MAJOR ARTICLE

Natural History of Human *Calicivirus* Infection: A Prospective Cohort Study

Barry Rockx,[1] Matty de Wit,[2] Harry Vennema,[1] Jan Vinjé,[3] Erwin de Bruin,[1] Yvonne van Duynhoven,[2] and Marion Koopmans[1]

[1]Diagnostic Laboratory for Infectious Diseases and Perinatal Screening and [2]Department of Infectious Disease Epidemiology, National Institute of Public Health and the Environment, Bilthoven, The Netherlands; and [3]Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill

We investigated the natural history of human *Calicivirus* infection in the community. Clinical information was obtained from 99 subjects infected with Norwalk-like viruses (NLV) and 40 subjects infected with Sapporo-like viruses (SLV) in a prospective, community-based cohort study. NLV infection was common in all age groups, whereas SLV infection was mainly restricted to children aged <5 years. Symptoms lasted for a median of 5 and 6 days for NLV and SLV infections, respectively. Disease was characterized by diarrhea during the first 5 days (87% of patients with NLV infection and 95% of patients with SLV infection) and vomiting on the first day (74% for NLV and 60% for SLV). Vomiting was less common in children aged <1 year (59% for NLV and 44% for SLV) than it was among children aged ⩾1 year (>75% for NLV and >67% for SLV). Overall, NLV was detected in 26% of patients up to 3 weeks after the onset of illness. This proportion was highest (38%) for children aged <1 year. SLV shedding subsided after 14 days. These data show that the durations of disease and viral shedding of caliciviruses are longer than has been described elsewhere. Therefore, the impact of these infections may have been underestimated.

Viral gastroenteritis is a common illness in humans, with high morbidity reported worldwide and substantial mortality reported in developing countries. In recent years, caliciviruses have emerged as an important cause of viral gastroenteritis in people of all age groups and the main cause of outbreaks of gastroenteritis in such institutions as nursing homes and hospitals [1–5]. Numerous outbreaks of *Calicivirus* infection have been linked to the consumption of food prepared by infected food-handlers [1].

The human caliciviruses have been divided into 2 genera on the basis of genome organization, morphology, and genetic and antigenic properties. Norwalk virus is the prototype strain for the genus "Norwalk-like viruses" (NLVs). These small, round-structured viruses are most commonly found in association with illness in humans [6]. Sapporo virus is the prototype strain for the genus "Sapporo-like viruses" (SLVs), which can infect humans as well.

The cloning and characterization of the Norwalk virus genome has allowed the development of molecular detection assays that have been applied in molecular epidemiological studies [4, 7–9] . By now, it has become apparent that the NLV genus is in fact a very diverse group of viruses and that multiple variants or genotypes cocirculate in the community [10]. Similarly, SLVs were found to consist of a group of related viruses [11].

Some studies involving volunteer subjects and numerous outbreak investigations have been performed to improve understanding of the course of infection and disease in healthy adult volunteers or in outbreaks of gastroenteritis [12–21]. These studies have resulted in the so-called "Kaplan criteria," which are considered

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Reprints or correspondence: Dr. Marion Koopmans, Diagnostic Laboratory for Infectious Diseases and Perinatal Screening, National Institute of Public Health and the Environment, PO Box 1, 3720 BA Bilthoven, The Netherlands (marion.koopmans@rivm.nl).

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highly indicative for caliciviral gastroenteritis outbreaks: an incubation period of 15–50 h, presence of acute symptoms (including vomiting) in more than one-half of cases and/or diarrhea, average duration of symptoms of 12–60 h, a high attack rate, and stool samples that test negative for bacterial pathogens.

However, little is known about the natural course of *Calicivirus* infections in the community. In a recently completed study from The Netherlands, caliciviruses were identified as one of the leading causes of illness, both in children and adults [22, 23]. In this article, we describe the natural history of NLV and SLV infections in humans.

