data collected using questionnaire we collected blood sera. Sera were collected from children admitted to paediatric wards or children in daily hospital at Department of Paediatrics and at Department of Infectious Diseases of UCHM. During regular blood sampling the rest of biochemical sample tube was taken for study. Sample was marked with code and sent to Central laboratory of UCHM where sera were separated and stored at −20 °C prior CMV IgG ELISA analysing.

Ethical approval was acquired from Ethical Committee (No. 229/18) at UCHM. The study was conducted according to Federal and Government regulations for Data Protection. All subjects and their samples were assigned a code used later in data analysis.

2.2. ELISA testing of CMV IgGs

Serology was performed within six months after taking blood sample. Sera were analyzed using CMV IgG kit from Diagnostic Automation, Cortez Diagnostics, USA. The antibody detection approach was based on indirect ELISA. Whole assay procedure was carried out according to manufacturer instructions. Briefly, sera, calibrator and control were diluted 1:40 in serum diluent. Then, 100 μL of diluted sera were added to each well. Plate was incubated three times. First with diluted sera only for 30 min, then with conjugate for 30 min and last time with chromogen solution for 15 min. Before each incubation wells were washed manually. After last incubation stop solution was added and plates were read on ELISA plate reader (BioRad, USA) at 450 nm filter. Measured optical density (O. D.) values of calibrator and samples were added to formula presented in the Manual and CMV G Index was calculated. If CMV G Index was less than 0.90 sera were interpreted as negative (1.1 IU/ml), between 0.91 and 0.99 sera was equivocal and needed to be retested, and if it was above 1.00 sample was seropositive (> 1.2 IU/ml).

2.3. Statistical analysis

Nominal data were presented using frequency and percentage. Frequencies of nominal variables were analyzed using chi-square (χ^2) test. The data distribution was determined using Shapiro-Wilk's W test. Continuous variables did not have normal distribution so Mann-Whitney *U* test was used for analysis and values were shown as median and interquartile range. Results were analyzed using the SPSS version 24.0 (SPSS, Chicago, IL) and computer program Excel (Microsoft Office Excel 2019). All tests were two-tailed and values of $P < 0.05$ were considered as statistically significant. Questions with missing data were excluded from analysis for each set of questions.

3. Results

This study included 253 subjects. Median of age was 6.0 (Range = 16) and it ranged from one to 18 years. There were 128 (50.6%) male children and 125 (49.4%) female children.

Overall CMV seroprevalence among paediatric population was 56.5% (χ^2 test = 4.30; df = 1; P = 0.038). In male children, CMV seroprevalence was 57.6% and in female children 55.5% (χ^2 test = 0.12; df = 1; P = 0.732).

Median age of seropositive children was seven vs. four of seronegative (Mann-Whitney Z = -3.16; P = 0.002). The CMV seroprevalence increased with age. The lowest was in early pre-school children 44.4% and highest was in adolescents 73.9% (χ^2 test = 11.12; df = 3; P = 0.011) (Fig. 1).

Risk factors associated with CMV serostatus were shown in Table 1. Factors associated with CMV seropositivity included: using of dirty cutlery and glasses (Fisher's exact = 8.22; df = 3; P = 0.039), everyday contact with animals (χ2 test = 5.93; df = 1; P = 0.015) and attendance at the summer school camp or sport camp (Fishers's exact = 4.54; df = 1; P = 0.04). On the other hand, place of residence (χ2 test = 0.07; df = 1; P = 0.788), number of household members (χ2 test = 1.38; df = 2; P = 0.501), presence of children younger than six years in household (χ2 test = 0.34; df = 1; P = 0.560), attendance at the kindergarten (χ2 test = 1.30; df = 1; P = 0.254) were not associated with higher CMV seroprevalence.

