MAJOR ARTICLE

Age-Specific Prevalence of Epstein–Barr Virus Infection Among Individuals Aged 6–19 Years in the United States and Factors Affecting Its Acquisition

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Background. Data on the age-specific prevalence of Epstein–Barr virus (EBV) infection are relevant for determining when to administer a prophylactic vaccine. Comparison of demographic groups could identify factors associated with its acquisition.

Methods. The National Health and Nutrition Examination Surveys (NHANES) examine a representative sample of the US population. Serum specimens from NHANES participants 6–19 years old were tested for EBV antibody by enzyme immunoassay (EIA). A random portion was also tested by indirect immunofluorescence (IFA). Prevalence estimates and risk-factor comparisons used demographic data and sampling weights in logistic regression models.

Results. Serum specimens collected between 2003 and 2010 from 9338 individuals participating in NHANES were tested. The concordance between EIA and IFA findings was 96.7%. The overall age-adjusted EBV antibody prevalence declined from 72% in 2003–2004 to 65% in 2009–2010 ($P = .027$). The prevalence in 2009–2010 by age group was as follows: 6–8 years, 50%; 9–11 years, 55%; 12–14 years, 59%; 15–17 years, 69%; and 18–19 years, 89%. Within each race/ethnicity group, younger age, health insurance coverage, higher household income, and education level were significantly associated with a lower prevalence of EBV antibody.

Conclusions. The EBV antibody prevalence declined in US individuals aged 6–19 years from 2003–2004 to 2009–2010, mainly because of the decrease among non-Hispanic white participants. The declining antibody prevalence over time and the consistently high observed prevalence among participants aged 12–19 years support broad use of EBV vaccine before 12 years of age.

Keywords. Epstein–Barr virus (EBV); age-specific EBV antibody prevalence; epidemiology of EBV infections.

Epstein–Barr virus (EBV) infection is very common in the United States. Most reviews state that >90% of adults are antibody positive by the age of 35 years. In a case-control study of multiple sclerosis, serum specimens collected between 1988 and 2000 and banked at the Department of Defense Serum Repository were tested [1]. Of the 166 control subjects (mean age, 24 years), 159 (96%) were EBV antibody positive.

In contrast, the prevalence of EBV antibodies among children is lower, varying from 20% to 80% depending on age and geographic location [2]. Factors implicated in early acquisition of primary EBV infection include geographic region [2], socioeconomic status [3–5], crowding or sharing a bedroom [5, 6], maternal education level [7], day care attendance [4], and school

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catchment area [5], but no national studies are available to define the relationship of age-specific acquisition of EBV infection to any of these factors.

There appears to be a complex interplay between age of acquisition of primary EBV infection, symptomatic versus asymptomatic infection, and the risk of EBV-associated cancers or autoimmune diseases [8–10]. Identifying demographic risk factors for age-specific acquisition of the virus and describing EBV infection disparities on the basis of race/ethnicity and/or country of birth will improve our current understanding of EBV infection in children and could inform appropriate prevention strategies.

In that regard, results of a phase 2 study of a prophylactic gp350 EBV vaccine suggested the possibility of a viable intervention [11]. This vaccine was shown to reduce the incidence of infectious mononucleosis, which is a clinically significant disease in more-developed countries because of its high attack rate, especially among young adults, and long duration of illness (median, 10 days) [12]. Besides infectious mononucleosis, EBV vaccine could potentially reduce the incidence of Hodgkin lymphoma, posttransplantation lymphoproliferative disorder, endemic Burkitt lymphoma, nasopharyngeal carcinoma, and even multiple sclerosis [13].

Our aims were to define the age-specific prevalence of EBV antibody among US individuals aged 6–19 years as a marker for acquisition of infection, which is helpful for selecting an appropriate age at which to administer a prophylactic EBV vaccine, and to identify risk factors for acquiring EBV infection earlier in life, which may predispose individuals to chronic EBV diseases. To accomplish these aims, we tested stored serum specimens for EBV antibody and analyzed linked demographic data collected during the following 4 National Health and Nutritional Examination Survey (NHANES) cycles: 2003–2004, 2005–2006, 2007–2008, and 2009–2010.