MATERIALS AND METHODS

Study design. Information on the natural course of *Calicivirus* infection was obtained through a community-based cohort study [23]. In brief, the study was performed in cooperation with the general practice network of The Netherlands Institute of Primary Health Care (NIVEL). This network consists of ~44 practices that cover 1% of the Dutch population and that represent the population with regard to age, sex, regional distribution, and degree of urbanization [22]. The study was a prospective cohort study with a nested case-control design. An age-stratified random sample was drawn from the population registered at general practices. Two consecutive cohorts were observed for 6 months each (4860 subjects total). Persons who fulfilled the case definition of gastroenteritis during follow-up were included in the nested case-control study. The case definition of gastroenteritis used in the present study was ⩾3 loose stools in 24 h, vomiting ⩾3 times in 24 h, loose stool with 2 additional symptoms, or vomiting with 2 additional symptoms. Additional symptoms included diarrhea, vomiting, nausea, fever, abdominal pain, abdominal cramps, and blood or mucus in stool. All cases kept a medical diary during the 4 weeks after the onset of symptoms. Healthy control subjects were selected for the same period and matched with cases by age and geographic location. Stool samples were submitted by cases (on days 1, 8, 15, and 22 after onset of symptoms) and control subjects (on days 1 and 8). An episode was considered to be new if it started after a symptom-free period of 2 weeks.

Informed consent was obtained from patients or their parents or guardians. The study was approved by and conformed to the guidelines for human experimentation of the Medical Ethical Committee of the Institute for Applied Scientific Research (TNO), Zeist, The Netherlands.

Detection of NLV and SLV. Both NLV and SLV were detected by reverse-transcriptase (RT)–PCR, as described elsewhere [4, 11]. In brief, viral RNA was extracted using the guanidinium thiocyanate–silica method [24] and assayed in a generic RT-PCR with primer pairs JV12/JV13 for NLV and JV33/SR80 for SLV. PCR products were analyzed by means of agarose gel electrophoresis and confirmed by Southern hybridization with a set of 5′-biotin–labeled probes.

Clinical information. Clinical information was obtained from medical diaries kept by cases or their caretakers on a daily basis for 4 weeks, starting on the day of onset of gastroenteritis. The diary contained questions about symptoms, such as fever (temperature, ⩾37.5°C), vomiting, nausea, abdominal pain, abdominal cramps, loose stool (more loose than usual), diarrhea (more often than usual), blood in stool, mucus in stool, and headache.

A severity score for gastroenteritis was created on the basis of these individual symptoms (table 1) [25], adapted from the clinical severity score for rotavirus gastroenteritis [26] by omitting treatment and dehydration. The severity scores for cases were compared with those for rotavirus-infected persons from the same cohort.

Table 1. Severity score for gastroenteritis in a study of human *Calicivirus* infection.

Symptom	Score
Fever[a]	2
Vomiting during 24 h[b]	
Once	1
2–4 times	2
⩾5 times	3
Duration of vomiting, days	
1	1
2	2
⩾3	3
Nausea	1
Abdominal pain	1
Abdominal cramps	1
No. of loose or frequent stools during past 24 h	
1–3	1
4–5	2
⩾6	3
Duration of loose or frequent stools, days	
1–4	1
5	2
6	3
Blood in stool	2
Mucus in stool	1
Headache	1
Maximum score	21

NOTE. The score is adapted from the clinical severity score for rotavirus gastroenteritis [26], with treatment and dehydration omitted.

[a] Temperature, >37.5°C.

[b] Maximum no. of episodes of vomiting in a 24-h period.

RESULTS

In the community-based study, 152 (19.7%) of 772 cases with gastroenteritis had NLV detected in the first or second stool sample, and 48 cases (6.2%) of SLV infection were observed. Thirty-five (4.5%) and 11 (1.4%) of 765 control subjects tested positive for NLV and SLV, respectively. All samples were also tested for a variety of bacteria, parasites, and other viruses, as described elsewhere [6, 23]. The positivity rates in cases were as follows: *Salmonella* species, in 0.4% of cases; *Campylobacter* species, in 1.3%; rotavirus, in 7.3%; adenovirus, in 3.8%; astrovirus, in 2%; *Giardia lamblia,* in 5%; and *Cryptosporidium* species, in 2%.