4. Discussion

In this study the CMV seroprevalence raised among children with respect to age and it was highest among adolescents (73.9%). Older children have higher CMV seroprevalence because they had more interpersonal contact, starting at kindergarten and later at school or sport camps. Source of virus in younger children is upper respiratory tract or urine.[9–11] Mouth kisses and sexual contact is the most common transmission pathway among adolescents.[2] In accordance to our study, Seale et al. found higher CMV seroprevalence in older children, but no difference in CMV seroprevalence among genders.[18]

There are few studies about CMV seroprevalence within the Balkan

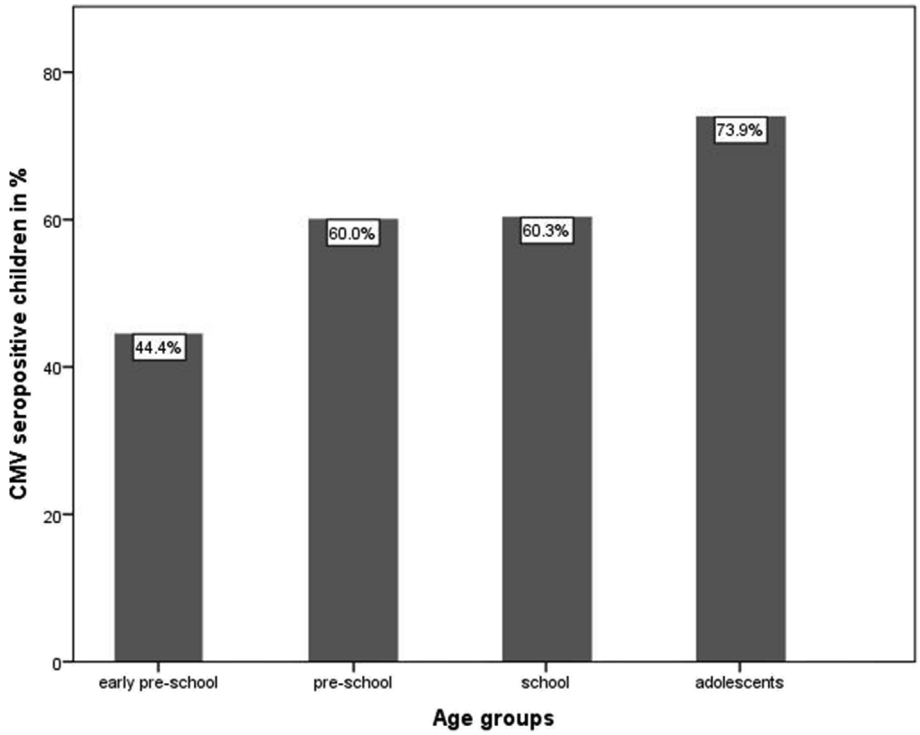

Fig. 1. CMV seroprevalence among children by age groups.

Table 1
The CMV serostatus by risk factors.

Variables		CMV positive N = 143 (%)	CMV negative N = 110 (%)	P
Using dirty cutlery and glasses	Never	18 (14.4)	15 (16.5)	0.039[b]
	Sometimes	90 (72)	52 (57.1)	
	Often	11 (8.8)	20 (22)	
	Very often	6 (4.8)	4 (4.4)	
Everyday contact with animals	Yes	65 (51.6)	31 (34.8)	0.015[a]
	No	61 (48.4)	58 (65.2)	
Attending summer school camp or sport camp	Yes	14 (11.2)	3 (3.3)	0.04[b]
	No	111 (88.8)	88 (96.7)	
Place of residence	Urban	46 (33.3)	32 (31.7)	0.788[a]
	Rural	92 (66.7)	69 (68.3)	
Number of household members	Less than 4	25 (19.8)	13 (13.8)	0.501[a]
	4–5	84 (66.7)	68 (72.4)	
	More than 5	17 (13.5)	13 (13.8)	
Presence of children younger than 6 years in household	Yes	50 (40)	40 (44)	0.560[a]
	No	75 (60)	51 (56)	
Attending kindergarten	Yes	62 (49.6)	38 (41.8)	0.254[a]
	No	63 (50.4)	53 (58.2)	