METHODS

Test Serum Specimens and Sampling Design

NHANES is conducted by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention. Since 1999, NHANES has been conducted as a continuous survey, with findings released every 2 years, that examines a nationally representative sample of the US noninstitutionalized civilian population and surveys about 5000 persons each year, using a complex multistage probability cluster sampling design [13]. Approximately 96% of interviewed individuals underwent a physical examination at the mobile examination center, and most of those examined consented to storage of their serum specimens. This study was approved by the Research Subjects Protection Program of the University of Minnesota and the NCHS Research Ethics Review Board. Participants provided written informed consent and assent for the storage of serum specimens and for future research, in addition to the interview and the physical examination.

Our study was based on individuals aged 6–19 years who were interviewed and examined during NHANES cycles 2003–2004, 2005–2006, 2007–2008, or 2009–2010 and had serum samples available for testing. At least 70% of individuals at every age (ie, 6–19 years) who received a physical examination had serum specimens available, except for those participating in the NHANES 2005–2006 cycle, among whom 65% had available samples.

NHANES Demographic and Questionnaire Data

We assessed those demographic factors that have been examined previously in NHANES serum samples for antibody prevalence to other herpesviruses, namely cytomegalovirus and herpes simplex virus type 1 [15–17]. The characteristics were sex, age (6–8, 9–11, 12–14, 15–17, and 18–19 years), race/ethnicity (non-Hispanic white, non-Hispanic black, and Mexican American), birthplace (United States, Mexico, and elsewhere), household income level (ratio of household income to the family's appropriate poverty threshold: low [<1.3], middle [1.301–3.500], or high [>3.5]), health insurance status (covered or not covered), household education level (the higher level of both parents in the categories less than high school, high school diploma including General Educational Development diploma, or more than high school), and crowding index (low [<0.5 person per room], middle [0.5–1 person per room], or high [>1 person per room]). Crowding index was the ratio of the number of persons in the household to the number of rooms in the house; the question about number of rooms was not asked during the NHANES 2009–2010 cycle. Comparisons between race/ethnicity groups were restricted to non-Hispanic whites, non-Hispanic blacks, and Mexican Americans because the proportion of Hispanic participants during the NHANES 2003–2004 and 2005–2006 cycles who were non–Mexican Americans was too small to produce reliable estimates [18].

Virology Laboratory Procedures

EBV Antibody Enzyme Immunoassay (EIA)

The presence of immunoglobulin G (IgG) antibody against EBV viral capsid antigen (VCA) was measured using semiquantitative EIA kits (Diamedix, Miami, FL). We chose this method because it has been continuously used in the clinical research virology laboratory of the University of Minnesota since 2002. We have tested >4000 samples in 158 runs with only 3 batch failures (failure rate, 1.9%) and have published results from 3 studies in which the antibody responses correlated closely with clinical findings in primary EBV infection and EBV load [12, 19, 20]. Briefly, 20 μL of sample was used for each assay. Specimens, calibrators, and controls were prediluted at a ratio of 1:21 before they were placed in the test wells. Cutoff

calibrator, control, and subject samples were diluted according to the specific test kit protocol, mixed briefly by vortex to ensure good distribution of the sample in the diluent, and transferred to the appropriately labeled wells and incubated. After the wells were washed, conjugate was added, followed by another incubation and wash step. Substrate was added and allowed to incubate before the addition of stop/color reagent. The plates were read at an absorbance of 450 nm, using a reference wavelength of 630 nm. EIA indices were calculated by hand, using the formulas in the kit package insert. Data were recorded as (1) positive (EIA index $\geq$ 1.10), negative (EIA index < 0.90), or equivocal (EIA index 0.90–1.09), and (2) the exact numeric EIA index. All positive and negative EIA results were included in the data analysis. Equivocal samples were not tested further, and subjects with equivocal results were not included in the data analysis because we had no way of determining whether they were incubating a primary EBV infection and were in the process of seroconverting or whether they had nonspecific reactivity in the antibody EIA.

Indirect Immunofluorescence Antibody Method

A random selection of samples was also tested for VCA IgG antibodies by an indirect immunofluorescence assay (IFA) to assess concordance of EIA findings with those of a second method. We chose IFA as our comparison method because the EBV IFA developed by the Henles [3, 21] was the criterion standard for EBV antibody determination until assays suitable for high throughput, such as EIAs, were perfected [22]. IFA antibodies were screened at a 1:10 serum dilution. We calculated the percentage positive agreement, the percentage negative agreement, and the concordance correlation coefficient.

