

ORIGINAL ARTICLE

A Population-Based Study of Primary Human Herpesvirus 6 Infection

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ABSTRACT

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BACKGROUND

Serologic studies indicate that human herpesvirus 6 (HHV-6) infects 90 percent of children by two years of age. Little is known about the acquisition, virologic course, and clinical manifestations of HHV-6 infection.

METHODS

We prospectively studied a cohort of 277 children from birth through the first two years of life to define the pattern of acquisition of HHV-6. The children's saliva was tested weekly for HHV-6 DNA with the use of the polymerase chain reaction. Parents maintained a daily log of signs and symptoms of illness in their children.

RESULTS

Primary HHV-6 infection occurred in 130 children, with cumulative percentages of 40 percent by the age of 12 months and 77 percent by the age of 24 months. The peak age of acquisition was between 9 and 21 months. The acquisition of HHV-6 was associated with female sex (adjusted hazard ratio, 1.7; 95 percent confidence interval, 1.2 to 2.4) and having older siblings (adjusted hazard ratio, 2.1; 95 percent confidence interval, 1.4 to 2.9). Among 81 children with a well-defined time of acquisition of HHV-6, 93 percent had symptoms, and 38 percent were seen by a physician. None had seizures. As compared with children who had other illnesses, those with primary HHV-6 infection were more likely to have fever ($P=0.003$), fussiness ($P=0.02$), diarrhea ($P=0.03$), rash ($P=0.003$), and roseola ($P=0.002$) and were more likely to visit a physician ($P=0.003$).

CONCLUSIONS

The acquisition of HHV-6 in infancy is usually symptomatic and often results in medical evaluation. Roseola occurs in a minority of patients, and febrile seizures are infrequently associated with primary HHV-6 infection. Older siblings appear to serve as a source of HHV-6 transmission.

HUMAN HERPESVIRUS 6 (HHV-6) IN- fects over 90 percent of people within the first two years of life.¹ Studies of febrile children in the emergency department indicate that primary HHV-6 infection accounts for a large proportion of visits and febrile seizures.² Despite the interest in HHV-6 as an important pathogen,³⁻⁵ no prospective, population-based study has evaluated the acquisition of HHV-6 outside the acute care setting. Thus, the full spectrum of illness and virologic aspects of primary HHV-6 infection remain unknown. We developed a noninvasive method of testing serially collected saliva specimens for HHV-6⁶ and used it prospectively in children from birth to two years of age to determine the pattern of acquisition and natural history of HHV-6 infection.

METHODS

This clinical research was conducted in accordance with guidelines for human experimentation specified by the institutional review board of Children's Hospital in Seattle. The institutional review boards of all involved hospitals approved the protocol. Between April 1997 and August 2001, pregnant women were recruited from obstetricians' offices. After the women had provided written informed consent, their infants were followed from birth until two years of age.

Parents were taught to collect saliva by placing five sterile, precut filter-paper strips (Sno strips) into their child's mouth until saturated⁶ and to wash their hands before collection and avoid touching the collection end of the strips to minimize contamination. Strips were then air-dried, bagged, and mailed to the laboratory weekly. Infants' serum (1 ml) was collected when blood was being drawn for other purposes.

Demographic information (e.g., sex and race or ethnic group) was collected by means of a standardized questionnaire at enrollment. Parents completed a daily symptom report for their child that offered the following choices: no symptoms, fever (temperature >38.0°C), cough, rhinorrhea, vomiting, diarrhea, rash (generalized), fussiness (above the baseline level for the child), seizure, and a visit to a physician (for illness). Roseola was defined post hoc as a febrile illness with a generalized rash on defervescence,⁷ with symptoms that lasted for at least two days and the appearance of rash on the day of or the day after defervescence. Throughout the study, parents were questioned about whether

and how long their child breast-fed and regularly attended child care in a group setting or a play-group (defined as scheduled group activities with other young children at least weekly).