Data from medical diaries on symptoms were complete for 111 cases with NLV infection and 45 cases with SLV infection. Dual infections were observed in 12 cases infected with NLV and 5 cases infected with SLV; these data were excluded from the analysis of clinical symptoms. Information on NLV and SLV shedding in follow-up stool samples, which were submitted at days 15 and 22 after the onset of illness, was available for 89 NLV-infected and 36 SLV-infected cases.

Ages affected. NLVs were detected frequently in cases from all age groups in the community cohort (figure 1). The proportion of gastroenteritis cases associated with NLV was highest in children (age, 0.5–17 years; proportion, 14%–19%) and in elderly patients (age, ⩾65 years; proportion, 13%). SLVs were detected only in younger children with gastroenteritis (with 19% in children aged <6 months [$P < .05$ by χ^2 test] and 5%–9% of children aged 0.5–11 years testing positive) and in the oldest age group (3% of cases in persons aged ⩾65 years). No SLV cases occurred in the 12–64-year-old age group.

Clinical symptoms. The clinical manifestations (table 2) reported by 99 cases with NLV infection were diarrhea (in 87% of patients) and vomiting (in 74%). These symptoms were accompanied by abdominal pain in 51% of patients; abdominal cramps, in 44%; nausea, in 49%; fever, in 32%; and the presence of mucus in stool, in 19%. No patients reported having bloody stools. Diarrhea was relatively more prevalent among NLV-infected children aged <1 year (in 95% of these children) than it was among NLV-infected children aged ⩾1 year (in 80%). A greater proportion of children aged 5–11 years (95%) and >12 years (82%) experienced vomiting, compared with 59% and 75% of children aged <1 and 1–4 years, respectively.

Symptoms found in SLV-infected cases (table 3) included diarrhea (in 95% of patients) and vomiting (in 60%). These symptoms were accompanied by abdominal pain in 45% of patients; abdominal cramps, in 35%; nausea, in 38%; fever, in 43%; and the presence of mucus in stool, in 13%. No patients reported having bloody stools. In children aged <1 year, the prevalences of vomiting (44%), abdominal cramps (22%), abdominal pain (11%), and nausea (22%) were lower than the prevalences among children aged 1–4 years (80%, 53%, 60%, and 47%, respectively), but these symptoms can be difficult to asses in infants.

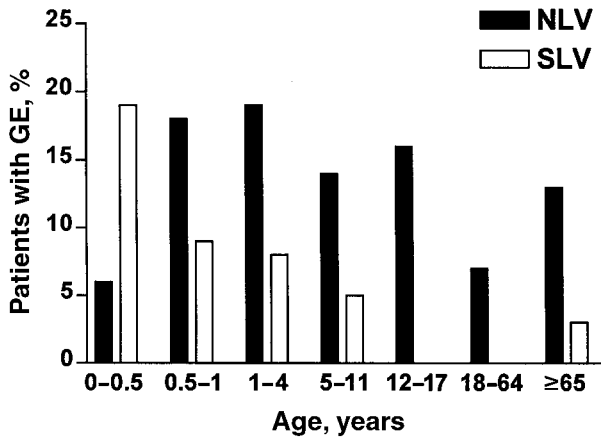

Figure 1. Proportion of patients with gastroenteritis (GE) associated with Norwalk-like virus (NLV) infection and Sapporo-like virus (SLV) infection, according to patient age.