The IFA method used commercial kits (Focus Laboratories, Cypress, CA). Briefly, 10 μL of serum was diluted 1:10 with phosphate-buffered saline. The diluted serum was then layered onto a slide containing the target (lymphocytes immortalized with EBV) and incubated. At the end of the incubation period, the slide was washed with phosphate-buffered saline to remove unbound antibody from the test well. The substrate wells were then layered with fluorescein-labeled anti-human IgG. After incubation the slide was washed again, dried, mounted with a coverslip, and examined by fluorescent microscopy. Positive cells were those with bright apple green cytoplasm in contrast to the negative control cells.

Western Blot Assay

Serum specimens with discordant EIA and IFA results were tested by Western blot using the method described by Grieger et al [23] and optimized for EBV as follows. EBV from a commercial H3PR1 cell lysate (EVO-12, Virusys, Taneytown, MD) was diluted in equal volumes of dilution buffer, resolved by sodium dodecyl sulfate gel (8% and 16%), and transferred to nitrocellulose membranes. The membranes were cut into strips and blotted with subjects' serum specimens in a 1:2000 dilution in 1% milk as the primary antibody. These strips were then treated with a secondary antibody, goat anti-Hu IgG in a 1:5000 dilution in 1% milk (PO214, Dako, Carpinteria, CA), and then treated with an enhanced chemiluminescence reagent (Pierce SuperSignal West Femto Maximum Sensitivity Substrate, Thermo-Fisher, Rockford, IL). Strips were exposed to photographic film (GeneMate, Bioexpress, Kaysville, UT) and developed for analysis. Analysis was performed by comparison of the derived bands to previously characterized bands measured in kilodaltons and to a known standard protein ladder, MagicMark XP (LC5602, Invitrogen, Carlsbad, CA).

Statistical Analysis

All EBV antibody prevalence estimates were calculated using NCHS examination weights to represent the civilian, noninstitutionalized US population 6–19 years old, using the Survey-Logistic procedure (version 9.3, SAS Institute). To examine the effect of missing data, we calculated adjusted weights by dividing the NCHS weights by the nonmissing proportion in a subject's age, sex, and race/ethnicity category [14]. These adjusted weights gave very similar antibody prevalence estimates well within the 95% confidence intervals (CIs) of estimates computed with the original NCHS weights, so only prevalence estimates based on NCHS weights are reported.

For comparisons between non-Hispanic whites, non-Hispanic blacks, and Mexican Americans, data from all 4 NHANES cycles were combined and NCHS sampling weights were multiplied by 0.25. Prevalence estimates from the combined data were predictive margins (back-transformed predicted means on the logit scale) from a single survey logistic regression model that adjusted for race/ethnicity, age, sex, household income, health insurance status, household education, NHANES cycle, and the interactions of race/ethnicity with age, sex, income, insurance, and education. Crowding index was not included as a predictor in this logistic model because 33% of crowding observations were missing, and neither the main effect nor interaction with race/ethnicity were significant; crowding index was added to the model only to estimate EBV antibody prevalence by crowding level. Birthplace was also omitted from the model because it was strongly confounded with race/ethnicity. Linear contrasts between fitted prevalence estimates were used to compare factor categories between cycles and to compare categories within a factor in each cycle.

For comparisons between the 2003–2004 and 2009–2010 NHANES cycles, sampling weights were multiplied by 0.5, and all data were used, with the following exceptions: in the comparison of racial/ethnic groups, the "Other Hispanic" group was omitted from the 2003–2004 cycle [18], and the "Other" group was omitted from both cycles. These comparisons were adjusted only for age; prevalence estimates were predictive

margins from survey logistic regression models for each demographic factor, with EBV status as the response and demographic factor, cycle, demographic factor–cycle interaction, and age as predictors. Individuals missing a particular demographic factor were omitted from the model for that factor only.

The SurveyLogistic procedure fits logistic regression models for discrete response survey data by using the maximum likelihood method and uses the Taylor series (linearization) method to estimate variance. All *P* values and CIs are based on these variance estimates. Differences with a *P* value of $< .05$ were deemed to be statistically significant.

