Varicella Seroprevalence in the U.S.: Data from the National Health and Nutrition Examination Survey, 1999–2004

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SYNOPSIS

Objective. We estimated the varicella seroprevalence among the U.S. population aged 6–49 years based on retested National Health and Nutrition Examination Survey (NHANES) specimens collected between 1999 and 2004—originally tested using a method unsuitable for detecting vaccine-induced immunity—and compared it with historical estimates.

Methods. We performed a confirmatory test suitable for detecting vaccine-induced immunity on all available specimens from 6- to 19-year-olds who originally tested negative (n=633), and on 297 randomly selected specimens that had tested positive. Retest results superseded original results for determining seroprevalence. We assessed seroprevalence for the entire sample aged 6–49 years (n=16,050) by participant demographic characteristics and compared it with historical estimates (NHANES 1988–1994).

Results. The percentage of false-negative results for the original test was higher for specimens from younger children (6–11 years of age: 27.5%; 12–19 years of age: 13.3%) and for specimens collected most recently (2001–2004: 26.0%; 1999–2000: 12.6%). The age-adjusted rate of varicella seroprevalence for 1999–2004 was 93.6% for 6- to 19-year-olds and 98.0% for adults aged 20–49 years compared with 90.0% and 98.1%, respectively, for 1988–1994. We found an increase in seropositivity between the survey periods, from 93.2% to 97.2% ($p<0.001$) among 12- to 19-year-olds. For children, non-Hispanic black ethnicity and younger age were associated with lower seroprevalence in both survey periods.

Conclusions. Varicella seroprevalence increased with age among children and was uniformly high in the U.S. adult population between 1999 and 2004. The original testing produced false-negative seroprevalence results among children's specimens collected between 1999 and 2004 from 6- to 19-year-olds.

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Varicella seroprevalence testing was included as part of the National Health and Nutrition Examination Survey (NHANES) for participants aged 6–49 years who provided sera from 1999 through 2004. These test results, which have been available for years as part of the publicly released NHANES datasets, were based on an enzyme immunoassay (EIA) method as specified in the original NHANES protocol. At the time this protocol was established, it was not known whether the EIA method, which was the same method that had been used successfully to detect disease-acquired immunoglobulin G (IgG) antibodies to varicella-zoster virus (VZV) in specimens from NHANES III participants (collected from 1988 through 1994),[1] could reliably detect vaccine-induced immunity. Empirical evidence has since demonstrated that the IgG response to vaccine is less robust than that to natural infection; only glycoprotein-based enzyme-linked immunosorbent assay (gpELISA) and the fluorescence antibody to membrane antigen (FAMA) assay are sufficiently sensitive to reliably detect seroconversion to the vaccine.[2–4]

Most of the children (and virtually all of the adults) with immunity to varicella in the NHANES 1999–2004 sample would have acquired immunity through disease rather than vaccination, as it took several years to establish the varicella vaccination program following the introduction of a live, attenuated varicella vaccine in the U.S. in 1996.[5] The vaccination program, which at that time targeted infants aged 12–18 months for routine vaccination and susceptible children aged 19 months to 12 years for catch-up vaccination,[5] achieved coverage levels of 25.8% in 1997 and 57.5% in 1999 among children aged 19–35 months.[6] Thus, instances of vaccine-induced immunity among NHANES 1999–2004 participants (for whom vaccination status was not collected), and possible instances of false-negative varicella seroprevalence test results, would most likely be found among the youngest children in the most recent survey years.

This study calculated false-negative rates for the original EIA test method by performing confirmatory testing on a sample of NHANES 1999–2004 specimens using a newer immunoassay method (gp-ELISA) with good sensitivity for detecting vaccine- and disease-induced immunity. The gp-ELISA results superseded original EIA results for determining seroprevalence. Varicella seroprevalence estimates were assessed by participant demographic characteristics and compared with historical estimates (NHANES III, 1988–1994). We examined predictors of seroprevalence between the two survey periods.

METHODS

Survey design and participants

The NHANES is conducted by the National Center for Health Statistics, Centers for Disease Control and Prevention (CDC) to provide statistics on the health and nutritional status of the U.S. civilian noninstitutionalized population through household interviews, standardized physical examinations, and the collection of biological samples in special mobile examination centers.[7] Sampling procedures for the stratified, multistage, probability cluster design used, as well as interview and examination procedures, have been described elsewhere.[8,9] This study was based on NHANES III (1988–1994) and the first six years of the continuous NHANES (1999–2004), after which varicella testing was dropped from the NHANES protocol. Non-Hispanic black and Mexican American people were oversampled in all years (1988–1994 and 1999–2004). NHANES 1999–2004 also included oversampling of low-income people and adolescents aged 12–19 years.

Laboratory testing

We performed varicella serological testing on specimens from participants aged 6 years and older for NHANES III but only for participants aged 6–49 years for NHANES 1999–2004. VZV IgG serology was initially performed for all years in question using an EIA method, which has been described previously.[10] Because vaccination status is not assessed in NHANES, all available specimens from participants aged 6–19 years whose sera had tested negative by EIA for NHANES 1999–2004 underwent follow-up testing using a glycoprotein antigen-based EIA (gp-ELISA) protocol to confirm the negative result. A simple random sample of 297 specimens that had tested positive by EIA testing was also selected for follow-up confirmation testing.