Duration of symptoms. Clinical symptoms associated with NLV infection lasted a median of 5 days. The median duration of symptoms was different for different symptoms (table 2). Diarrhea lasted longest, with a median duration of 4 days (figure 2*A*); however, durations of up to 28 days (the end of the reporting period) were observed. Vomiting (figure 2*B*), nausea, and fever primarily occurred on the first day of illness. The median duration of the overall symptoms decreased with age: 6 days in children aged <1 year, compared with 4 days in children aged 1–4 years, 5 days in children aged 5–11 years, and 3 days in patients aged ⩾12 years.

SLV infection was generally associated with clinical symptoms that lasted a median of 6 days. Diarrhea was most common during the first days of infection, with a median duration of 5 days (table 3). Vomiting, nausea, and fever were primarily observed during the first 2 days of illness (data not shown).

The median severity score for both NLV- and SLV-infected patients was 6 (range, 0–12 and 0–15, respectively). This was lower than the score for rotavirus-infected patients in the present study (median severity score, 8 [range, 0–14]; data not shown). A severity score of ⩾7 occurred in 44% of the NLV-infected cases; 31% of the NLV-infected cases had a severity score of 4–6, and 25% of the cases had mild illness (i.e., a severity score of ⩽3.)

Viral shedding. The duration of viral shedding was determined by RT-PCR of follow-up stool samples obtained on days 1, 8, 15, and 22 after onset of illness. Virus could be detected on the first day of sampling in the stool samples of 78% of all NLV-infected cases and 89% of SLV-infected cases. Shedding of NLV up to 22 days after the onset of illness was observed in 26% of the cases (figure 3). In 10% of cases, NLV could be detected from day 8 up to day 22 (14 days), but not

Table 2. Symptoms of and symptom durations for Norwalk-like virus infection, according to age group, for patients with gastroenteritis in a community cohort in The Netherlands.

	Symptom					
Patient age, variable	Diarrhea	Vomiting	Fever	Nausea[a]	Abdominal pain[a]	Abdominal cramp[a]
<1 year (n = 37)						
Percentage of patients	95	59	24	30	11	41
Duration, days						
Median	6	2	1	2	1	2
QR	6	2	1	1	0	2
Range	28	7	9	6	2	6
1–4 years (n = 32)						
Percentage of patients	84	75	40	53	63	35
Duration, days						
Median	3	1	1	1	1	1
QR	5	0	0	1	2	1
Range	27	5	6	4	11	3
5–11 years (n = 19)						
Percentage of patients	74	95	48	76	90	52
Duration, days						
Median	1	1	1	1	4	2
QR	3	0	0	1	7	2
Range	7	3	2	5	18	9
⩾12 years (n = 11)						
Percentage of patients	91	82	45	55	91	82
Duration, days						
Median	2	1	1	2	2	2
QR	2	0	1	2	2	2
Range	21	3	2	6	10	10
All (n = 99)						
Percentage of patients	87	74	32	49	51	44
Duration, days						
Median	4	1	1	1	2	2
QR	6	1	1	1	4	2
Range	28	7	9	6	18	10

NOTE. QR, quartile range.

[a] Difficult to determine in children aged <5 years.

on day 1. In the remaining cases (12%), virus was detected only in the samples obtained on days 8 and/or 15. The percentage of cases that continued to shed NLV at day 15 and/or day 22 was highest in newborns aged <1 year (47% and 38%, respectively; figure 3). Duration of shedding decreased in all age groups. Long-term NLV shedding was not associated with increased severity of disease or prolonged duration of clinical symptoms (data not shown).

The duration of SLV shedding was shorter than the duration of NLV shedding. In 89% of the cases, virus could be detected on day 1, decreasing to 14% by day 15. After 22 days, no SLV shedding was observed (figure 4). Duration of viral shedding was not associated with prolonged duration of clinical symptoms.