RESULTS

We received surplus serum samples from 9344 NHANES subjects. Of these, 46 samples (0.5%) gave equivocal EIA results, and 6 (0.06%) had an insufficient quantity to test; these 52 observations were excluded, leaving 9292 observations (unweighted total). The concordance between EIA and IFA findings was 96.7%. Seven of 216 randomly selected samples tested by both EIA and IFA differed qualitatively, yielding a discordance rate of 3.2% (exact 95% CI, 1.3%–6.6%). Six were IFA positive and EIA negative, whereas 1 was EIA positive and IFA negative.

Table 1. Demographic Factors Associated With Epstein–Barr Virus (EBV) Antibody (Ab) Prevalence, by Race/Ethnicity—National Health and Nutrition Examination Survey Cycles 2003–2004, 2005–2006, 2007–2008, and 2009–2010

	White, Non-Hispanic		Black, Non-Hispanic		Mexican American	
Demographic Factor	Subjects, No.[a]	EBV Ab Prevalence, %[b] (95% CI)	Subjects, No.[a]	EBV Ab Prevalence, %[b] (95% CI)	Subjects, No.[a]	EBV Ab Prevalence %[b] (95% CI)
Total	2461	64 (58–69)	2469	88 (85–91)	2586	88 (84–90)
Sex						
Male	1267	62 (56–67)^{A}	1251	87 (83–91)^{A}	1297	87 (82–90)^{A}
Female	1194	66 (60–71)^{A}	1218	90 (86–92)^{A}	1289	89 (85–91)^{A}
Age, y						
6–8	440	48 (40–56)^{A}	412	80 (73–85)^{A}	490	80 (74–84)^{A}
9–11	487	55 (48–62)^{B}	443	83 (78–88)^{A}	517	83 (76–87)^{A,B}
12–14	542	58 (50–65)^{B}	602	89 (85–92)^{B}	648	87 (82–90)^{B,C}
15–17	609	70 (64–75)^{C}	603	91 (87–94)^{B}	575	91 (87–94)^{C,D}
18–19	383	83 (78–87)^{D}	409	94 (91–96)^{C}	356	94 (89–97)^{D}
Household income to poverty level ratio						
Low (≤1.3)	723	72 (66–77)^{A}	1195	92 (89–94)^{A}	1325	92 (90–94)^{A}
Middle (1.301–3.5)	842	63 (56–69)^{B}	925	88 (83–92)^{B}	979	87 (83–91)^{B}
High (>3.5)	896	56 (50–63)^{C}	349	84 (77–90)^{B}	282	81 (74–87)^{C}
Health insurance status						
Insured	2237	66 (61–70)^{A}	2224	84 (82–86)^{A}	1826	84 (81–87)^{A}
Not insured	224	62 (54–69)^{A}	245	92 (86–95)^{B}	760	90 (86–93)^{B}
Household education level						
Less than high school	188	72 (61–80)^{A}	631	91 (87–94)^{A}	1222	91 (89–93)^{A}
High school or GED diploma	566	63 (56–69)^{A,B}	599	89 (84–92)^{A,B}	580	86 (80–91)^{B}
More than high school	1707	56 (52–60)^{B}	1239	85 (80–89)^{B}	784	84 (80–87)^{B}
Crowding index[c]						
Low (<0.5)	430	61 (54–68)^{A}	266	87 (81–91)^{A}	78	90 (82–94)^{A}
Middle (0.5–1.0)	1309	65 (59–72)^{A}	1494	89 (84–92)^{A}	1293	89 (85–92)^{A}
High (>1.0)	84	69 (49–84)^{A}	312	83 (75–89)^{A}	649	93 (90–95)^{A}

Within each racial/ethnic group, categories within a demographic factor were compared by pairwise linear contrasts. Rates with no superscripted capital letters in common are significantly different ($P < .05$), whereas rates that share superscripted capital letters are not significantly different. Comparison of every demographic category between race/ethnicity groups (comparisons within each row) revealed that Ab prevalence rates for whites were all significantly lower than corresponding rates for blacks and Mexican Americans, which did not differ significantly.

Abbreviations: CI, confidence interval; GED, General Educational Development.

[a] Data are unweighted counts of individuals surveyed.

[b] Data are adjusted for age, income, insurance, education, survey cycle and for interactions between race-ethnicity and age, income, insurance, and education, unless otherwise indicated.

[c] Defined as the no. of persons per room. Prevalence estimates were determined from a separate logistic model with the same adjustors, plus crowding index and interaction between crowding index and race/ethnicity.

