MAJOR ARTICLE

Seroepidemiology and Risk Factors for Sporadic Norovirus/Mexico Strain

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Risk factors associated with transmission of sporadic norovirus (NV; formerly Norwalk-like virus)/Mexico strain were identified in a seroepidemiological study conducted in rural Mexico. Acquisition of Mexico strain IgA antibodies was age-related; 34% of 1–4-year-olds were seropositive, compared with 81% of adults ($P < .001$). After 12 months, 42% of 1–4-year-olds showed a seroresponse to Mexico strain, compared with 27% of adults ($P < .01$). Personal and domestic hygiene measures, such as hand washing, general cleanliness of the mother's clothing, and the type of room assigned for cooking were significantly associated with odds of a seroresponse. For infants, having a dog in or near the home was a risk factor for seroresponse ($P < .01$), whereas, for older children, the mother's involvement in agricultural activities was a risk factor ($P < .001$). This study provides initial evidence of risk factors associated with sporadic NV infection. Data indicate some similarities to risk factors associated with outbreaks of NV infection.

Human caliciviruses (HuCVs) are major causes of sporadic and epidemic nonbacterial gastroenteritis in children and adults, in both developed and developing countries [1]. HuCVs previously were thought to cause only mild and self-limiting illness, but they are now being associated with severe gastroenteritis [1].

HuCVs, of the family Caliciviridae, are classified into 2 genera—noroviruses (NVs; formerly Norwalk-like viruses [strains include Mexico, Norwalk, Snow Mountain, and Hawaii]) and sapoviruses (formerly Sapporoviruses [strains include Sapporo/82, Hou/90, and Lon/92]) [2]. As molecular epidemiological techniques develop, more genetic diversity has been detected. This has limited the development of simple, economical, and reliable assays to detect calicivirus infections. At present, diagnosis is based on polymerase chain reaction amplification and sequencing of the P (protruding) domain fragment. Immunoassays for detection of antigens in stools have not yet been standardized for routine diagnosis because of this antigenic variability [3]. Although a calicivirus strain that causes both animal and human disease has never been detected, the possibility of a zoonotic reservoir for HuCVs has been suggested by several authors [4–7].

The worldwide importance of HuCVs as the causative agent in outbreaks of gastroenteritis has been well documented [1, 2]. Of 90 selected nonbacterial outbreaks reported in the United States during 1996–1997, NVs were detected in 96% of outbreaks [8]. NVs are estimated to be responsible for illness in ~23 million persons annually in the United States [9]. NVs were associated with >87% of gastroenteritis outbreaks in The Netherlands [10], and HuCVs (mostly NVs) were detected in 60% of outbreaks in Sweden [11]. NV has been associated with >85% of all nonbacterial outbreaks of gastroenteritis reported in 10 countries across Europe during 1995–2000 [12].

The role of HuCV in sporadic nonbacterial gastroenteritis has only recently become apparent [1, 13].

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Black et al. [14] had found that children in the United States acquired serum antibodies to NVs during the first 5–15 years of life, whereas children in Bangladesh were infected earlier. In Mexico, 13% of diarrheal episodes among children <2 years old were associated with HuCV [15]. In Finland, among children <2 years old, HuCV was detected in 29% of cases of gastroenteritis, making it as common as rotavirus [16]. Studies indicate that, unlike cases of rotavirus infection, seroprevalence of NVs among adults is similar to that reported among children [10, 16]. In a community-based study of all ages, Marshall et al. detected NVs in 11% of cases of gastroenteritis [13].

The production of recombinant NV particles [17] and the development of rapid and sensitive EIAs using NV particles as antigens [18] have enabled seroepidemiological studies to be conducted. The recombinant NV particles contain both the constant S (shell) domain, which defines the group, and the variable P domain, which defines the strain. Although the immune response is directed against both domains, current assays predominantly detect antibodies to the variable P domain; thus, there is no single assay that reliably detects infections by group. Children acquire antibodies to NVs at an early age, and prevalence of antibodies to NVs increase with age and can reach 100% by adulthood [19, 20]. Seroprevalence to NVs varies between countries [19, 21] and even between neighboring communities [22]. This pattern of seroprevalence is observed in both developed and developing countries [20, 23–28].