DISCUSSION

In the present study, we investigated the natural history of human *Calicivirus* infections in the community. We showed that NLV infection was commonly associated with gastroenteritis in all age groups in the community, as has been suggested elsewhere [6, 8, 20, 27, 28], whereas SLV infection was mainly restricted to children aged <5 years. Symptoms due to NLV and SLV infections were observed for a median of 5 and 6 days,

Table 3. Symptoms of and symptom durations for Sapporo-like virus infection, according to age group, for patients with gastroenteritis in a community cohort in The Netherlands.

	Symptom					
Patient age, variable	Diarrhea	Vomiting	Fever	Nausea[a]	Abdominal pain[a]	Abdominal cramp[a]
<1 year (n = 18)						
Percentage of patients	94	44	50	22	11	22
Duration, days						
Median	5	2	2	4	1	2
QR	6	5	3	1	1	1
Range	21	7	7	4	2	3
1–4 years (n = 15)						
Percentage of patients	93	80	33	47	60	53
Duration, days						
Median	6	2	2	2	4	3
QR	3	1	4	1	3	3
Range	18	21	17	3	6	5
5–11 years (n = 6)						
Percentage of patients	100	67	50	67	83	33
Duration, days						
Median	2	1	2	1	1	2
QR	3	0	1	0	0	1
Range	7	2	3	2	3	3
All (n = 40)						
Percentage of patients	95	60	43	38	45	35
Duration, days						
Median	5	2	2	2	2	3
QR	6	1	3	2	3	2
Range	21	21	17	4	6	5

NOTE. Only 1 patient was aged ≥12 years and is therefore not separately presented in table. QR, quartile range.

[a] Difficult to determine in children aged <5 years.

respectively, which is longer than the durations described elsewhere [12, 16, 29]. The prevalence of vomiting in both NLV-infected and SLV-infected cases was lower in children aged <1 year than it was in children aged ≥1 year. NLV could be detected in stool samples up to 3 weeks after the onset of illness in more than one-quarter of the cases. SLV shedding generally subsided after 14 days.

Several studies of volunteer subjects and epidemiological studies have been performed to get an insight into the course of disease associated with NLV infection [12, 14–18, 30]. These studies have focused either on healthy adults or on at-risk communities involved in outbreaks of acute nonbacterial gastroenteritis, and they have shown that manifestations of disease lasted for 24–72 h after an incubation period of 12–48 h. Symptoms associated with NLV infection included diarrhea, vomiting, abdominal cramps, and nausea. We confirm that NLV and SLV infections are usually mild, and the clinical severity score for these infections was lower than that for rotavirus infection [20]. The clinical manifestations associated with NLV infections appear to be similar in both naturally occurring and experimentally induced disease [12]. However, in contrast to studies reported elsewhere [16, 13, 31], our data suggest that there is a higher prevalence of diarrhea among young children (age, <5 years), whereas a greater proportion of children aged ≥5 years experienced vomiting, abdominal cramps, and abdominal pain. A surprising finding in the present study was the relatively long duration of symptoms (median for NLV infection, 5 days), whereas illness was generally believed to last 12–60 h [15, 16]. Disease was characterized by diarrhea during the first 5 days, but durations of diarrhea of up to 28 days after onset were reported. Vomiting, nausea, and fever occurred on the first day of disease. The large range in the durations of symptoms might be explained by differences in the clinical manifestations of the different virus types, because multiple genotypes cocirculate in the community [10].

With regard to the duration of symptoms, differences between the findings of our study and of studies reported elsewhere may be because our study focused on infection in the