POLYMERASE CHAIN REACTION

Saliva specimens were tested for HHV-6 DNA by means of the polymerase chain reaction (PCR) as previously described.^{5,8} Briefly, DNA was extracted from the collection ends of the five Sno strips. Qiagen ATL Buffer and Qiagen protease were added to each sample and incubated overnight. Then, Qiagen AL Buffer was added to each sample and the sample was incubated for 10 minutes. The rest of the procedure followed Qiagen's single-column extraction protocol except that the sample was run through the spin column three times at an initial spin speed of 8000 rpm. DNA was eluted into 100 μ l of Qiagen AE Buffer, and 20 μ l of the solution was tested for HHV-6 DNA with the use of a real-time quantitative fluorescent-probe PCR assay as previously described.^{5,8} The detection of 1 copy of HHV-6 DNA per reaction, or 80 copies per milliliter of saliva, was considered to indicate positivity. HHV-6 DNA identified by PCR was then further typed as A or B.⁵

SEROLOGIC STUDIES

IgM and IgG antibodies against HHV-6 were detected by means of Western blotting with the use of a method adapted from Black and colleagues⁹ and Yamamoto and colleagues.¹⁰ Purified HHV-6 antigen (ABI) was separated on sodium dodecyl sulfate-polyacrylamide gel by electrophoresis and then transferred to polyvinylidene fluoride (PVDF) strips. Three PVDF strips were incubated with each serum sample: strip 1 was incubated with test serum in 4 percent goat serum, and strips 2 and 3 were incubated with test serum pretreated with IgG-rheumatoid factor adsorbent (Meridian Bioscience). Horseradish-peroxidase-labeled goat antihuman secondary antibodies (Chemicon) were then added to the strips. Immunoreactive bands were visualized with the use of a chemiluminescent substrate sensitive to horseradish peroxidase (Pierce) as described previously.¹¹ Seroconversion was defined by the detection of serum IgM at any age or by the detection of IgG in a child who had previously undetectable levels of IgG or who was at least 18 months of age.

STATISTICAL ANALYSIS

SPSS (version 11.5) and SAS (version 8.02) statistical-software programs were used for analyses. On

the basis of observed patterns of salivary HHV-6 DNA, primary HHV-6 infection was defined as a positive PCR result followed within 28 days by at least one positive test at any level and within 42 days by at least one finding of 800 copies of HHV-6 per milliliter or more, by a positive PCR result followed within 28 days by a finding of at least 800 copies per milliliter, or by an initial result of at least 800 copies per milliliter followed by a finding of at least 800 copies per milliliter at the next measurement. This strategy ensured that children with fewer follow-up data had to have relatively higher levels of HHV-6 DNA to be counted as infected. Positive specimens that did not meet the definition of primary infection were excluded (treated as missing). In the case of 13 children (15 specimens) the final specimens were positive but the date of acquisition could not be determined; for these children, follow-up data were included only through the date on which the last negative specimen was obtained.

The age at the time of primary HHV-6 infection was analyzed with the use of Kaplan–Meier survival analysis and Cox regression. The time of acquisition was calculated as the midpoint between the last negative and the first positive saliva specimen. (Estimates of acquisition rates were nearly identical when the time of the first positive specimen was used for the time of acquisition.) Data on children who did not acquire HHV-6 were censored at the time of the last negative saliva specimen.

Factors associated with the acquisition of HHV-6 were examined by means of Cox regression with backward elimination for the final model selection. Because information on the use of breast-feeding or participation in a child-care group or a playgroup was not available for some children, sensitivity analyses were performed in which missing values were designated as either “yes” or “no.” None of these transformed variables were significantly associated with the acquisition of HHV-6. Children who acquired HHV-6 were followed significantly longer than those who did not. Survival analysis was based on the assumption that children who discontinued follow-up before the acquisition of HHV-6 did so for reasons unrelated to their risk of acquisition. We find this assumption plausible and do not suspect that there was bias in our evaluation of risk factors for HHV-6.