NV-associated outbreaks of nonbacterial gastroenteritis have been related to the consumption of feces-contaminated food [29, 30] and water [31, 32], to food handlers [33], and to person-to-person transmission [34, 35]. Knowledge of risk factors for sporadic HuCV infection is however limited. A study from Chile found that lower socioeconomic status and increasing age were risk factors for infection with both the Norwalk and Mexico strains, whereas consumption of seafood was a risk factor for infection with the Norwalk strain, and crowded living conditions and consumption of uncooked vegetables were risk factors for infection with the Mexico strain [20]. The aim of the longitudinal study described here was to investigate the seroepidemiology of NV/Mexico strain (NV-Mx) and to identify the risk factors associated with sporadic infection in a rural Mexican population.

SUBJECTS AND METHODS

Study design. A longitudinal seroepidemiological study was conducted between November 1993 and January 1995 in Tecozautla, Hidalgo State, Mexico. This is a semidesert region 130 km north of Mexico City. The municipality of Tecozautla has >4800 households, with 50%–60% involved in farming. To investigate the seroepidemiology of NV-Mx and associated risk factors, 135 individuals/age group (1–4 years, 5–9 years, 10–14 years, and ⩾15 years) were randomly selected from 414 households involved in a study of diarrheal disease risk factors in rural Mexico. The 414 households had been randomly selected following a census of all households in the villages surrounding Tecozautla (all individuals aged >1 year living in each household were invited to participate). Informed, verbal consent to participate in the study was obtained from all individuals. The study was approved by the ethical committees of the London School of Hygiene and Tropical Medicine and National Institute of Medical Sciences and Nutrition, Mexico. A field doctor was available for consultation and treatment, where appropriate throughout the study.

Data and sample collection. Finger-prick blood samples were obtained in vacutainers (Becton Dickinson) at the start of the study (November 1993) and 1 year later. By use of knowledge of local practices gained during previous studies in the area, open and structured household interviews were conducted to finalize the list of socioeconomic and hygiene factors and possible routes of transmission to be studied and to improve the structuring of questions. In spring 1994, during the dry season, a questionnaire on socioeconomic and hygiene factors and possible routes of transmission was completed with the head female in each household. Variables included occupation of the head of the household (HH) and his wife, number of individuals per bedroom, location of the kitchen, frequency of consumption of vegetables and meat (consumption data over the course of 7 days was obtained on 2 separate occasions during the course of the study), source of vegetables, hand washing practices, site of defecation, and presence of animals near or in the house. At the same time, a blood sample was obtained for determination of initial titer of IgA antibodies. A second visit (June–July 1994) was made to households, and information was obtained for variables that, it was considered, could change over time or be seasonal and were of particular interest. These included frequency of consumption of vegetables and meat, drinking water source, source of vegetables, and washing vegetables.

Serologic testing. The vacutainers were transported vertically on ice to the field laboratory, where the serum was separated by centrifugation and stored in vials at −20°C. Batches of serum samples were periodically transferred to the main laboratory in Mexico City, where samples were also stored at −20°C. Serum IgA antibodies to NV-Mx were detected by use of ELISA using recombinant NV-Mx capsid antigen (1 μg/mL) [18, 36]. Serum samples were diluted 1:100. An alkaline phosphatase rabbit anti–human IgA conjugate (Dako) at a 1:1000 dilution was used. The optical density of samples was read in an automatic EIA reader (Bio-Rad).

To minimize interplate variations, batches of serum samples from 13 individuals were randomly selected, and the initial and final serum samples for the selected individuals, together with control serum samples with negative (n), medium (m) and

high (h) optical density values were assayed in duplicate on the same plate. Intra- and interassay variations were monitored by use of Shewhart charts [37], to compare accumulated optical density readings for each control sample with readings from the current day. The coefficient of variation for control samples was >10% on 6% of plates, which were retested. Optical density values were corrected for intra- and interplate variations by multiplying by a correction factor (CF) [38] calculated by use of the following equation:

$$CF = \frac{\left(\frac{\text{True OD}_n}{\text{Actual OD}_n} + \frac{\text{True OD}_m}{\text{Actual OD}_m} + \frac{\text{True OD}_h}{\text{Actual OD}_h}\right)}{3},$$

where True OD is the mean optical density over all plates, and Actual OD is the optical density on a particular plate.

Outcome measures. The seroepidemiology of NV-Mx was described in terms of seropositivity, seroresponse, and geometric mean of the optical density. An individual was defined as seropositive if the corrected optical density was greater than the true OD_n of the negative control plus 2 SD (cutoff point, 0.252). Seroprevalence of NV-Mx was defined as the proportion of seropositive individuals in a particular age group. Seroresponse was defined as a ⩾50% increase in corrected optical density value from the first to the final serum sample. The geometric mean was the mean of the corrected optical density values for any named group of individuals. A total of 33 individuals with an initial corrected OD ⩾1.00 were excluded from all the statistical analyses, because these readings were at the maximum limits for the automatic EIA reader (i.e., it was not possible to determine whether these individuals had a seroresponse).