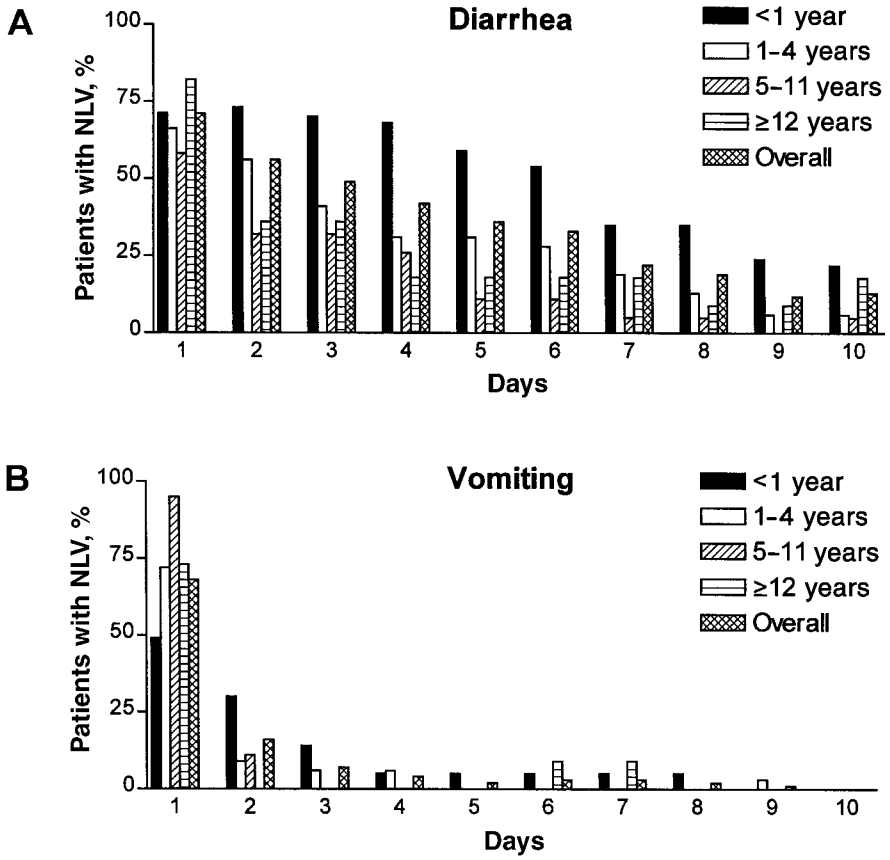

Figure 2. Proportion of Norwalk-like virus (NLV) cases associated with diarrhea *(A)* or vomiting *(B)* in patients aged <1, 1–4, 5–11, and ⩾12 years

community, whereas other studies have mainly focused on symptoms associated with outbreaks or experimental infections in adults. Those studies described the clinical manifestations for 1 virus type and in an age group for which we found a shorter duration.

SLV is thought to be a common cause of viral gastroenteritis in infants and young children [20, 32]. However, little is known about the clinical manifestations of SLV apart from the findings of studies of gastroenteritis outbreaks that occurred in infants' homes [33, 34]. Symptoms associated with SLV infection included diarrhea, vomiting, and fever; these symptoms usually lasted for a median of 6 days (for any symptoms reported). The progression of the disease was similar to that of NLV infection. The prevalence of fever was higher among SLV-infected patients than it was among NLV-infected patients.

Despite the high level of NLV infection in children, a considerable proportion of adults still get infected. Serological studies have shown that antibodies against NLV are obtained early in life, reaching a prevalence close to 100% at the age of 9–10 years [26, 35–37], and the antibodies remain present throughout the person's life [35, 36, 38]. These prevalent antibodies do not appear to correlate with protective immunity in adults, given that symptomatic NLV infection of adults is quite common. In studies of experimental infections of healthy humans, the presence of antibodies was not associated with protection against infection [19, 21]. However, the duration of symptoms decreased with age, which suggests that adults have partial protection or a generally better-developed immune system.