The gp-ELISA protocol for detecting VZV IgG was developed by the staff of the National VZV Laboratory at CDC using VZV glycoprotein preparations developed by Merck & Co. and made available under a material transfer agreement. Lentil-lectin-purified glycoprotein antigens derived from VZV-infected human fibroblast cells were coated on the wells of a 96-well microtiter plate and subsequently incubated with a diluted test specimen. Control (normal tissue) antigen (also obtained from Merck & Co.) was prepared from uninfected fibroblasts, plated separately into different wells, and incubated with test serum to account for any nonspecific antibody reactivity. After unbound serum components are removed by washing, anti-human IgG antibody:alkaline phosphatase conjugate was added to the wells and incubated. Colorimetric substrate

for the enzyme was added to the wells and incubated for a sufficient time to permit color development and then chemically stopped, and optical density was determined using a spectrophotometer at a wavelength of 405 nanometers.

For VZV gp-ELISA test results, adjusted mean test optical density readings were recorded (average optical density from two normal tissue control wells was subtracted from the average optical density from two test antigen wells). Positive test cutoffs were established empirically by analyzing operator and test variation of results from 16 serum specimens (eight positive, eight negative) tested twice a day (two different operators) on three different days. Internal standards (strong positive, weak positive, and negative) were run on each test plate.

The CDC gp-ELISA testing method has been validated against a number of laboratory-based VZV serologic methods and performs comparably to all of them for detecting antibodies from natural infection with VZV (Unpublished data, CDC, Division of Viral Diseases, 2008). Additionally, the CDC gp-ELISA test is more sensitive than the EIA test for detecting vaccine-induced immunity[11] (Unpublished data, CDC, Division of Viral Diseases, 2008). Final results from the gp-ELISA testing were linked to the NHANES 1999–2004 datasets and compared with the original EIA test results to calculate the false-negative percent of the EIA test. All specimens that originally tested negative via EIA but were found to be positive by gp-ELISA were classified as original false-negative tests and reclassified as positive tests for analyses estimating seroprevalence. Specimens that originally tested negative and also tested gp-ELISA negative or indeterminate were considered to be true negatives.

Statistical analysis

The proportion of specimens with false-negative varicella IgG test results for varicella seroprevalence was calculated for the sample of specimens with a negative EIA test result that underwent follow-up confirmatory testing using the gp-ELISA method. From these specimens, the percent of false-negative tests was calculated by dividing the number of specimens with a gp-ELISA positive result by the total number with an EIA negative result, multiplied by 100. Comparison of VZV serological tests (EIA vs. gp-ELISA) was conducted on the original unweighted data. Differences were assessed using a Chi-square test, with $p<0.05$ considered significant.

We examined seroprevalence by age at the time of survey (using the NHANES standard age groupings of 6–11, 12–19, 20–29, 30–39, and 40–49 years of age), race/ethnicity, and variables previously examined in NHANES III (e.g., gender, birth outside the U.S., and poverty).[3] Race/ethnicity was obtained by self-report and classified as non-Hispanic white, non-Hispanic black, or Mexican American. Those who were not classified as one of these three groups were placed in the "other" racial/ethnic group. Because of small sample size in this other racial/ethnic group, these individuals were only included in the estimates for the total population. Prevalence estimates were weighted to represent the U.S. population, to account for oversampling in specific demographic subgroups, and for nonresponse to the household survey and physical examinations. Prevalence estimates for NHANES III and NHANES 1999–2004 were compared, both unadjusted by age group and age adjusted to the 2000 U.S. population by the direct method.

Standard error (SE) estimates were calculated using the Taylor series linearization method in SUDAAN® to account for the complex sample design.[8] The exact binary method was used to calculate 95% confidence intervals (CIs).[3,12] A t-statistic with the combined SE was used to test differences in prevalence between NHANES III and NHANES 1999–2004. We considered $p<0.05$ to be significant. Analysis focused on people aged 6–19 years who were most likely to have been impacted by the varicella vaccination program.

Potential demographic predictors of varicella seroprevalence were initially assessed using a t-statistic from a general linear contrast procedure in SUDAAN, without correction for multiple comparisons. Multivariate modeling analyses using logistic regression were performed stratified by age (6–19 years and 20–39 years) because some predictor variables were relevant only to the adult population and because the epidemiology of varicella for children who have grown up primarily in the post-vaccine era is likely to be different than that of adults. Those aged 40–49 years were excluded because varicella seroprevalence approached 100% for this group. The adult population was also stratified by gender, as the variable parity only pertains to females. Statistical significance for variables in the models was determined using the Satterthwaite adjusted F-statistic ($p<0.05$).

RESULTS

Study population: NHANES III (1988–1994)

Of the 19,935 people aged 6–49 years who were selected into NHANES III, 17,079 (86%) were interviewed, 15,934 (93% of those interviewed) underwent a physical examination as part of the NHANES protocol, and 14,393 (90% of those examined) had sera tested for

antibodies to VZV. Only 82% of examined children aged 6–11 years had sera tested, compared with 90%–94% among the remaining age groups. Percent sera tested among those examined was 91% among non-Hispanic white, 88% among non-Hispanic black, and 92% among Mexican American people ($p<0.001$). Fewer people born in the U.S. (90%) compared with those born outside the U.S. (91%, $p<0.05$), and fewer people living below the federal poverty level (FPL, 90%) compared with those living at or above the FPL (91%, $p<0.05$) had their sera tested. The percentage of people with sera tested among those examined did not vary by gender.