Statistical analysis. To investigate possible risk factors for sporadic NV-Mx, seroresponse was adopted as the outcome measure. New variables were created where risk factors had changed between visits, to minimize information bias (e.g., 5% variation for water source between visits), and categories were ranked according to predicted risk; for example, for water, the lowest risk was having water piped to the house at both visits, and the highest risk was having an unprotected water source at both visits. In the univariate analysis, the prevalence of seroresponse, the odds ratio of a seroresponse for a given variable (adjusted only for age, sex, and baseline corrected optical density value), 95% confidence intervals, and likelihood ratio test (LRT) *P* values were determined for each risk factor. Those variables found to be significant ($P < .10$, LRT), together with age, sex, and baseline titer, were included in the multivariate logistic model. Multiple logistic regression was used to assess the association between each significant risk factor and the outcome measure (i.e., seroresponse). In the multivariate analysis, a hierarchical approach using a conceptual framework was adopted [39], with significant risk factors divided into 2 groups: (1) socioeconomic variables, which were adjusted for each other, and (2) hygiene-related variables, which were adjusted for each other and for the significant socioeconomic risk factors. Separate multivariate analyses were performed for 1–4-year-olds, 5–14-year-olds, and ⩾15-year-olds. Adjusted odds ratios, 95% confidence intervals, and LRT *P* values were calculated for all multivariate analyses. Allowance was made for clustering by household, by use of the Huber method, which adjusts the SEs. All statistical analyses were performed by use of STATA software (version 5; StataCorp). Sample-size calculations indicated that a sample size of at least 135 individuals was required in each age group to ensure detection of a 20% difference in seroresponse to NV-Mx with 80% power and a significance level of 5%.

RESULTS

IgA antibody response to NV-Mx. Acquisition of NV-Mx antibodies in the study population was age related. The geometric mean optical density of NV-Mx antibodies accordingly increased with age (figure 1). The proportion of the study population with low optical density values (<0.20) was 55% (73/133) for 1–4-year-olds, 25% (64/261) for 5–14-year-olds, and 13% (14/111) for ⩾15-year-olds. Conversely, the proportion of the study population with high optical density values (0.40–0.99) increased with age (16%, 25%, and 58% respectively).

Of the 505 individuals included in the present study, 55% were seropositive for NV-Mx at the start of the study, and 66% were seropositive for NV-Mx at its conclusion, 1 year later. One-third of 1–4-year-olds were initially seropositive, compared with >80% of ⩾15-year-olds ($P < .001$) (table 1). After 12 months, 56% of the children originally in the 1–4-year-old group were seropositive. There was no change in the percentage of seropositive adults over the period of study ($P > .10$). The percentage of individuals demonstrating a seroresponse during the 12-month period of the study was similarly age related; a

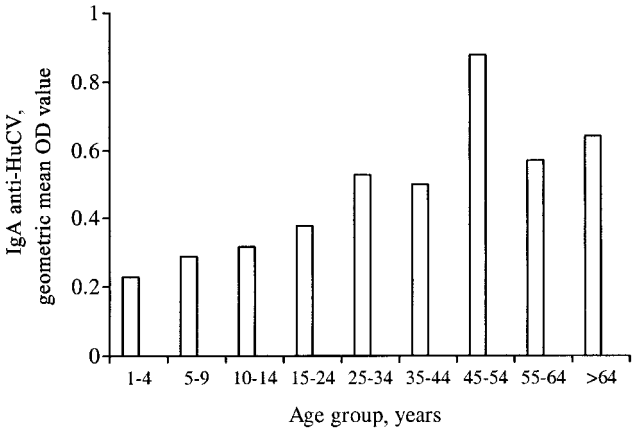

Figure 1. Age-related acquisition of norovirus/Mexico strain IgA antibodies. HuCV, human calicivirus; OD, optical density.

Table 1. Seropositivity and seroresponse to norovirus/Mexico strain among the study population.

Age group, years	Total	Seropositivity		Seroresponse
		Initial	Final	
1–4	133	45 (34)	75 (56)	56 (42)
5–14	261	145 (56)[a]	169 (65)	86 (33)[b]
⩾15	111	90 (81)[a]	90 (81)[a]	30 (27)[c]

NOTE. Data are no. (%) of subjects. All *P* values were calculated by use of the χ^2 test and indicate comparisons between age groups.