Not all subjects answered on each question in survey.
[a] $\chi 2$ test was used.
[b] Fisher's exact test was used.

region. The study carried out by Vilibic-Cavlek et al. in Croatia found seroprevalence of 90.7% among dialyzed patients.[19] Also, the study from Kosovo among childbearing-aged women found CMV seroprevalence of 96.2%.[20] Recently, the study performed on pregnant women in Tuzla and Mostar in Bosnia and Herzegovina found high CMV seroprevalence of 93% and 92%, respectively.[21,22] In accordance to this, we could presume that CMV seroprevalence among paediatric population in Bosnia and Herzegovina would be same as in low SES and developing countries from Asia or Africa.[5,6] Additionally, Jansen et al. found that by the age of three almost all children in countries with low SES were CMV seropositive whereas CMV seroprevalence varies between 40 and 60% among developed European countries.[7] Similar to our results, our recent study performed among patients at UCHM revealed that CMV seroprevalence was increased with age from 50.8% in children aging between one and five years, up to 97.7% in > 65 age group. Furthermore, above mentioned recent study showed overall seroprevalence of 81.4% among all patients and gender difference in patients older than 20 years were not demonstrated.[22] Also, results from our study did not show difference in CMV seroprevalence related to the gender among children and the overall CMV seroprevalence among paediatric population was 56.5% showing for the first time valuable results paediatric population in Bosnia and Herzegovina. Furthermore, we can conclude that CMV seroprevalence among paediatric population in Bosnia and Herzegovina is higher than in most western European countries, but lower than seroprevalence in Asian and African countries.

We did not find any difference between CMV serostatus and children living in urban or rural area, so in Bosnia and Herzegovina there are no varieties among living environments. This thesis was also confirmed through studies in Poland and Germany.[23,24]

Another risk factor that we assessed is number of household members. The study done by Korndewal et al. in the Netherlands showed that number of household members did not have impact on CMV seroprevalence, same as we found in our study.[8] On the other hand, Fowler et al. found that children coming from families with three or more household members have higher risk of acquiring congenital CMV infections.[25] Crowding in living space should be associated with higher seroprevalence because there is more interpersonal contact but most of studies did not relate it to higher risk for CMV infections.

This study showed higher CMV seroprevalence among children that had pets or domestic animals in their living environment. Currently, in literature there is no information about CMV seroprevalence and exposure to animals. One research in Kirkuk noted some connections between seroprevalence and contact with animals. However, results were not statistically significant even if they showed higher

seroprevalence 92.8% vs. 90.4% in women that had contact with animals in their living environment.[26] Animal CMV differs from human CMV and transmission is not possible.[27] Conversely, one research group found that baboon CMV can replicate in human fibroblasts[28] implicating cross-species transmission. We assume that people who had animals had warmer personality and had more interpersonal contacts with other people so they had bigger chance of acquiring CMV infection.

Attending summer school camp or sports camp means more interpersonal contact. Common toilets, bathrooms, sharing cutlery and glasses are some of risk factors for CMV infection associated with staying at group facilities.[2] In our study we found higher CMV seroprevalence among children that attended sports camp more than six months. On the other side, Stadler et al. did not find higher CMV seroprevalence among adolescents attending camps, even so this study was done only on male subjects.[29]

CMV is found in human saliva, therefore, we can expect higher seroprevalence among children that use cutlery and glasses after someone.[2] Our study proved that, children that used dirty cutlery and glasses had higher CMV seroprevalence. Contrary, Stadler et al. did not find connection between this risk factor and CMV infection.[29]

Major strength of this research is that we acquired representative sample for southern region of Bosnia and Herzegovina because all children from this region gravitate to UCHM. We acquired age-specific data related to prevalence of CMV that can be later used to determine ideal time for children CMV vaccination. Weakness of this study is small sample size. Another weakness is that we only examined children at hospitals and they may be susceptible to infections because they were admitted to hospital for different reasons. We could not examine sexual behaviour of adolescents because most of them refused to answer those questions and we had insufficient data.