Study population: 1999–2004

In NHANES 1999–2004, 22,036 people aged 6–49 years were selected into the survey, 18,433 (84%) were interviewed, 17,672 (96% of those interviewed) underwent a physical examination as part of the NHANES protocol, and 16,050 (91% of those examined) had sera tested for antibodies to VZV. The lowest percentage of people with sera tested was in the youngest age group (6–11 years, 83%), but no other consistent trends with age after age 11 were observed (range: 91%–95%). The percentage of people with sera tested of those examined was slightly lower among non-Hispanic black people (89%) vs. non-Hispanic white people (91%) or Mexican Americans (92%) ($p<0.001$), and among those born in the U.S. vs. those born outside the U.S. (91% vs. 92%, $p<0.01$). The percentage of people with sera tested among those examined did not vary by gender or poverty level.

Comparison of VZV serological testing: EIA vs. gp-ELISA

Specimens from a total of 649 NHANES 1999–2004 participants aged 6–19 years tested negative by EIA, of which surplus sera were available for 633 participants for confirmatory testing. The gp-ELISA results indicated that 142 (22.4%) of these specimens were positive for VZV antibodies, while 390 (61.6%) were confirmed to be negative. The remaining 101 (16.0%) specimens had an indeterminate result by gp-ELISA (Table 1). The proportion of specimens that tested false negative increased over time, from 12.6% for specimens collected in 1999 and 2000 to 26.0% for specimens collected from 2001 to 2004 ($p<0.001$). The proportion of specimens that tested false negative was twice as high for specimens from the 6- to 11-year age group (27.5%) compared with specimens from the 12- to 19-year age group (13.3%) ($p<0.001$). Surplus sera were available for confirmatory testing for all but three of the 300 randomly selected specimens that originally tested positive for VZV antibodies by EIA. Of these, 296 (99.7%) also tested positive by gp-ELISA; one specimen tested indeterminate. Using gp-ELISA results changed the estimated seroprevalence estimate for 6- to 11-year-olds to 88.9%, compared with 83.9% for the original test results.

Changes in varicella seroprevalence: NHANES 1999–2004 vs. NHANES III

6- to 19-year-olds. As shown in Table 2, seroprevalence did not change between the survey periods among those aged 6–11 years (86.0% in NHANES III vs. 88.9%

Table 1. Proportion of NHANES specimens testing false negative for varicella immunity, by time period of specimen collection and age

Variables	*EIA test negative*	*Gp-ELISA test result*			*False negative (percent)*[a]
		Positive	*Negative*	*Indeterminate*	
Total	633	142	390	101	22.4
Time period					
1999–2000	167	21	129	17	12.6
2001–2004	466	121	261	84	26.0
Age (in years)					
6–11	407	112	223	72	27.5
12–19	226	30	167	29	13.3

[a]Percent false negative = (number positive by gp-ELISA divided by number negative by EIA test) times 100.

NHANES = National Health and Nutrition Examination Survey

EIA = enzyme immunoassay

gp-ELISA = glycoprotein-based enzyme-linked immunosorbent assay

in NHANES 1999–2004, $p=0.10$) with the exception of an increase among non-Hispanic black children, from 81.5% to 87.3% ($p<0.01$). Seropositivity among 12- to 19-year-olds increased from 93.2% (NHANES III) to 97.2% (NHANES 1999–2004) ($p<0.001$). This increase was evident in all three racial/ethnic groups, with the largest increase found for non-Hispanic black children, from 88.7% to 95.5% ($p<0.001$). In the age-adjusted analyses, seroprevalence among 6- to 19-year-olds increased between the survey periods from 90.0% (NHANES III) to 93.6% (NHANES 1999–2004) ($p<0.001$). Age-adjusted seroprevalence increased between the survey periods among both males and females, among those living below the FPL and at or above the FPL, and among those born in the U.S. This pattern of results was consistent across racial/ethnic groups, although statistical significance varied.

20- to 49-year-olds. As shown in Table 3, between the survey periods, seroprevalence increased for 20- to 29-year-olds (from 95.5% to 97.3%, $p<0.05$), decreased for 30- to 39-year-olds (98.9% to 97.3%, $p<0.001$), and did not change significantly for 40- to 49-year-olds. No difference was found between survey periods in the age-adjusted analyses overall or for any racial/ethnic group, with the exception of a slight decline among Mexican Americans, from 99.0% (NHANES III) to 97.8% (NHANES 1999–2004) ($p<0.05$). This decline was associated with country of birth; seroprevalence declined for Mexican Americans born outside the U.S., from 98.8% (NHANES III) to 96.6% (NHANES 1999–2004) ($p<0.01$), but did not decline among Mexican Americans born in the U.S.