Analyses of symptoms associated with primary HHV-6 infection included only the 81 children for whom the time of acquisition of HHV-6 was well defined — that is, only those whose last negative

test and first positive test for salivary HHV-6 were no more than 14 days apart. No descriptive characteristics were found to differ significantly between the 81 children who were included in the analysis and the 49 children who were excluded. To identify HHV-6–associated symptoms while taking into account PCR sampling frequency, we evaluated the period spanning from two weeks before to one week after the first detection of HHV-6 DNA for symptoms.

To determine whether symptoms were associated with HHV-6 acquisition, we compared 80 of the 81 children for whom the time of acquisition was well defined with 80 children age-matched to the day, drawn at random from the 147 children who had no apparent acquisition of HHV-6 and for whom there were complete symptom data at the age that the corresponding patient had primary HHV-6 infection. Differences were examined with the use of conditional logistic regression.

To determine which symptoms were associated with primary HHV-6 infection rather than other infectious illnesses, we compared symptoms that occurred during the acquisition of HHV-6 with those occurring during the closest episode of illness before and after acquisition in the 81 children with a well-defined time of HHV-6 acquisition. Generalized estimating equations with a logit link were used to test the hypothesis that symptoms during primary HHV-6 infection differed from those during other illnesses.

The duration of symptoms occurring during primary HHV-6 infection and at other, control times in the 81 children with a well-defined time of acquisition was compared with the use of the Wilcoxon signed-rank test and a case-crossover design.¹² Control episodes were random episodes of illness during a period other than that in which primary HHV-6 infection occurred. For each symptom, only children who had the symptom in both periods were included in the analysis.

To identify correlates of febrile primary HHV-6 infection as compared with afebrile infection, we performed multivariate logistic regression using data from the 81 children with a well-defined time of acquisition of HHV-6. All reported P values are two-sided.

RESULTS

Of the 277 infants enrolled in the study, 46 percent were girls, 80 percent were white, and 52 percent

had at least one sibling (Table 1). The median follow-up was 60 weeks (range, 1 to 113), and a median of 33 saliva samples per child (range, 1 to 106) were available for testing. Follow-up was longer for children who were infected with HHV-6 (median, 79 weeks; range, 14 to 113) than for those who were not (median, 39 weeks; range, 1 to 107; $P < 0.001$ by the Mann-Whitney U test). Similarly, more samples were available from children who became infected with HHV-6 (median, 51; range, 5 to 106) than from those who did not become infected (median, 16; range, 1 to 105). At least one sample was available from 219 infants within the first four weeks after delivery (79 percent).

PRIMARY HHV-6 INFECTION

Primary HHV-6 infection, defined by the onset of persistent salivary levels of HHV-6 DNA, occurred in 130 children. The incidence of infection was highest between 9 and 21 months of age (Fig. 1), with a cumulative incidence of 40 percent by 12 months of age and 77 percent (95 percent confidence interval, 67 to 88) by 24 months of age. In three infants, HHV-6 DNA was detected during the first week of life, suggesting in utero infection. In an additional six infants, the first sample was also positive for HHV-6 DNA; however, they were all older than two

weeks of age at the time. Hence, whether they acquired HHV-6 in utero or during the perinatal period is unclear. These nine children were not included in analyses of symptoms, because the timing of acquisition was imprecise.

We analyzed the dynamics of salivary HHV-6 DNA in several ways. To examine salivary HHV-6 DNA shedding immediately after primary infection, we summarized weekly saliva results for the 81 patients for whom the time of acquisition was well defined — that is, no more than 14 days between the last negative and the first positive saliva samples (Fig. 2A). To analyze shedding during the year after primary infection, we summarized monthly saliva results for all 130 children who acquired HHV-6 (Fig. 2B). The salivary viral level tended to be low at week 1 (median, 1700 copies per milliliter) and to increase to 100,000 copies per milliliter by week 8 (Fig. 2A). Salivary viral loads remained detectable at moderately high levels for at least 12 months, peaking at a median of about 130,000 copies per milliliter at month 3, then decreasing to a fairly constant median of about 40,000 copies per milliliter after month 7 (Fig. 2B). Rising viral levels were reflected in the increasing frequency of positive samples over time. In the first 4 weeks after the initial detection of HHV-6, approximately one third of patients had