[a] $P \leqslant .001$.
[b] $.05 < P < .10$.
[c] $.01 < P \leqslant .05$.

significantly higher percentage of 1–4-year-olds had seroresponses, compared with adults ($P = .014$, χ^2 test) (table 1).

Characteristics of the study population. The demographic and socioeconomic characteristics of the study population are shown in table 2. More than one-half of all individuals ate meat <2 times/week, lived in houses with concrete floors and asbestos roofs, and shared a bedroom with ⩾4 other members of the household. Between 50% and 60% of individuals lived in homes in which the HH was a farmer, and >10% lived in homes in which the wife of the HH was involved in agricultural activities.

The hygiene-related characteristics of the study population are shown in table 3. For most individuals, water was piped to their house or a neighbor's house, water for drinking was stored, rather than being taken directly from the tap on demand, and water was not boiled before drinking. The site for cooking varied slightly between age groups. The dishes were washed in a sink in the homes of >60% of individuals. Most individuals obtained their vegetables from within the study area, where crops are irrigated with borehole water, with only a small number reporting to have obtained, on at least 1 occasion, vegetables from other areas. At least 25% of individuals reported occasionally eating uncooked vegetables without washing them. A quarter of individuals lived in households where the wife's clothing was described by the interviewer as dirty. All individuals reported washing their hands in relation to at least 1 daily activity (for >70% of individuals, this referred to washing hands or having their hands washed before eating food). Additional hand washing activities were reported by more than one-half of all individuals. The most frequent additional activity was hand washing after defecation. Statistical interaction between the number of daily hand washing activities and other significant risk factors was investigated. There was a significant interaction with hand washing technique, land ownership, and location of the kitchen, but these associations were not significant after adjustment for all other significant risk factors. Nearly 80% of individuals lived in houses where a dog was observed near or inside the home.

Risk factors related to seroprevalence. Risk factors for seroprevalence for NV-Mx showed some association with age, sex, hygiene, and socioeconomic status. Seroprevalence increased with age among 1–4-year-olds, but not in older age groups. Among 5–14-year-olds, seroprevalence was greater among girls than boys. Seroprevalence was lower among 1–4-year-olds whose parents owned land. Seroprevalence was higher among 5–14-

Table 2. Demographic and socioeconomic characteristics of Mexican study population, by age group.

Variable, category	Age group, years		
	1–4	5–14	⩾15
Sex			
Male	66 (50)	133 (51)	41 (37)
Female	67 (50)	128 (49)	70 (63)
Material of floor			
Concrete	73 (55)	163 (63)	86 (77)
Earth	57 (43)	92 (35)	20 (18)
Other	2 (2)	6 (2)	5 (5)
Material of roof			
Asbestos	82 (62)	153 (59)	63 (57)
Concrete	36 (27)	74 (28)	31 (28)
Other	15 (11)	34 (13)	17 (15)
No. of individuals/bedroom			
<3	13 (10)	44 (17)	45 (40)
3	19 (14)	41 (16)	14 (13)
4	16 (12)	40 (15)	19 (17)
⩾5	85 (64)	136 (52)	33 (30)
Occupation of HH			
Farmer	67 (50)	154 (59)	64 (58)
Trade or handicrafts	45 (34)	78 (30)	36 (32)
Other	21 (16)	29 (11)	11 (10)
Occupation of wife of HH			
HW	88 (67)	195 (76)	96 (87)
HW and agriculture	13 (10)	12 (5)	2 (2)
Agriculture	31 (23)	50 (19)	12 (11)
Own or rent land			
No	56 (42)	88 (34)	33 (30)
Yes	77 (58)	173 (66)	78 (70)
No. of times red meat was consumed during two 7-day periods			
0	49 (37)	92 (35)	34 (31)
1	43 (32)	113 (43)	34 (31)
2	26 (20)	32 (12)	26 (23)
3–14	15 (11)	24 (9)	17 (15)
No. of times chicken was consumed during two 7-day periods			
0	31 (23)	53 (20)	25 (22)
1	41 (31)	90 (35)	24 (22)
2	38 (29)	76 (29)	32 (29)
3–14	23 (17)	42 (16)	30 (27)

NOTE. Data are no. (%) of subjects. HH, head of household; HW, housework.

Table 3. Hygiene-related characteristics of Mexican study population, by age group.