Differences in seroprevalence by demographic factors within the survey period

6- to 19-year-olds. For each survey period, we used multivariate logistic models to assess the significance of the association of various demographic cofactors with varicella seroprevalence. In a model that included age, race/ethnicity, and gender, in both surveys adolescents aged 12–19 years were more likely to be varicella seropositive compared with children aged 6–11 years (NHANES III: odds ratio [OR] = 2.1, 95% CI 1.5, 3.0; and NHANES 1999–2004: OR=4.5, 95% CI 3.4, 6.1). While a significant interaction was found between gender and race/ethnicity for NHANES III, with Mexican American males being more likely to be seropositive (OR=1.7, 95% CI 1.1, 2.6) compared with Mexican American females, no such interaction was found for NHANES 1999–2004. For both surveys, non-Hispanic black people were less likely to be seropositive than non-Hispanic white people (NHANES III: OR=0.5, 95% CI 0.3, 0.8; and NHANES 1999–2004: OR=0.7, 95% CI 0.5, 1.0). Neither poverty index nor country of birth was associated with varicella seroprevalence for either survey.

20- to 39-year-olds. As shown in Table 4, we again used multivariate logistic regression models to assess significance of various cofactors and varicella seroprevalence among adults aged 20–39 years. Adults aged 40–49 years were not included because their seroprevalence approached 100%. Models were stratified on gender to look at birth history among females. In a multivariate logistic regression model that included age, race/ethnicity, foreign birth, and marital status (for males) or parity (for females), for both males and females aged 20–39 years, age and race/ethnicity were significantly associated with higher varicella seroprevalence for NHANES III. In NHANES III, both males and females aged 30–39 years were more likely to be seropositive for varicella than those aged 20–29 years (male-specific model: OR=2.3, 95% CI 1.0, 5.1; female-specific model: OR=3.0, 95% CI 1.4, 6.7).

Among males in NHANES III, Mexican American males were more likely to be seropositive than non-Hispanic white males (OR=2.9, 95% CI 1.0, 8.3), while among females, non-Hispanic black females were significantly less likely to be seropositive than non-Hispanic white females (OR=0.5, 95% CI 0.2, 0.9). In NHANES III, men who were married (OR=2.7, 95% CI 1.2, 5.7) and women who had delivered a live-born infant (OR=4.1, 95% CI 2.3, 7.2) were more likely to be seropositive. After adjusting for parity, marital status was no longer a significant predictor of varicella seroprevalence for females in NHANES III.

A history of live birth (OR=3.5, 95% CI 1.4, 8.9) and non-Hispanic black race/ethnicity (OR=0.4, 95% CI 0.2, 1.0) were the only significant predictors of seropositivity for NHANES 1999–2004 among women. Among males in both surveys, those born outside the U.S. were less likely to be seropositive (NHANES III: OR=0.3, 95% CI 0.1, 0.8; NHANES 1999–2004: OR=0.4, 95% CI 0.2, 0.7). This was the only significant predictor for males aged 20–39 years in NHANES 1999–2004.

DISCUSSION

Confirmatory testing of NHANES specimens for varicella seroprevalence by gp-ELISA determined that the original EIA test produced false-negative results for 22.4% of specimens from participants aged 6–19 years collected between 1999 and 2004. As one would expect given the timing of implementation of the varicella vaccination program, the percentage of false-negative EIA test results increased over time between 1999 and

Table 2. Varicella seroprevalence among 6- to 19-year-olds by demographic characteristics and NHANES period