Table 1. Demographic Characteristics of Children and Their Association with HHV-6 Acquisition.*

Characteristic	Total (N=277)	Acquisition of HHV-6 before Follow-up Visit		Hazard Ratio for Acquisition of HHV-6 (95% CI)	
		Yes (N=130)	No (N=147)	Unadjusted	Adjusted†
At baseline					
Female sex — no./total no. (%)	126/274 (46)	66/128 (52)	60/146 (41)	1.5 (1.1–2.1)	1.7 (1.2–2.4)
White race — no./total no. (%)‡	208/260 (80)	100/123 (81)	108/137 (79)	1.0 (0.7–1.6)	—
≥1 Older sibling in household — no./total no. (%)	144/277 (52)	71/130 (55)	73/147 (50)	1.9 (1.3–2.7)	2.1 (1.4–2.9)
Family income >\$35,000/yr — no./total no. (%)§	223/247 (90)	107/115 (93)	116/132 (88)	—	—
Mother's age at delivery — yr	33.2±5.0	33.5±4.7	32.9±5.2	1.0 (0.96–1.04)	—
At study completion					
Breast-fed — no./total no. (%)	212/222 (95)	123/127 (97)	89/95 (94)	1.4 (0.5–3.8)	—
Attended group child care — no./total no. (%)	73/217 (34)	44/128 (34)	29/89 (33)	0.9 (0.6–1.2)	—
Attended playgroup — no./total no. (%)	123/214 (57)	77/125 (62)	46/89 (52)	1.0 (0.7–1.5)	—

* All families but one included two parents. Plus-minus values are means ±SD. CI denotes confidence interval.

† Values were adjusted for all other variables with hazard ratios in that column.

‡ Race was assigned by the children's parents.

§ Family income was not evaluated with Cox hazard regression owing to the small number of families with an income of \$35,000 or less.

at least one saliva sample in which HHV-6 was undetectable, whereas between 4 and 12 weeks after acquisition, 507 of 512 saliva samples (99 percent) had detectable levels of HHV-6 DNA (Fig. 2A). Among the 130 children with primary HHV-6 infection, samples from 113 had sufficient DNA available for typing, and all were found to be type B.

The nearly invariable persistence of HHV-6 in saliva after infection provided a powerful approach for defining primary HHV-6 infection. However, HHV-6 was occasionally detected in saliva samples but was not immediately followed by persistent shedding and thus did not meet the definition of acquisition. Such samples were obtained from 50 children and accounted for 72 of the 1725 positive samples (4 percent). Sixty-four of these 72 samples (89 percent) were single positive samples followed by multiple negative ones. The other eight specimens from four children consisted of two positive samples followed by a gap in data or multiple negative specimens before the next positive specimen. The level of HHV-6 DNA in the 72 such positive specimens was low; 82 percent had fewer than 1000 copies of DNA per milliliter, as compared with 13 percent of the samples that met the definition of primary HHV-6 infection ($P < 0.001$). Forty-seven of these 72 positive samples (65 percent) were from 32 children who later had persistent salivary shedding of HHV-6. The median time from the excluded sample to the sample that met the definition for acquisition was 70 days. The remaining 25 positive samples were from 18 children who were not found

to have acquired HHV-6. Our classification of these 72 positive samples as falsely positive allowed us to optimize the specificity of our definition of primary HHV-6 infection. The total effect of the reclassified samples was small, since they constituted only 72 of the 3695 negative salivary samples we analyzed (2 percent).