Variable, category	Age group, years		
	1–4	5–14	⩾15
Source of DW			
Piped to house, yard, or neighbor	110 (83)	218 (84)	98 (88)
Public tap on 1 or both visits	9 (7)	19 (7)	3 (3)
Other source on 1 or both visits	14 (10)	24 (9)	10 (9)
Boil DW			
Yes	27 (20)	38 (15)	23 (21)
No	106 (80)	223 (85)	88 (79)
Store DW before use			
Yes	123 (92)	240 (92)	105 (95)
No	10 (8)	21 (8)	6 (5)
Room where cooking performed			
Specific room in house	50 (38)	138 (54)	74 (67)
Communal or bedroom in house	56 (43)	87 (34)	25 (23)
Lean-to outside house	24 (19)	32 (12)	11 (10)
Site for washing dishes			
Sink (kitchen or yard)	86 (65)	169 (65)	78 (70)
Bucket in kitchen	16 (12)	36 (14)	17 (15)
Bucket in yard	31 (23)	56 (21)	16 (15)
Source of vegetables			
Study area	124 (93)	238 (91)	97 (87)
Study area and other	9 (7)	23 (9)	14 (13)
Wash vegetables			
Always use chemical or soap	33 (25)	56 (22)	38 (34)
Always use water	58 (44)	129 (49)	45 (41)
Occasionally do not wash	42 (31)	76 (29)	28 (25)
Clothing of wife of HH			
Clean	28 (21)	65 (25)	32 (29)
Normal	70 (53)	137 (52)	59 (53)
Dirty	35 (26)	59 (23)	20 (18)
No. of hand washing activities			
1	67 (50)	85 (33)	13 (12)
⩾2	66 (50)	176 (67)	98 (88)
Usual hand washing technique			
Water only	44 (33)	67 (26)	19 (17)
Water plus soap	60 (45)	125 (48)	66 (59)
Water plus detergent	29 (22)	68 (26)	26 (24)
Site of defecation			
All use toilet	110 (82)	141 (54)	48 (43)
⩾1 outside and far from house	10 (8)	76 (29)	37 (33)
⩾1 outside and near house	13 (10)	44 (17)	26 (24)
Dog near or in house			
No	28 (21)	60 (23)	19 (17)
Yes	105 (79)	201 (77)	92 (83)

NOTE. Data are no. (%) of subjects. DW, drinking water; HH, head of household.

year-olds who lived in houses in which cooking was performed inside rather than outside the house, in which drinking water was obtained from a public tap rather than a household/neighbor's tap, and in which the mother's clothing was described as normal or dirty. Seroprevalence was also higher among those living in a household where ⩾1 individual defecated in the open air and near the house.

Risk factors related to annual incidence of seroresponse. Risk factors for seroresponse to sporadic NV-Mx are shown in table 4 and come from age-specific multivariate models (1–4-year-olds, 5–14-year-olds, and ⩾15-year-olds, respectively). More 5–14-year-old girls (38%) than boys (27%) had a seroresponse to NV-Mx antibodies. Individuals with a corrected OD of <0.40 had a higher annual incidence of seroresponse than did individuals with a corrected OD of ⩾0.4. Increased consumption of meat was associated with a decrease in the odds of a seroresponse to NV-Mx antibodies.

In households where the wife of the HH reported her occupation as wholly or partially agricultural work, adults and 5–14-year-olds had significantly higher odds of a seroresponse to NV-Mx antibodies than did individuals who lived in households where the wife of the HH reported housework as her main occupation. Crowded living conditions were associated with an increase in the odds of seroresponse among 1–4-year-olds ($P < .01$, test for trend), but not in the older age groups.

There was an increase in the odds of a seroresponse among 1–4-year-olds and adults living in households in which the food was cooked on an open fire in a lean-to outside the house, rather than on a gas stove in a room within the house. Observation by the interviewer that the clothes of the wife of the HH were dirty was associated with a slight increase in the odds of a seroresponse among individuals in all age groups. Reporting hand washing for >1 daily activity was associated with an 82% reduction in the odds of a seroresponse among 1–4-year-olds. Observation of a dog near or in the house during the interview was associated with a >7-fold increase in the odds of a seroresponse among 1–4-year-olds.

DISCUSSION

To our knowledge, this is the first study to investigate the seroepidemiology and risk factors associated with sporadic HuCV infection. The results presented here give an initial insight into the transmission of sporadic NV-Mx infection in a community setting in rural Mexico.