	Total		Race/ethnicity					
			Non-Hispanic white		Non-Hispanic black		Mexican American	
Characteristic	*NHANES III 1988–1994 Seroprevalence Percent (95% CI)*	*NHANES 1999–2004 Seroprevalence Percent (95% CI)*	*NHANES III 1988–1994 Seroprevalence Percent (95% CI)*	*NHANES 1999–2004 Seroprevalence Percent (95% CI)*	*NHANES III 1988–1994 Seroprevalence Percent (95% CI)*	*NHANES 1999–2004 Seroprevalence Percent (95% CI)*	*NHANES III 1988–1994 Seroprevalence Percent (95% CI)*	*NHANES 1999–2004 Seroprevalence Percent (95% CI)*
Age-specific								
6–11 years	86.0	88.9	87.4	89.2	81.5	87.3[a]	89.8	89.5
	(83.0, 88.6)	(86.7, 90.8)	(83.5, 90.6)	(85.9, 92.0)	(78.1, 84.5)	(85.2, 89.1)	(87.7, 91.7)	(86.7, 91.8)
N	2,678	2,715	693	711	872	925	993	908
12–19 years	93.2	97.2[b]	94.1	97.7[a]	88.7	95.5[b]	93.9	97.5[b]
	(91.5, 94.6)	(96.5, 97.8)	(91.8, 96.0)	(96.7, 98.5)	(85.2, 91.6)	(94.2, 96.6)	(92.4, 95.1)	(96.4, 98.3)
N	2,875	6,362	734	1,639	1,009	2,037	986	2,264
Age-adjusted								
Total	90.0	93.6[b]	91.2	94.0[c]	85.6	91.9[b]	92.1	94.0[c]
	(88.4, 91.5)	(92.4, 94.6)	(89.2, 92.9)	(92.4, 95.4)	(82.7, 88.1)	(90.8, 93.0)	(90.7, 93.3)	(92.5, 95.3)
N	5,553	9,077	1,427	2,350	1,881	2,962	1,979	3,172
Gender								
Male	90.1	93.9[a]	90.6	94.4[a]	86.1	92.2[b]	93.4	94.3
	(87.8, 92.1)	(92.8, 94.9)	(87.9, 92.9)	(93.0, 95.7)	(83.0, 88.7)	(90.4, 93.7)	(92.0, 94.6)	(92.5, 95.8)
N	2,702	4,576	682	1,183	907	1,509	985	1,596
Female	90.0	93.2[c]	91.8	93.6	85.1	91.7[b]	90.8	93.7[c]
	(87.9, 91.8)	(91.5, 94.6)	(89.3, 93.9)	(91.1, 95.5)	(81.6, 88.2)	(90.1, 93.0)	(88.9, 92.5)	(91.9, 95.2)
N	2,851	4,501	745	1,167	974	1,453	994	1,576
Poverty index								
<100% FPL	90.7	94.4[c]	93.2[d]	95.5[d]	83.7	93.4[b]	92.7	93.8
	(88.3, 92.8)	(92.3, 96.1)	(87.4, 96.9)	(90.0, 98.4)	(79.6, 87.2)	(91.8, 94.8)	(90.0, 94.9)	(91.2, 95.9)
N	2,015	2,837	200	372	821	1,118	883	1,152
≥100% FPL	90.4	93.4[c]	91.5	93.8	87.6	90.6	92.3	93.8
	(88.3, 92.3)	(92.1, 94.6)	(89.0, 93.5)	(92.1, 95.3)	(84.4, 90.4)	(88.3, 92.5)	(90.5, 94.0)	(92.1, 95.2)
N	3,052	5,550	1,158	1,863	917	1,576	844	1,744
Birthplace								
Non-U.S.	90.5	93.5	NA[e]	NA[e]	NA[e]	94.2[d]	92.0	93.3
	(83.8, 95.1)	(90.3, 95.9)				(88.7, 97.6)	(85.7, 96.1)	(89.2, 96.2)
N	633	1,179				147	418	818
U.S.	89.9	93.7[b]	91.2	94.0[c]	85.5	91.9[b]	92.1	94.4[c]
	(88.2, 91.5)	(92.5, 94.7)	(89.2, 93.0)	(92.4, 95.4)	(82.7, 88.1)	(90.8, 92.9)	(90.3, 93.6)	(92.8, 95.7)
N	4,902	7,897	1,398	2,271	1,800	2,814	1,552	2,354

[a] $p<0.01$ for comparison between NHANES III and NHANES 1999–2004 within characteristic
[b] $p<0.001$ for comparison between NHANES III and NHANES 1999–2004 within characteristic
[c] $p<0.05$ for comparison between NHANES III and NHANES 1999–2004 within characteristic
[d] Relative standard error of the estimate is greater than standards of statistical reliability (>30%), but estimate is based on at least 10 negative people.
[e] Sample size too small for a stable estimate
NHANES = National Health and Nutrition Examination Survey
CI = confidence interval
FPL = federal poverty level
NA = not applicable

Table 3. Varicella seroprevalence among adults aged ≥20 years by demographic characteristics and NHANES period