The positive agreement was 99.4% (167/168); the negative agreement was 87.5% (42/48). All 7 serum specimens that gave discordant results by EIA and IFA were negative or indeterminate by Western blot, which suggested that their positive IFA or EIA results were either false positives or that the subjects were in the early phase of primary EBV infection and had not yet made a definitive antibody response.

The EBV antibody prevalence among participants aged 6–19 years was compared between non-Hispanic whites, non-Hispanic blacks, and Mexican Americans, using combined data from all 4 NHANES cycles. A total of 8137 subjects were evaluated, after omission of 1155 (12%) whose race/ethnicity was classified as "Other Hispanic" or "Other." In these 3 race/ethnic groups, 438 (5%) were missing data on household income, 62 (0.8%) were missing data on insurance status, 222 (3%) were missing data on parents' education level, 4 were missing data on birthplace, and 1816 (22%) were missing data on crowding index. EBV antibody prevalences, adjusted for age, sex, family income, health insurance status, and parents' education level, are shown for non-Hispanic whites, non-Hispanic blacks, and Mexican Americans in Table 1.

The age-adjusted EBV antibody prevalence was 64% (95% CI, 58%–69%) among non-Hispanic whites, significantly lower than the prevalence of 88% (95% CI, 85%–91%) among non-Hispanic blacks and of 88% (95% CI, 84%–90%) among Mexican Americans. Within all 3 racial/ethnic groups, the adjusted EBV antibody prevalence increased with age and decreased with household income and education levels (Table 1 and Figure 1). Among non-Hispanic blacks and Mexican Americans, those with health insurance coverage had significantly lower prevalence estimates than those without such coverage; there was no difference among whites. The adjusted EBV antibody prevalence did not differ by sex and was not associated with crowding index. In every level of every demographic category, antibody prevalence for whites was significantly lower than corresponding estimates for non-Hispanic blacks and Mexican Americans, which did not differ significantly (Table 1).

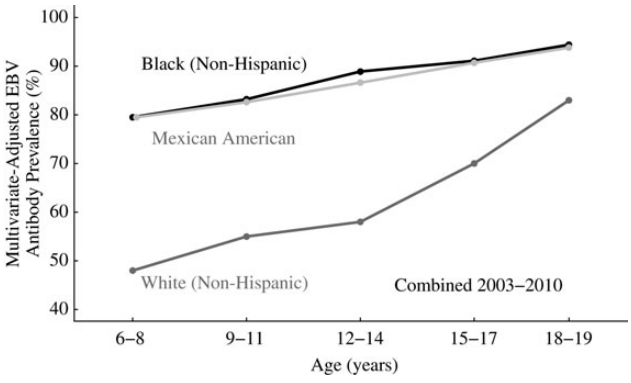

Figure 1. Epstein–Barr virus (EBV) antibody prevalence, by race/ethnicity and age, based on combined National Health and Nutrition Examination Survey data from 2003 through 2010. Prevalence estimates are adjusted for age, sex, household income, health insurance status, and parents' education level.

To assess changes in the EBV antibody prevalence over time, NHANES 2009–2010 data were compared with NHANES 2003–2004 data, using all 4998 available subjects in both surveys; only comparisons by racial/ethnic groups omitted individuals whose race/ethnicity was classified as "Other Hispanic" (2003–2004 only) or "Other" (Table 2). In these 2 NHANES cycles, 299 subjects (6%) were missing data on family income, 44 (1%) were missing data on insurance status, 129 (2.6%) were missing data on parents' education level, and 3 were missing data on birthplace; data on crowding index were not available for 2009–2010 and were missing for 30 subjects (1%) in 2003–2004.

The age-adjusted EBV antibody prevalence declined from an average of 72% during 2003–2004 to 65% during 2009–2010 (Table 2). This decrease was due mostly to the significant decrease among non-Hispanic whites, from 64% to 51% overall, whereas values for neither non-Hispanic blacks nor Mexican Americans changed significantly (Table 2 and Figure 2). The prevalence among non–Mexican American Hispanics during 2009–2010 did not differ from the prevalence among Mexican Americans. Substantial decreases in prevalence were seen among non-Hispanic whites aged 6–11 years, with moderate decreases among non-Hispanic whites aged 12–17 years; changes among non-Hispanic blacks and Mexican Americans were small and inconsistent (Figure 2). After adjustment for age, there were also significant decreases in the EBV antibody prevalence among females, participants aged 9–11 years and those aged 15–17 years, participants born in the United States, and participants with health insurance coverage (Table 2).