Advances in diagnostic techniques are allowing the burden of disease attributable to HuCV to be addressed. Although they are not appropriate for use in routine public health laboratories, techniques such as those used in the present study, which detect

Table 4. Risk factors for seroresponse to sporadic norovirus/ Mexico strain.

Age group, variable, category	Prevalence (%)	OR (95% CI)[a]	*P*
1–4-year-olds			
Sex			
Male	26/65 (40)	1.00	.43
Female	28/65 (43)	1.35 (0.67–2.69)	
Initial corrected OD value			
<0.20	34/73 (47)	1.00	.04
0.20–0.39	16/35 (46)	1.05 (0.47–2.31)	
0.40–0.99	4/22 (18)	0.25 (0.07–0.90)	
No. of days ate chicken			
0	15/31 (48)	1.00	.026
1	23/41 (56)	1.48 (0.49–4.42)	
2	9/35 (26)	0.34 (0.11–1.07)	
3–14	7/23 (30)	0.51 (0.14–1.79)	
Own or rent land			
No	15/54 (28)	1.00	.013
Yes	39/76 (51)	2.73 (1.17–6.38)	
No. of individuals/bedroom			
<3	4/13 (31)	1.00	.072
3	5/19 (26)	1.61 (0.32–8.23)	
4	7/16 (44)	3.69 (0.83–16.45)	
⩾5	38/82 (46)	4.75 (1.11–20.16)	
Room used for cooking			
Room in house[b]	39/106 (37)	1.00	.061
Lean-to outside	15/24 (63)	3.31 (1.04–10.59)	
Clothing of wife of HH			
Clean	10/28 (36)	1.00	.19
Normal	26/70 (37)	1.22 (0.35–4.26)	
Dirty	18/32 (56)	3.53 (0.84–14.82)	
No. of hand washing activities			
1	33/65 (51)	1.00	.001
⩾2	21/65 (32)	0.18 (0.06–0.57)	
Usual hand washing technique			
Water only	16/44 (36)	1.00	.004
Water plus soap	25/60 (42)	3.01 (0.94–9.67)	
Water plus detergent	13/26 (50)	4.95 (1.26–19.39)	
Dog seen near or in house			
No	4/27 (15)	1.00	.002
Yes	50/103 (49)	7.61 (2.02–28.69)	
5–14-year-olds			
Sex			
Male	35/131 (27)	1.00	.014
Female	48/126 (38)	1.99 (1.18–3.35)	
Initial corrected OD value			
<0.20	24/64 (38)	1.00	.017
0.20–0.39	46/128 (36)	0.91 (0.47–1.73)	
0.40–0.99	13/65 (20)	0.36 (0.16–0.83)	
No. of days ate meat			
0	35/92 (38)	1.00	.045
1	35/109 (32)	0.69 (0.38–1.27)	
2	5/32 (16)	0.25 (0.08–0.77)	
3–14	8/24 (33)	1.07 (0.39–2.95)	
Wife's occupation			
HW only	58/195 (30)	1.00	.034
Involves agriculture[c]	25/62 (40)	2.04 (1.05–3.97)	
Clothing of wife of HH			
Clean or normal	58/198 (29)	1.00	.074
Dirty	25/59 (42)	1.83 (0.93–3.60)	
⩾15-year-olds			
Sex			
Male	13/40 (33)	1.00	.34
Female	17/69 (25)	0.63 (0.26–1.51)	
Initial corrected OD value			
<0.40	19/45 (42)	1.00	.002
0.40–0.69	10/44 (23)	0.35 (0.13–0.94)	
0.70–0.99	1/20 (5)	0.06 (0.01–0.38)	
Wife's occupation			
HW only	23/95 (24)	1.00	.025
Involves agriculture[c]	7/14 (50)	4.79 (1.25–18.38)	
Room used for cooking			
Room in house[b]	23/98 (23)	1.00	.025
Lean-to outside	7/11 (64)	6.81 (1.01–46.12)	
Clothing of wife of HH			
Clean	6/31 (19)	1.00	.098
Normal	17/59 (29)	3.63 (1.10–12.00)	
Dirty	7/19 (37)	3.84 (0.94–15.66)	

NOTE. The tables shows results from 3 separate models based on the age groups 1–4-year-olds, 5–14-year-olds, and ⩾15-year-olds. All *P* values were calculated by use of the likelihood ratio test. CI, confidence interval; HH, head of household; HW, housework; OR, odds ratio.

[a] ORs were adjusted for significant risk factors within the appropriate level of the conceptual framework.

[b] The category "room in house" combines the categories "specific room in house" and "communal or bedroom in house".