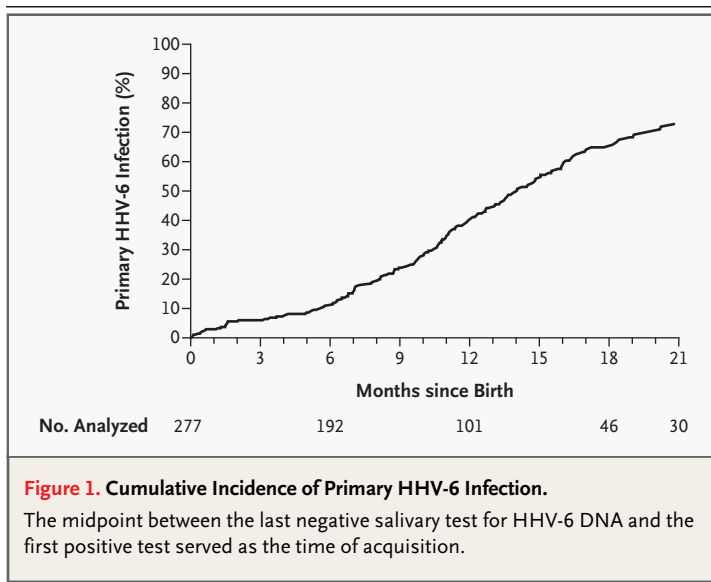
A total of 183 serum specimens were obtained from 159 children for HHV-6 antibody testing. Serologic results were available near the time of primary HHV-6 infection in the case of 7 of the 130 children who were identified as having HHV-6 infection on the basis of salivary PCR. In all seven, serum HHV-6 IgM and IgG were both detected. An eighth child had evidence of seroconversion in the absence of detection of salivary HHV-6. In this two-year-old child, HHV-6 IgM was detected, but serum HHV-6 IgG and salivary HHV-6 DNA were not; follow-up samples were not available.

FACTORS ASSOCIATED WITH HHV-6 ACQUISITION

Female sex and having at least one older sibling were independently associated with the acquisition of HHV-6 (Table 1). The age of the mother at the time of delivery, breast-feeding, and playgroup or child-care attendance were not significantly associated with the acquisition of HHV-6. In addition, the season did not appear to be associated with acquisition. Of the 130 children who acquired HHV-6, 33 (25 percent) did so during the fall, 38 (29 percent) during the summer, 30 (23 percent) during the spring, and 29 (22 percent) during the winter ($P = 0.68$). To evaluate further the potential of seasonality, we also compared the risk of acquisition of HHV-6 over time according to the season of birth. Examination of resulting Kaplan–Meier plots showed no significant difference among the four survival curves ($P = 0.64$).

CLINICAL MANIFESTATIONS OF PRIMARY HHV-6 INFECTION

Of the 81 children with a well-defined time of acquisition of HHV-6, 93 percent had symptoms. Fever, fussiness, and rhinorrhea were present in most children at the time of HHV-6 acquisition (in 57 percent, 69 percent, and 65 percent, respectively), whereas cough, diarrhea, and rash occurred less frequently (in 33 percent, 26 percent, and 31 percent, respectively). None of the 81 children had seizures at the time of acquisition. Roseola, a clinical syndrome that is relatively specific for HHV-6 infection, occurred in 19 of the 81 children (23 percent).