Altogether, our study is the first cross-sectional study presenting the CMV seroprevalence among paediatric population in Bosnia and Herzegovina, and confirming previously shown potential risk factors for the CMV infections. This data can be starting point for future research and comparison of such data to the results obtained in other southeast European countries. These data cannot be generalized for whole country due to the relatively small number of subjects and without including medical centres in different parts of Bosnia and Herzegovina. The CMV seroprevalence was higher than in developed European countries and it was probably result of poor hygiene and low SES in which children are growing up, but at the same time it should raise awareness of CMV infections as potential public health problem that might be solved by systematic educational approach.

Author contributions

All authors contributed to the conception and design of this study, were involved in the interpretation of the data, and the development and approval of the manuscript.

Ethical considerations

All procedures followed were in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. As this was a prospective study, every patient was informed and gave consent for data and blood sample collecting, any potentially-identifying patient information was omitted.

Ethical approving number 229/18 at UCHM, Mostar, Bosnia and Herzegovina.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial and profit sectors.

Declaration of competing interest

None declared.

Acknowledgments

We thank to medical staff at Department of Paediatrics, Department of Infectious Diseases and Central laboratory of UCHM for helping in collecting data for the study.

References

1. Drew WL. Herpes viruses. In: Ryaned. *Sherris Medical Microbiology*. vol. 4. McGraw-Hill Medical; 2004:555–576.
2. Pass RF. Cytomegalovirus. In: Fieldsed. *Fields Virology*. vol. 5. Philadelphia: Lippincott; 2001:2675–2706.
3. Galea G, Urbaniak SJ. Cytomegalovirus studies on blood donors in North-East Scotland and a Review of UK data. *Vox Sang.* 1993;64:24–30.
4. Hecker M, Qiu D, Marquardt K, Bein G, Hackstein H. Continuous cytomegalovirus seroconversion in a large group of healthy blood donors. *Vox Sang.* 2004;86:41–44.
5. Pultoo A, Meetoo G, Pyndiah MN, Khittoo G. Seroprevalence of cytomegalovirus infection in Mauritian volunteer blood donors. *Indian J Med Sci.* 2001;55:73–78.
6. Kothari A, Ramachandran VG, Gupta P, Singh B, Talwar V. Seroprevalence of cytomegalovirus among voluntary blood donors in Delhi, India. *J Health Popul Nutr.* 2002;20:348–351.
7. Jansen MA, van den Heuvel D, Bouthoorn SH, et al. Determinants of ethnic differences in cytomegalovirus, epstein-barr virus, and herpes simplex virus type 1 seroprevalence in childhood. *J Pediatr.* 2016;170:126–134.
8. Korndewal MJ, Mollema L, Tcherniaeva I, et al. Cytomegalovirus infection in The Netherlands: seroprevalence, risk factors, and implications. *J Clin Virol.* 2015;63:53–58.
9. Pass RF, Little EA, Stagno S, Britt WJ, Alford CA. Young children as a probable source of maternal and congenital cytomegalovirus infection. *N Engl J Med.* 1987;316:1366–1370.
10. Adler SP. Molecular epidemiology of cytomegalovirus: viral transmission among children attending a day care center, their parents, and caretakers. *J Pediatr.* 1988;112:366–372.