	Total		Race/ethnicity					
			Non-Hispanic white		Non-Hispanic black		Mexican American	
Characteristic	*NHANES III 1988–1994 Seroprevalence Percent (95% CI)*	*NHANES 1999–2004 Seroprevalence Percent (95% CI)*	*NHANES III 1988–1994 Seroprevalence Percent (95% CI)*	*NHANES 1999–2004 Seroprevalence Percent (95% CI)*	*NHANES III 1988–1994 Seroprevalence Percent (95% CI)*	*NHANES 1999–2004 Seroprevalence Percent (95% CI)*	*NHANES III 1988–1994 Seroprevalence Percent (95% CI)*	*NHANES 1999–2004 Seroprevalence Percent (95% CI)*
Age-specific (in years)								
20–29	95.5	97.3[a]	96.3	98.0[a]	94.1	95.2	97.1	95.4
	(94.0, 96.7)	(96.5, 97.9)	(94.5, 97.6)	(97.1, 98.7)	(92.0, 95.7)	(92.6, 97.0)	(95.9, 98.0)	(92.5, 97.3)
N	3,280	2,454	848	1,058	1,032	482	1,258	692
30–39	98.9	97.3[b]	99.1[c,d]	97.7[a]	97.5	96.4	99.7[c,d]	97.8[a,c]
	(98.2, 99.3)	(96.6, 97.9)	(98.1, 99.6)	(96.8, 98.4)	(96.3, 98.4)	(93.9, 98.1)	(99.2, 99.9)	(95.2, 99.2)
N	3,116	2,277	993	1,080	1,041	467	951	509
40–49	99.6[c]	99.2	99.8[c,d]	99.2[c]	98.8[c,d]	98.6[c,d]	99.9[c,d]	99.9[c,d]
	(99.1, 99.9)	(98.7, 99.6)	(99.0, 100.0)	(98.5, 99.7)	(97.7, 99.4)	(97.3, 99.4)	(99.4, 100.0)	(99.4, 100.0)
N	2,444	2,242	877	998	746	528	719	574
Age-adjusted (in years)								
20–49	98.1	98.0	98.5	98.3	96.9	96.8	99.0	97.8[a]
	(97.6, 98.5)	(97.6, 98.3)	(97.9, 98.9)	(97.9, 98.7)	(96.2, 97.6)	(95.5, 97.9)	(98.6, 99.3)	(96.5, 98.7)
N	8,840	6,973	2,718	3,136	2,819	1,477	2,928	1,775
Gender								
Male	98.1	97.4	98.4	98.0	96.4	96.0	98.8	97.2
	(97.5, 98.7)	(96.8, 98.0)	(97.5, 99.1)	(97.2, 98.5)	(95.2, 97.4)	(93.5, 97.8)	(98.1, 99.3)	(95.3, 98.5)
N	4,079	3,199	1,223	1,436	1,238	681	1,450	820
Female	98.1	98.5	98.6	98.7[c]	97.4	97.5	99.2	98.5[c]
	(97.3, 98.7)	(98.0, 98.9)	(97.6, 99.2)	(97.9, 99.2)	(96.3, 98.3)	(96.0, 98.5)	(98.7, 99.6)	(97.2, 99.3)
N	4,761	3,774	1,495	1,700	1,581	796	1,478	955
Poverty index								
<100% FPL	97.3	98.1	98.8[c,d]	99.4[c,d]	96.5	97.3[c]	98.9	96.7[a,c]
	(95.8, 98.4)	(96.9, 98.9)	(97.0, 99.7)	(98.0, 99.9)	(94.8, 97.8)	(94.7, 98.8)	(98.1, 99.5)	(94.1, 98.4)
N	2,089	1,357	236	391	796	369	957	476
≥100% FPL	98.3	97.8	98.5	98.1	97.1	96.5	99.1	98.1[c]
	(97.7, 98.8)	(97.5, 98.2)	(97.8, 99.0)	(97.6, 98.6)	(96.4, 97.7)	(95.1, 97.7)	(98.7, 99.5)	(96.5, 99.1)
N	6,053	5,127	2,380	2,574	1,781	986	1,649	1,156
Birthplace								
Non-U.S.	97.0	96.5	96.6[c,d]	98.8[c,d]	91.6[c]	92.7	98.8	96.6[e]
	(95.7, 98.0)	(95.3, 97.5)	(90.1, 99.3)	(96.6, 99.8)	(82.5, 96.9)	(88.2, 95.8)	(98.1, 99.3)	(94.7, 97.9)
N	2,266	1,842	118	178	204	155	1,648	1,141
U.S.	98.3	98.3	98.6	98.3	97.4	97.3	99.3[c]	99.8[c,d]
	(97.8, 98.7)	(97.9, 98.6)	(98.0, 99.0)	(97.8, 98.7)	(96.8, 97.9)	(95.9, 98.4)	(98.7, 99.7)	(99.4, 100.0)
N	6,549	5,131	2,598	2,958	2,597	1,322	1,279	634

NOTE: Specimens testing negative for age group 20–49 years for NHANES 1999–2004 were not retested using the gp-ELISA test, which is more sensitive for detecting vaccine-induced immunity than the test performed.

[a] $p<0.05$ for comparison between NHANES III and NHANES 1999–2004 within characteristic

[b] $p<0.001$ for comparison between NHANES III and NHANES 1999–2004 within characteristic

[c] Relative standard error of the estimate is greater than standards of statistical reliability (>30%).

[d] Estimate may be unstable based on <10 negative people.

[e] $p<0.01$ for comparison between NHANES III and NHANES 1999–2004 within characteristic

NHANES = National Health and Nutrition Examination Survey

CI = confidence interval

FPL = federal poverty level

NA = not applicable

gp-ELISA = glycoprotein-based enzyme-linked immunosorbent assay

Table 4. Predictors of varicella seroprevalence in U.S. females and males aged 20–39 years: comparison of NHANES III (1988–1994) and NHANES 1999–2004

	NHANES III (1988–1994)		*NHANES 1999–2004*	
Characteristics	N	*Odds ratio (95% CI)*	N	*Odds ratio (95% CI)*
Males				
Age (in years)				
30–39	1,351	2.3 (1.0, 5.1)[a]	874	0.7 (0.4, 1.2)
20–29	1,463	Ref.	942	Ref.
Race/ethnicity				
Non-Hispanic black	898	0.6 (0.3, 1.2)	406	0.5 (0.3, 1.1)
Mexican American	1,078	2.9 (1.0, 8.3)[a]	521	0.9 (0.5, 1.8)
Non-Hispanic white	802	Ref.	889	Ref.
Birthplace				
Non-U.S.	789	0.3 (0.1, 0.8)[a]	457	0.4 (0.2, 0.7)[b]
U.S.	1,989	Ref.	1,359	Ref.
Marital status				
Ever married	1,577	2.7 (1.2, 5.7)[a]	896	1.5 (0.7, 3.1)
Never married	1,201	Ref.	920	Ref.
Females				
Age (in years)				
30–39	1,614	3.0 (1.4, 6.7)[b]	1,020	1.0 (0.5, 2.4)
20–29	1,636	Ref.	1,141	Ref.
Race/ethnicity				
Non-Hispanic black	1,134	0.5 (0.2, 0.9)[a]	448	0.4 (0.2, 1.0)[a]
Mexican American	1,098	1.9 (0.7, 5.0)	594	0.8 (0.4, 1.7)
Non-Hispanic white	1,018	Ref.	1,119	Ref.
Birthplace				
Non-U.S.	699	0.7 (0.4, 1.5)	450	0.6 (0.3, 1.1)
U.S.	2,551	Ref.	1,711	Ref.
Parity				
≥1 live births	2,385	4.1 (2.3, 7.2)[c]	1,504	3.5 (1.4, 8.9)[b]
No live births	865	Ref.	657	Ref.