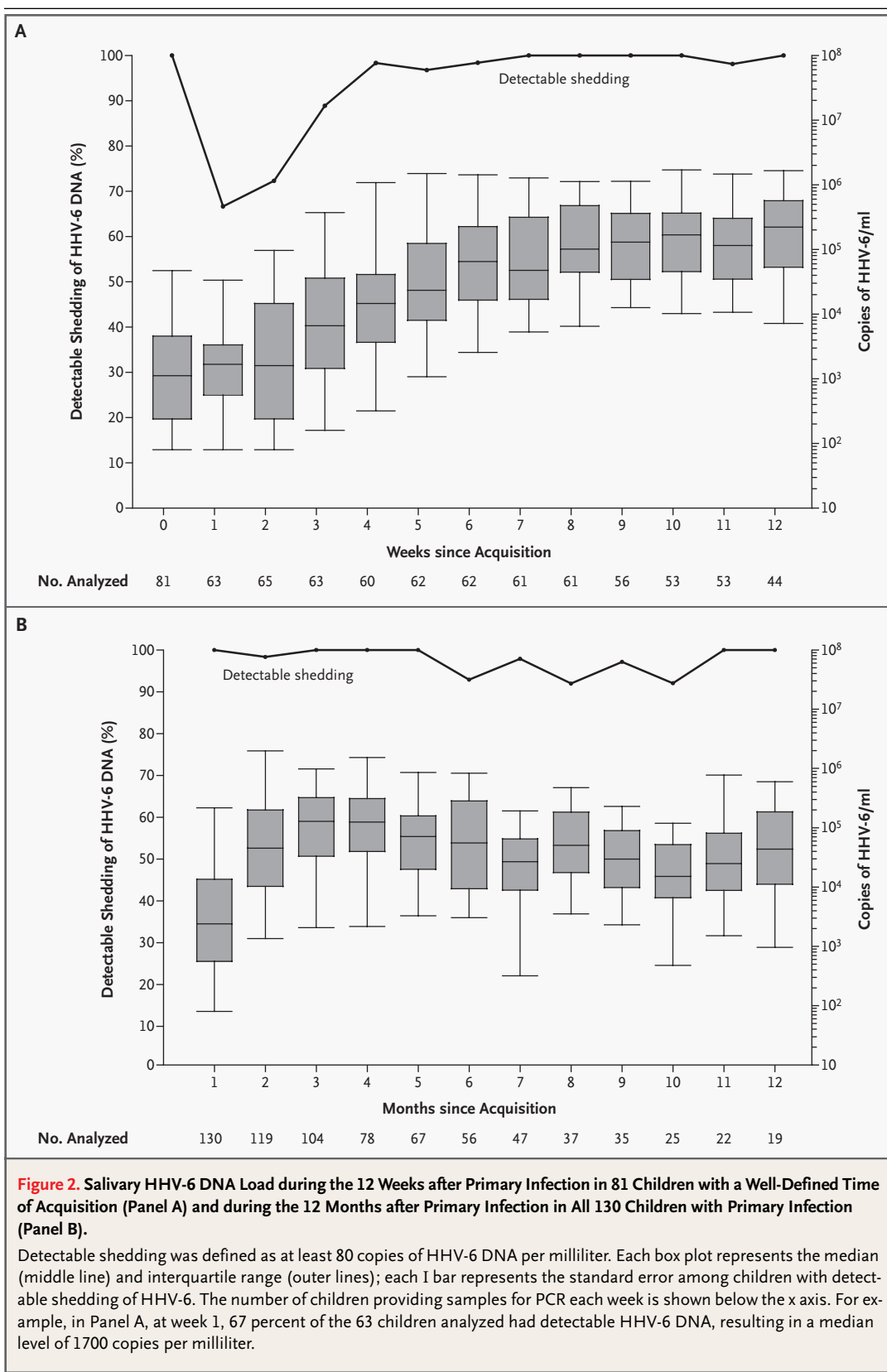


Table 2. Incidence of Symptoms and Visits to a Physician among Children with Primary HHV-6 Infection and Age-Matched Controls without HHV-6 Infection.

Variable	HHV-6- Positive (N=80)	HHV-6- Negative (N=80)	P Value
	% (95% CI)*		
Any symptom	94 (88–99)	71 (61–81)	0.004
Fever	58 (47–68)	14 (6–21)	<0.001
Fussiness	70 (60–80)	46 (35–57)	0.009
Rhinorrhea	66 (56–77)	46 (35–57)	0.02
Cough	34 (23–44)	33 (22–43)	0.87
Vomiting	8 (2–13)	5 (0–10)	0.53
Diarrhea	26 (17–36)	11 (4–18)	0.05
Rash	31 (21–41)	8 (2–13)	0.002
Roseola	24 (14–33)	3 (0–6)	0.003
Seizure	0 (0–2)	0 (0–2)	—
Visit to physician	39 (28–49)	19 (10–27)	0.009

* CI denotes confidence interval.