11. Staras SAS, Dollard SC, Radford KW, Flanders WD, Pass RF, Cannon MJ. Seroprevalence of cytomegalovirus infection in the United States, 1988–1994. *Clin Infect Dis.* 2006;43:1143–1151.
12. Jordan MC, Rousseau WE, Noble GR, Steward JA, Chin TD. Association of cervical cytomegaloviruses with venereal disease. *N Engl J Med.* 1973;288:932–934.
13. Kotton CN. CMV: prevention, diagnosis and therapy. *Am J Transplant.* 2013;13:24–40.
14. Horwitz CA, Henle W, Henle G, et al. Clinical and laboratory evaluation of cytomegalovirus-induced mononucleosis in previously healthy individuals. Report of 82 cases. *Medicine.* 1986;65:124–134.
15. Burbelo PD, Issa AT, Ching KH, et al. Highly quantitative serological detection of anti-cytomegalovirus (CMV) antibodies. *Virol J.* 2009;6:45.
16. Nelson CT, Istas AS, Wilkerson MK, Demmler GJ. PCR detection of cytomegalovirus DNA in serum as a diagnostic test for congenital cytomegalovirus infection. *J Clin Microbiol.* 1995;33:3317–3318.
17. Plotkin SA, Boppana SB. Vaccination against the human cytomegalovirus. *Vaccine.* 2018;37:7437–7442.
18. Seale H, MacIntyre CR, Gidding HF, Backhouse JL, Dwyer DE, Gilbert L. National seroosurvey of cytomegalovirus in Australia. *Clin Vaccine Immunol.* 2006;13:1181–1184.
19. Vilibić-Čavlek T, Kolarić B, Bogdanić M, Tabain I, Beader N. Herpes group viruses: a seroprevalence study in hemodialysis patients. *Acta Clin Croat.* 2017;56:255–261.
20. Pribakovic JA, Katanic N, Radevic T, et al. Serological status of childbearing-aged women for Toxoplasma gondii and cytomegalovirus in northern Kosovo and Metohija. *Rev Soc Bras Med Trop.* 2019;52:e20170313.
21. Porobic-Jahic H, Skokic F, Ahmetagic S, Piljic D, Jahic R, Petrovic J. Cytomegalovirus infection in pregnancy - our experiences. *Med Arch.* 2019;73:149–153.
22. Arapovic J, Rajic B, Pati S, et al. Cytomegalovirus seroprevalence and birth prevalence of congenital CMV infection in Bosnia and Herzegovina: a single-center experience. *Pediatr Infect Dis J.* 2020;39:140–144.
23. Lachmann R, Loenenbach A, Waterboer T, et al. Cytomegalovirus (CMV) seroprevalence in the adult population of Germany. *PloS One.* 2018;13 e0200267-e0200267.
24. Siennicka J, Dunal-Szcepaniak M, Trzcinska A, Godzik P, Rosinska M. High seroprevalence of CMV among women of childbearing age implicates high burden of congenital cytomegalovirus infection in Poland. *Pol J Microbiol.* 2017;65:425–432.
25. Fowler KB, Pass RF. Risk factors for congenital cytomegalovirus infection in the offspring of young women: exposure to young children and recent onset of sexual activity. *Pediatrics.* 2006;118:e286–e292.
26. Aljumaili ZKM, Alsamarai AM, Najem WS. Cytomegalovirus seroprevalence in women with bad obstetric history in Kirkuk, Iraq. *J Infect Publ Health.* 2014;7:277–288.
27. Marsh AK, Ambagala AP, Perciani CT, et al. Examining the species-specificity of Rhesus Macaque cytomegalovirus (RhCMV) in cynomolgus Macaques. *PloS One.* 2015;10:e0121339.
28. Michaels MG, Jenkins FJ, St George K, Nalesnik MA, Starzl TE, Rinaldo CR. Detection of infectious baboon cytomegalovirus after baboon-to-human liver xenotransplantation. *J Virol.* 2001;75:2825–2828.
29. Stadler LP, Bernstein DI, Callahan ST, et al. Seroprevalence of cytomegalovirus (CMV) and risk factors for infection in adolescent males. *Clin Infect Dis.* 2010;51:e76–81.