[a] $p<0.05$ for p-value from t-test for beta coefficient = 0

[b] $p<0.01$ for p-value from t-test for beta coefficient = 0

[c] $p<0.001$ for p-value from t-test for beta coefficient = 0

NHANES = National Health and Nutrition Examination Survey

CI = confidence interval

Ref. = referent group

2004. That the percentage of false-negative results was also significantly higher for younger children than for older children likely reflects a higher proportion of children in the younger group having vaccine-induced immunity. The gp-ELISA results were posted as surplus specimen data on the NHANES website in March 2009.

Results indicate that seroprevalence among both children and adults has remained relatively stable across the two survey periods; age-adjusted seroprevalence of IgG antibody to VZV was 93.6% for 6- to 19-year-olds and 98.0% for adults aged 20–49 years (NHANES 1999–2004) compared with 90.0% and 98.1%, respectively, for NHANES 1988–1994. For children, non-Hispanic black race/ethnicity and younger age were associated with increased risk for susceptibility to varicella in both survey periods. For adults, history of live birth for females and being born in the U.S. for males were the two predictors of seroprevalence that reached statistical significance in the logistic models for the more recent NHANES period. Being married was a significant predictor of seroprevalence for men in the earlier survey period but not for the later survey period.

Varicella is a highly contagious disease caused by VZV and is transmissible by contact, respiratory secretions, and aerosolized spread.[13] Prior to vaccine licensure in 1995, there were approximately four million cases of varicella each year in the U.S., which resulted in 11,000 to 13,500 hospitalizations, and 100 to 150

deaths annually.[13] A live, attenuated varicella vaccine became available to the public sector in May 1996, and in July 1996 it was recommended by the Advisory Committee on Immunization Practices (ACIP) for all children aged 12–18 months (one dose), susceptible children by age 13 (two doses), people in contact with those at high risk for severe disease (family members of immunocompromised people), and adults at high risk for exposure (two doses: health-care workers, teachers, day care or institutional employees, military members, college students, adolescents and adults in households with children, international travelers, and nonpregnant women of childbearing age).[5] In June 2006, ACIP voted for a universal second dose for children <13 years of age.[13]

Varicella vaccine induces humoral and cell-mediated immunity in more than 95% of vaccine recipients,[5] and its overall effectiveness is estimated to be 70%–90% for children in post-licensure studies and clinical trials against all disease, and >95% against moderate to severe disease.[13] Vaccine coverage among children aged 19–35 months increased from 25.8% in 1997 to 87.5% in 2004.[14] Population-based disease surveillance data from two communities showed cases of varicella decreased by 90% from 1995 to 2005.[15] Additionally, varicella hospitalizations and ambulatory visits have declined dramatically among all age groups in the U.S. since the introduction of the varicella vaccination program.[16,17]

As the childhood vaccination program is implemented, one might expect to see an increase in seroprevalence among the youngest children with a decline in seroprevalence in slightly older children in the absence of a fully implemented catch-up program, due to declining circulation of varicella in communities and a concomitant decrease in likelihood of exposure. That such a decline was not seen for those aged 12–19 years in this study should not be viewed as refuting this expectation. The data available for this analysis do not provide a sufficient test of how the vaccination program implementation affected seroprevalence rates, as only the 6- and 7-year-old participants from the 2003 and 2004 data collection years can be considered as having grown up in the "post-vaccine" era. Data from future NHANESs are needed to evaluate the impact of the vaccine on seroprevalence. However, varicella testing was not included in the NHANES protocol for specimens collected from 2005 through 2008.

Analysis of varicella seroprevalence estimates based on the original EIA test results for varicella IgG antibodies (as opposed to the gp-ELISA results presented in this article) would have suggested a decline in seroprevalence among those aged 6–11 years between the two survey periods (86.0% to 83.9%), but this decline would not have reached statistical significance.

Because commercially available varicella seroprevalence tests are based on EIA methods, these findings highlight the importance of maintaining accurate vaccination records, as a negative result from such a test does not necessarily indicate that the child is susceptible. In the absence of vaccination records, the commercially available assays for varicella seroprevalence currently in use are undoubtedly leading to false-negative results among vaccinated people without disease history and resulting in the unnecessary vaccination of such people (e.g., testing of vaccinated health-care workers). Though there are no safety concerns associated with vaccinating people who are already seropositive, it does result in wasted resources. Commercially available tests that more reliably detect vaccine-induced immunity would be beneficial.

Limitations

This study had several limitations. Analyses of more detailed age groups or selected population characteristics within racial/ethnic subgroups were limited due to small sample sizes and, in some cases, 100% seroprevalence among people of selected strata. Several of the estimates presented, especially among adults, were unstable with very high relative SEs (>30%) and based on small numbers of varicella-negative people as noted in our tables, and should be interpreted with caution. We limited our analyses to characteristics likely to be important in the epidemiology of varicella to minimize the potential of identifying statistically significant, yet spurious associations due to multiple comparisons.[18] A comparison of specimens with EIA false-negative results between vaccinated and unvaccinated individuals could not be performed, as vaccination status is not assessed in NHANES.