[c] The category "involves agricultural work" combines the categories "housework and agricultural work" with "only agricultural work".

serum IgA antibodies to NV-Mx by ELISA using recombinant NV-Mx capsid antigen [18, 36], permit an initial assessment of the role of HuCV in both sporadic and epidemic infection. HuCV is being increasingly associated with sporadic nonbacterial acute gastroenteritis in children and adults [1].

Seroprevalence to NV-Mx increased with age in the present study. NV-Mx was shown to be acquired at an early age (34% seroprevalence among 1–4-year-olds), and, by adulthood, >80% of individuals were seropositive. Not surprisingly, in all age groups, individuals with a low initial baseline corrected

optical density had greater odds of a seroresponse than did individuals with a higher initial baseline optical density. That >40% of the susceptible population seroconverted after 1 year demonstrates the high incidence of community-acquired NV-Mx infection in this setting. These findings confirm results from previous studies that NVs such as Norwalk strain and Mexico strain are acquired in early childhood, with seroprevalence reaching 70%–100% in adulthood, in both developed and developing countries [20, 23–28]. In the present study, we observed a high prevalence of diarrhea—15% among 1–4-year-olds, 5.7% among 5–14-year-olds, and 3.4% among adults (authors' unpublished data). Although we do not know the proportion of NV diarrhea in this population, it is likely to be an important cause, since children <5 years of age had an annual incidence of seroresponse of 40%. Cohort studies done in underprivileged communities in Mexico City showed an annual seroresponse to rotavirus of 70%, with one-third of cases associated with diarrhea [40]. Similarly, of all calicivirus infections detected, one-third were associated with diarrhea [41].

Agricultural work or tending to livestock by the wife of the HH was associated with increased odds of a seroresponse among 5–14-year-olds and adults. Possession or use of land for agricultural purposes by the family was associated with increased odds of a seroresponse among 1–4-year-olds. These results suggest that contact with either livestock or the land has a role to play in transmission of NV-Mx. Interestingly, the presence of a dog near or inside the house was also a significant risk factor for a seroresponse to NV-Mx among 1–4-year-olds. Although there are no studies demonstrating zoonotic transmission of caliciviruses, several authors have proposed that zoonotic reservoirs may exist [4–7]. An outbreak of gastroenteritis among staff and residents of a nursing home for the elderly in the United States began 24 h after the owner's dog had been sick. Serological evidence suggested that the calicivirus isolated from 1 of the cases may be capable of infecting dogs, as well as humans [42]. Van de Poel et al. raised the possibility of an animal reservoir for HuCV [7]. Enteric caliciviruses have also been detected in calves and dogs [5, 43–45]. Although canine calicivirus shares distinguishing amino acid motifs found in the polymerase and capsid genes of HuCV, it is clearly distinct from HuCV, and no isolation of canine calicivirus in human has been reported [46].

Personal and domestic hygiene were important risk factors in the present study, despite a high proportion of households having access to piped drinking water. Hand washing, the room where cooking was performed, and the cleanliness of the clothing of the wife of the HH were all found to be significant risk factors. Cleanliness of the clothes of the wife of the HH was a risk factor for all ages, with significantly higher odds of a seroresponse associated with dirty clothes. Some daily hand washing was reported by all individuals, with the most common activity being washing hands or having their hands washed before eating food. Reporting an additional hand washing activity was protective for 1–4-year-olds, with a >80% reduction in the odds of a seroresponse to NV-Mx. Interestingly, the most frequent additional activity reported was hand washing after defecating. When hand washing occurs may be more important than whether soap is used. The results are comparable with a recent meta-analysis of studies relating hand washing to the risk of infectious intestinal and diarrheal disease, in which hand washing was associated with a reduction of 42%–47% in diarrheal disease [47]. Cooking in a covered area outside the house, as opposed to within the house, was associated with a significant increase in odds of a seroresponse among 1–4-year-olds and adults. The interpretation of this result is not simple; cooking outside the house may be associated with lower standards of hygiene or may be a general indicator of lower socioeconomic status.