SLV infection was more common among children aged <1

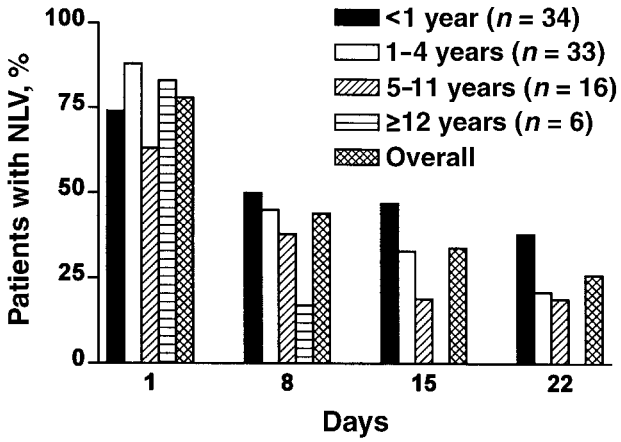

Figure 3. Duration of Norwalk-like virus (NLV) shedding in stool, according to age group.

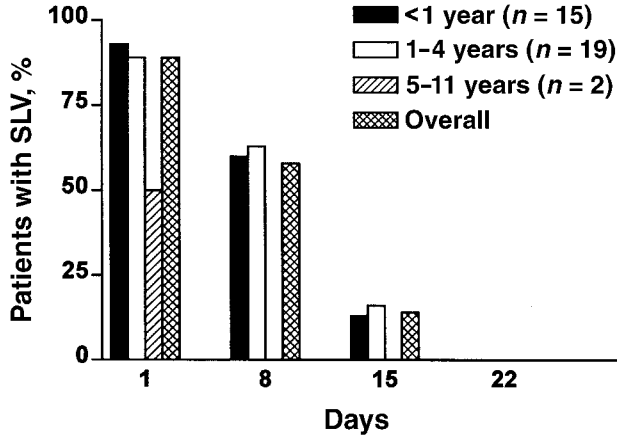

Figure 4. Duration of Sapporo-like virus (SLV) shedding in stool, according to age group.

year than it was among children aged ⩾1 year. Again, a high prevalence of antibodies against SLV in adults has been described elsewhere [32, 39], and SLV infections were rarely detected in people aged >12 years, which suggests the induction of protective immunity after exposure to the viruses during childhood. However, the high prevalence of SLV infection in children aged <6 months would suggest the absence of maternal antibodies. The apparent absence of protective immunity against NLV and its presence for SLV may be explained by the number of virus types. Because SLV comprises only 4 subtypes, people are more likely to be challenged by a virus type for which immunity has already developed. For NLV, this will be less likely because of the diversity of viruses that cocirculate in the community [10] (authors' unpublished data) and the regular emergence of formerly unknown types (authors' unpublished data).

For both NLV and SLV, it may be important to test multiple stool samples obtained at 1-week intervals, because we demonstrated that it was possible to test too early: 22% and 11% of the NLV and SLV cases, respectively, were not initially detected. In total, 28% of the NLV-infected cases were found to shed virus up to 22 days after the onset of illness. The duration of NLV shedding may be even longer, but samples were not available from later in the course of infection. Long-term shedding correlated with age but not with increased severity of disease or prolonged duration of clinical symptoms. However, this long-term shedding, accompanied by the relatively large group of asymptomatic infections, illustrates the high transmission potential of these viruses. Most patients will resume normal activities after resolution of symptoms (median, 5 days), acting as human reservoirs for secondary spread. This might explain the relatively high incidence of NLV infection in the community. In studies reported elsewhere, information on NLV shedding was collected through experimental infections of volunteers; the experimental infections were limited to infection with Norwalk virus. In those studies, virus was detected directly by means of electron microscopic evaluation or by detection of virus antigen via enzyme immunoassays; virus was detected up to 20 days after infection in 10% of cases [40]. In the subjects in our study, the higher incidence of viral shedding found at day 22 may be attributed to the fact that molecular detection of virus is more sensitive or that viral shedding may differ for different virus types. This issue will be addressed in a follow-up study that uses quantitative PCR.

In conclusion, the present study describes the natural history of human *Calicivirus* infection in the community and may help determine the burden of illness due to human *Calicivirus* infections. Our findings suggest that the burden of illness may be underestimated when findings are based on historic data on the duration of illness.

Acknowledgments

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