That the more recently collected specimens had increased false-negative results might suggest the possibility that the older samples could have experienced great degradation. This is normally an issue associated with repeated freeze-thaw cycles of specimens, which does not apply to the NHANES specimens used in this study. NHANES specimens are aliquoted at the outset and carefully controlled to minimize the number of freeze-thaw events. Additionally, even 10 or more freeze-thaw cycles would not significantly degrade IgG antibody in a serum specimen. Moreover, the comparison of conventional EIA methods to gpELISA and FAMA using specimens newly isolated from vaccinated people reveals a pronounced difference in sensitivity;

EIA methods using crude antigen preparations are universally too insensitive to reliably detect seroconversion to vaccine.[11]

CONCLUSIONS

The varicella vaccination program has reduced the disease burden in the U.S.[13] It remains to be seen whether it has negatively impacted the susceptibility of children, as had been predicted at vaccine introduction. Therefore, as the circulation of the virus is reduced further, it is important to continue to monitor the impact of varicella vaccination on VZV seroprevalence, especially as the vaccinated cohorts enter adolescence and adulthood. Thus, future rounds of NHANES should include testing for varicella seroprevalence along with ascertainment of vaccination status. Efforts to implement the existing vaccination policy recommendations to achieve >90% vaccine coverage among young children should be continued. Such efforts will help avoid increased susceptibility as circulation of the virus continues to decline.

The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention or the Department of Health and Human Services.

REFERENCES

1. Kilgore PE, Kruszon-Moran D, Seward JF, Jumaan A, Van Loon FP, Forghani B, et al. Varicella in Americans from NHANES III: implications for control through routine immunization. J Med Virol 2003;70 Suppl 1:S111-8.
2. Gershon AA, Piomelli S, Karpatkin M, Smithwick E, Steinberg S. Antibody to varicella-zoster virus after passive immunization against chickenpox. J Clin Microbiol 1978;8:733-5.
3. Hammond O, Wang Y, Green T, Antonello J, Kuhn R, Motley C, et al. The optimization and validation of the glycoprotein ELISA assay for quantitative varicella-zoster virus (VZV) antibody detection. J Med Virol 2006;78:1679-87.
4. Maple PA, Gray J, Breuer J, Kafatos G, Parker S, Brown D. Performance of a time-resolved fluorescence immunoassay for measuring varicella-zoster virus immunoglobulin G levels in adults and comparison with commercial enzyme immunoassays and Merck glycoprotein enzyme immunoassay. Clin Vaccine Immunol 2006;13:214-8.
5. Prevention of varicella: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 1996;45(RR-11):1-25.
6. Centers for Disease Control and Prevention (US). Immunization coverage in the U.S. National Immunization Survey (NIS)—children (19–35 months), data tables 1997 and 1999 [cited 2009 May 2]. Available from: URL: http://www.cdc.gov/vaccines/stats-surv/imz-coverage.htm#nis
7. Gunter EW, McQuillan G. Quality control in planning and operating the laboratory component for the Third National Health and Nutrition Examination Survey. J Nutr 1990;120 Suppl 11:1451-4.
8. Research Triangle Institute. SUDAAN®: Version 7.0. Research Triangle Park (NC): Research Triangle Institute; 1996.
9. Jones JL, Kruszon-Moran D, Wilson M, McQuillan G, Navin T, McAuley JB. Toxoplasma gondii infection in the United States: seroprevalence and risk factors. Am J Epidemiol 2001;154:357-65.
10. Forghani B, Schmidt NJ, Dennis J. Antibody assays for varicella-zoster virus: comparison of enzyme immunoassay with neutralization, immune adherence hemagglutination, and complement fixation. J Clin Microbiol 1978;8:545-52.
11. Schmid SD, Jumaan AO. Impact of varicella vaccine on varicella-zoster virus dynamics. Clin Microbiol Rev 2010;23:202-17.
12. Collett D. Modelling binary data. London: Chapman and Hall; 1991.
13. Marin M, Guris D, Chaves SS, Schmid S, Seward JF. Prevention of varicella: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2007;56 (RR-4):1-40.
14. Luman ET, Ching PL, Jumaan AO, Seward JF. Uptake of varicella vaccination among young children in the United States: a success story in eliminating racial and ethnic disparities. Pediatrics 2006;117:999-1008.
15. Guris D, Jumaan AO, Mascola L, Watson BM, Zhang JX, Chaves SS, et al. Changing varicella epidemiology in active surveillance sites—United States, 1995–2005. J Infect Dis 2008;197 Suppl 2:S71-5.
16. Galil K, Brown C, Lin F, Seward J. Hospitalizations for varicella in the United States, 1988 to 1999. Pediatr Infect Dis J 2002;21:931-5.
17. Zhou F, Harpaz R, Jumaan AO, Winston CA, Shefer A. Impact of varicella vaccination on health care utilization. JAMA 2005;294:797-802.
18. Daniel WW. Analysis of variance. In: Daniel WW. Biostatistics: a foundation for analysis in the health sciences. 7th ed. New York: John Wiley & Sons, Inc.; 1999. p. 314-7.