The onset of roseola followed the initial detection of salivary HHV-6 by less than one week in 11 of these children (58 percent), by one to two weeks in 6 (32 percent), and by no more than three weeks in 2 (11 percent). Thirty-one children with primary infection (38 percent) were evaluated by a physician for an illness near the time of acquisition of HHV-6.

To determine whether the acquisition of HHV-6 was significantly associated with symptoms, we compared children who had well-defined primary HHV-6 infection with age-matched controls who never acquired HHV-6. Children with primary HHV-6 infection were more likely than controls to have fever, fussiness, rhinorrhea, diarrhea, rash, and roseola and were more likely to have seen a physician for illness (Table 2).

Among the 81 children with a well-defined time of acquisition of HHV-6, primary HHV-6 infection accounted for 118 of 1831 episodes of illness (6 percent), 50 of 403 episodes of fever (12 percent), and 41 of 407 visits to a physician for illness (10 percent). To differentiate symptoms associated with primary HHV-6 infection from those due to other infectious illnesses, we compared the period of HHV-6 acquisition with other periods of illness in these 81 children. Episodes of illness were defined by the presence of fever, rhinorrhea, cough, vomit-

ing, diarrhea, or rash. Of the 81 children, 68 were ill and had one or more of these symptoms during primary HHV-6 infection, whereas 73 had such an episode of illness a median of 52 days before the acquisition of HHV-6 and 79 had one a median of 33 days after acquisition. Fever, fussiness, diarrhea, rash, roseola, and visits to a physician were all more common during primary HHV-6 infection than during control periods of illness (Fig. 3). There was no significant difference between groups in the frequency of rhinorrhea, cough, vomiting, or seizure.

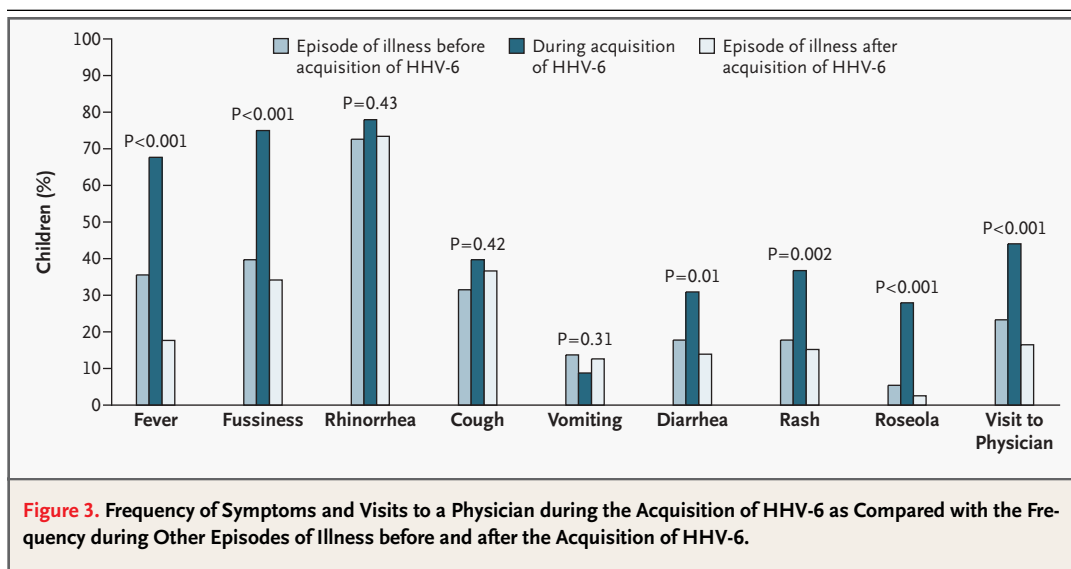
In general, the median duration of symptoms was longer during primary HHV-6 infection than during other randomly selected illnesses: 9 days (interquartile range, 4 to 15) as compared with 3 days (interquartile range, 2 to 7; $P < 0.001$). Similarly, fever lasted a median of three days (interquartile range, two to four) during primary HHV-6 infection, as compared with two days (interquartile range, one to two) during other illnesses ($P < 0.001$). Results were not appreciably different when control illnesses just before and just after primary infection were examined (data not shown).