These results coincide with previous findings from outbreaks, suggesting that improving hygiene behaviors, such as hand washing, could be an important intervention to reduce the transmission of NV-Mx. Hand hygiene was promoted after a hospital outbreak of gastroenteritis [48]; however, although environmental samples were analyzed by use of reverse-transcription polymerase chain reaction (30% positive), investigators did not measure hand contamination. A study using cauliflower mosaic virus DNA as a marker of transmission of pathogens traced transmission through a children's home, after the DNA marker was placed on sensitized objects [49]. Hand contact with contaminated areas was identified as the major factor leading to spread of the marker. Hand washing and surface cleaning were found to decrease the spread of the marker. The source of an outbreak of gastroenteritis after a wedding was traced to a kitchen assistant who, on the eve of the reception, had vomited in a sink used for preparing vegetables [50]. The sink was disinfected with chlorine and the next day was used to prepare a potato salad, which was identified as the vehicle of infection among the guests. Several studies have demonstrated that environmental contamination with HuCVs exists both during and after outbreaks [47, 51, 52]. Curtis et al. [53] suggested that primary barriers to transmission of pathogens can be more important than secondary barriers, such as hand washing. In the present study, a slight interaction between hand washing, land ownership, and location of the kitchen may be indicative of the burden of pathogen contamination in the environment. Less contact with the land or cooking in a room within the house reduced the importance of hand washing as a barrier to transmission of NV-Mx. However, when land was owned by the individual's family or when cooking was performed in a lean-to outside the main house, hand washing, as a secondary barrier to transmission, was associated with a reduction in odds of a seroresponse. Consumption of meat, included as a proxy measure of socioeconomic status, was asso-

ciated with a reduction in the odds of a seroresponse to NV-Mx antibodies, suggesting that, in this population, higher socioeconomic status may be synonymous with improved hygiene.

Crowded living conditions were a risk factor, with the odds of seroconversion increasing among 1–4-year-olds living in overcrowded conditions. Although it is not possible to determine whether crowding was indicative of person-to-person transmission via the fecal-oral route or aerosol transmission or both, this result supports findings from studies of outbreaks indicating that a close proximity with others increases the risk of transmission of NV. An NV outbreak among 2 opposing football teams indicated person-to-person contact [54]. The teams had not shared food or drinks and had no contact off the playing field. The only common denominator was that they had played together. Food could not be implicated in an outbreak of gastroenteritis following a meal in a hotel [55]; however, the closer guests were sitting to a guest who had vomited during the wedding the greater their risk of illness. No one eating in a separate restaurant in the hotel reported illness. Chadwick et al. also suggested aerosol transmission during a hospital outbreak [56].

Consumption of uncooked vegetables was not associated with an increase in the odds of a seroresponse to NV-Mx antibodies, whereas a study conducted in Chile [20] identified consumption of uncooked vegetables as a risk factor for seropositivity. However, in Santiago, 1 of the 2 cities used in the Chilean study, there was extensive use of untreated sewage to irrigate crops, whereas the Mexican population studied here used borehole water to irrigate vegetables.

The present study has a number of strengths. This is one of the first studies to measure seroresponse to NV antibodies in a community setting. It is also the first study to measure risk factors associated with sporadic HuCV infection and to allow for potential confounding factors in the associations explored. There are also a number of limitations to this study. First, seroresponse was not related to disease. This would have required measurement of incidence of diarrheal disease and identification of pathogens. The study population was very dispersed and a longitudinal design with weekly visits (to enable 7-day recall) was outside the scope of the study. Some data on diarrheal disease were collected, and prevalence of diarrheal disease with 7-day recall was calculated. Second, a larger sample size would have given the study more statistical power but was not possible, because of financial constraints. Third, some variables, particularly hand washing variables, may have been affected by recall bias. The use of soap was associated with increased odds of a seroresponse. People can overreport "good" behaviors, rather than reporting what they actually do in practice. Although the question "When do you wash your hands during the day?" was designed to reduce the possibility of recall bias, the question asking how hands were washed may have been leading. Some research suggests that the use of soap may be not as important as the action of rubbing hands together while washing [57]. Information bias should also be considered, because of possible changes over time in some variables. The possibility of bias was minimized by including a second visit to households and collecting information for variables that, it was considered, could change over time and were of particular interest.

The risk factors for sporadic NV-Mx infection identified in the present study suggest several possible routes of transmission for NV-Mx, although a causal relationship between each risk factor and seroresponse remains to be established. Transmission of sporadic NV was associated with personal and domestic hygiene, agricultural work, and a possible zoonotic reservoir (i.e., dogs). Data indicate some similarities to risk factors associated with outbreaks of NV infection. The overall incidence of seroresponse to NV-Mx observed in the present study was high. Further study of the risk factors and routes of transmission for HuCVs is essential if measures are to be taken to reduce the occurrence of sporadic infection and decrease the importance of HuCV infection in sporadic nonbacterial gastroenteritis among children and adults, in both developed and developing countries.

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