Comparing age-adjusted EBV antibody prevalence within levels of each demographic factor, trends seen in 2003–2004 and 2009–2010 were the same as those seen from the combined data: non-Hispanic blacks and Mexican Americans had significantly higher prevalence estimates than non-Hispanic whites, prevalence increased with age, and decreased with health insurance, household income, and household education (Table 2). Prevalence also decreased with crowding index in 2003–2004.

DISCUSSION

A striking finding of this study was the significant decline in the age-adjusted prevalence of EBV antibody between 2003–2004 and 2009–2010. This decrease was mainly driven by a lower EBV antibody prevalence among non-Hispanic white participants. A similar trend has been reported for herpes simplex type 1 [17, 24] and type 2 [24]. Interestingly, a Japanese study also showed that the age-specific EBV antibody prevalence was trending downward [25]. Among 5–7-year-old Japanese children, the EBV antibody prevalence decreased from 88% during 1975–1979 to 59% during 1996–1998. Reasons for this

Table 2. Differences Between National Health and Nutrition Examination Survey (NHANES) Cycles 2003–2004 and 2009–2010 in Age-Adjusted Epstein–Barr Virus (EBV) Antibody (Ab) Prevalence Estimates for the US Population Aged 6–19 Years

Demographic Factor	NHANES 2003–2004		NHANES 2009–2010		*P*
	Subjects, No.[a]	EBV Ab Prevalence, % (95% CI)	Subjects, No.[a]	EBV Ab Prevalence, % (95% CI)	
Total	2849	72 (67–77)	2149	65 (62–69)	.027
Sex					
Male	1437	70 (64–75)[A]	1412	64 (59–68)[A]	.095
Female	1130	75 (69–80)[A]	1019	67 (62–72)[A]	.045
Race/ethnicity					
White, non-Hispanic	735	64 (58–70)[A]	672	51 (47–55)[A]	<.001
Black, non-Hispanic	1037	88 (85–90)[B]	433	85 (82–87)[B]	.111
Mexican American	877	88 (85–91)[B]	644	86 (82–89)[B]	.334
Other Hispanic[b]	. . .	. . .	257	81 (75–85)[B]	
Age, y					
6–8	395	57 (46–67)[A]	472	50 (44–56)[A]	.278
9–11	439	67 (60–73)[A,B]	508	55 (48–63)[A,B]	.029
12–14	769	65 (57–73)[A]	429	59 (54–65)[B]	.214
15–17	738	78 (73–83)[B,C]	467	69 (63–75)[C]	.018
18–19	508	84 (78–89)[C]	273	89 (81–94)[D]	.337
Birthplace					
United States	2525	71 (66–76)[A]	1932	64 (61–67)[A]	.019
Mexico	206	97 (93–98)[B]	101	93 (80–97)[B]	.213
Other	118	77 (62–87)[A]	113	79 (62–89)[A,B]	.861
Household income to poverty level ratio					
Low (≤1.3)	1226	85 (80–89)[A]	895	80 (74–85)[A]	.133
Middle (1.301–3.5)	986	71 (66–76)[B]	694	66 (60–71)[B]	.168
High (>3.5)	515	57 (49–66)[C]	383	50 (46–54)[C]	.125
Health insurance status					
Insured	2304	71 (65–75)[A]	1853	64 (60–68)[A]	.049
Not insured	508	82 (76–87)[B]	289	77 (68–83)[B]	.222
Household education level					
Less than high school	771	89 (83–93)[A]	539	88 (83–92)[A]	.498
High school graduate or GED diploma	715	76 (68–82)[B]	466	73 (67–78)[B]	.822
More than high school	1282	66 (60–71)[C]	1096	57 (52–61)[C]	.010
Crowding index[c]					
Low (<0.5)	321	62 (54–70)[A]	. . .	. . .	
Middle (0.5–1.0)	1937	72 (68–76)[B]	. . .	. . .	
High (>1.0)	561	83 (73–90)[C]	. . .	. . .	

Each demographic factor was modeled separately. Within each NHANES cycle, categories within a demographic factor were compared by pairwise linear contrasts. Rates with no superscripted capital letters in common are significantly different ($P < .05$), whereas rates that share superscripted capital letters are not significantly different.