FACTORS ASSOCIATED WITH FEVER DURING HHV-6 ACQUISITION

Because fever occurred in only 57 percent of children who acquired HHV-6 and because fever is a clinical sign often prompting medical evaluation in young children, we explored correlates of febrile primary HHV-6 infection. Children with febrile HHV-6 infection were more likely to be older than those who were afebrile: 43 of 46 children with fever were older than six months, as compared with 23 of 35 without fever (93 percent vs. 66 percent, $P = 0.004$). The association between febrile infection and age remained significant after adjustment for covariates (adjusted odds ratio, 7.2; 95 percent confidence interval, 1.7 to 30.7) (see Table 1 in the Supplementary Appendix, available with the full text of this article at www.nejm.org).

DISCUSSION

Our prospective, population-based study yielded information on the clinical and virologic aspects of primary HHV-6 infection. We found that most children with primary HHV-6 infection had symptoms and almost half visited a physician, whereas roseola accounted for a minority of cases and seizures were not reported. Salivary levels of HHV-6 DNA were relatively low early during primary infection,



increased in the subsequent weeks, and remained at high levels during the months of follow-up. Having siblings, but not attending group child care, was associated with the acquisition of HHV-6, suggesting that intimate contact is probably involved in the transmission of the virus.

The cumulative incidence of primary HHV-6 infection in this population was 77 percent by two years of age. The majority of children with primary HHV-6 infection had symptoms such as fever, fussiness, diarrhea, rash, and roseola. In contrast to the results of studies performed in emergency departments,^{2,13} in which fever was an inclusion criterion, we found that 43 percent of children with primary HHV-6 infection were afebrile, 7 percent were asymptomatic, and children older than six months were more likely than younger children to have fever with primary infection. Also in contrast to emergency department–based studies, in which seizures occurred in as many as 13 percent of children with primary HHV-6 infection,² no child in our study had a seizure near the time of acquisition of HHV-6. Despite this finding, primary HHV-6 infection appears to cause a clinical illness that is relatively worrisome to parents, and perhaps physicians, since 20 percent of the emergency visits for fever were due to primary HHV-6 infection² and substantially more children with primary HHV-6 infection than children with other types of illnesses saw a physician.

We used a new, nonserologic method to detect the acquisition of the virus that was based on the development of sustained, high levels of salivary

HHV-6 DNA. Salivary shedding appeared to follow the onset of symptoms, and initial values tended to be low, rising and then plateauing over time. A salivary test for HHV-6 could be useful in the diagnosis of primary HHV-6 infection if combined with other approaches such as a plasma test for HHV-6 DNA.

Monitoring this cohort of infants for HHV-6 infection from a single geographic region required intensive parental involvement without remuneration. It is possible that in other populations, epidemiologic and pathologic features of HHV-6 could be different and that factors such as breast-feeding or group child care could play a role in acquisition. It is also possible that our method of testing saliva may have missed some cases of HHV-6 infection. The amount of saliva collected on Sno strips is small and less than that obtained by expectoration.⁶ The reduction of salivary viral levels to below the limit of detection in a number of children in the first weeks after their initial positive specimen may indicate that weekly sampling with Sno strips missed the onset of infection in some children. When developing a method to screen infants for this study, we were aware that the sensitivity of Sno-strip samples was less than that of expectorated samples from adults.⁶ This lower sensitivity was balanced, however, by a lower frequency of PCR inhibition. In addition, the use of Sno strips enabled specimens to be collected by the children's parents. Though limited, the serologic data and data we obtained regarding the onset of viral shedding in children with HHV-6–associated roseola support the usefulness

of our methods for determining the approximate time of infection.

Primary HHV-6 infection frequently results in visits to physicians. Better diagnostic methods and an effective, safe therapy may improve care for the large number of children who acquire this infection during their first two years of life.

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