Abbreviations: CI, confidence interval; GED, General Educational Development.

[a] Data are unweighted counts of individuals surveyed.

[b] Data for the 2003–2004 cycle are not shown because the proportion of Hispanic participants who were non–Mexican Americans was too small to produce reliable estimates.

[c] Defined as the no. of persons per room. Data on the no. of rooms per house were not collected during the 2009–2010 cycle.

decline in antibody prevalence have not been determined but could include changes in breastfeeding patterns, child care practices, day care attendance, and efforts to improve hygiene, such as the use of alcohol-based hand sanitizers in day care facilities and schools.

Another important finding was that antibody prevalence across all age groups was substantially higher among non-Hispanic blacks and Mexican Americans, who had essentially the same high age-specific antibody prevalence. The greatest disparity in antibody prevalence was among the younger

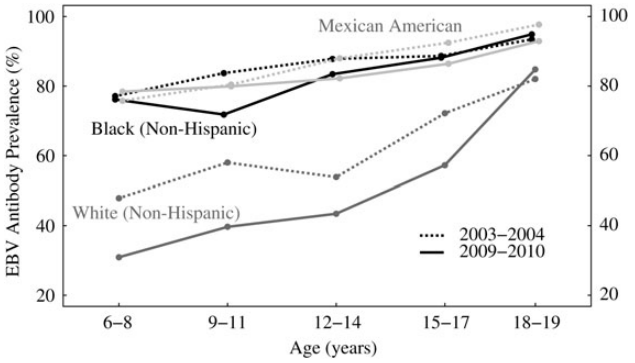

Figure 2. Epstein–Barr virus (EBV) antibody prevalence, by race/ethnicity and age, from the 2003–2004 (dashed lines) and 2009–2010 (solid lines) National Health and Nutrition Examination Surveys.

participants, especially those aged 6–8 years. Family environment and/or social practices may differ among white families and families of other races/ethnicities, which could account for this disparity in antibody prevalence. Interestingly, the difference in antibody prevalence between whites and individuals with other racial/ethnic backgrounds diminished during the teenage years.

Factors significantly associated with lower age-specific antibody prevalence were white race, younger age, having health insurance coverage, having a higher household income, and higher household education level. This prompts the question of whether it is better to acquire primary EBV infection earlier or later in life. Some would say earlier, because preadolescent children do not commonly experience infectious mononucleosis, whereas adolescents and young adults do. However, some data suggest that early acquisition of EBV is harmful. A multinational study showed that children with multiple sclerosis were significantly more likely than matched controls to be infected with EBV [26]. Among Kenyan infants, younger age at the time of primary EBV infection was associated with higher level of EBV viremia throughout infancy, leading the investigators to speculate that these infants were at higher risk for endemic Burkitt lymphoma [27]. If early acquisition of primary EBV infection is a risk factor for chronic EBV-associated diseases, identifying and ameliorating factors responsible for acquisition of the virus before adolescence may be an important public health step in disease prevention. This is a fertile area for future research.

A major limitation is that we have only 1 serum sample per subject, and therefore our study is a sequence of 4 cross-sectional snapshots of a dynamically changing epidemiologic picture. Also, demographic data were limited to what was collected by the surveys, and we had no opportunity to ask additional questions or collect follow-up specimens. Finally, we had no samples to test from children younger than 6 years.

Strengths included a sampling strategy carefully designed to be representative of the entire noninstitutionalized US civilian population; a large sample size; inclusion of serum specimens collected over an 8-year period (2003–2010), permitting evaluation of changes in antibody prevalence over time; and use of a robust antibody EIA, which produced equivocal results in only 0.5% of cases and had findings that were 96.7% concordant with findings of the former criterion standard, IFA.

Recommending an age to administer a prophylactic EBV vaccine is complicated by the difference in age-specific antibody prevalence we found among non-Hispanic whites versus non-Hispanic blacks and Mexican Americans. If one chooses to vaccinate the oldest age group in which the age-specific antibody prevalence is ≤50%, our data suggest that this would be <6 years for non-Hispanic blacks and Mexican Americans but could be up to 12 years for non-Hispanic whites. Ultimately, targeting all children in the United States <12 years old to receive EBV vaccine may be warranted, especially because of the decreasing age-specific antibody prevalence over time, as well as the need to confer protection during the period of highest risk for clinically significant disease.

Notes

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