Epidemiologic and Clinical Features of Other Enteric Viruses Associated with Acute Gastroenteritis in American Indian Infants

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Objective To investigate the viral etiology, through the use of molecular methods, of acute gastroenteritis (AGE), which is a considerable public health burden in Native American infants.

Study design From March 2002 through February 2004, AGE and non-diarrheal stools were collected from Navajo and White Mountain Apache infants who received placebo during a rotavirus vaccine trial. Case (n = 247) and control (n = 344) specimens were tested for enteric adenovirus, astrovirus, norovirus, rotavirus, and sapovirus with real-time polymerase chain reaction. The odds of AGE were compared with population-averaged logistic regression models.

Results In 65% of the cases of AGE (161/247), at least one virus was detected; norovirus (n = 80, 32%) and rotavirus (n = 70, 28%) were the most common. A virus was detected in 38% of control specimens (132/344). Detection of "any virus" was associated with AGE (OR = 3.22; 95% CI, 2.11-4.91), as was detection of norovirus (OR = 2.00; 95% CI, 1.22-3.26) and rotavirus (OR = 2.69; 95% CI, 1.52-4.79).

Conclusion This study highlights the significant burden of viral AGE in American Indian infants and identifies pathogen targets for future prevention efforts in this population. *(J Pediatr 2012;161:110-5)*.

Acute gastroenteritis (AGE) causes approximately 1.76 million deaths each year and is one of the leading causes of death in children <5 years of age in the developing world.[1] In developed countries, AGE is rarely fatal, but remains a frequent reason for hospitalization and outpatient visits.[2] From 2000 to 2003, an annual average of 130 000 to 150 000 AGE hospitalizations were estimated to have occurred in children <5 years of age in the United States.[3]

Historically, American Indian (AI) and Alaska Native (AN) children had disproportionately high rates of AGE,[4] particularly compared with those in the general US population (262.6 and 154.7 diarrhea-associated hospitalizations per 10 000 infants, respectively).[5] These trends suggest that AI/AN children may be exposed to infectious enteric pathogens earlier in infancy, may be exposed to higher number or concentration of enteric pathogens, may be more susceptible to infection by enteric pathogens, or may develop more severe symptoms resulting in hospitalization given equal infection rates.

Rates of AGE in AI/AN children living in the southwestern United States ranked as some of the highest compared with rates in AI/AN in other areas of the United States.[5,6] Two of the largest tribes in the southwestern United States are the Navajo and Apache tribes. In studies of AGE etiology conducted before 1985 in Navajo and White Mountain Apache children, the pathogens most commonly associated with diarrhea were the same as those in populations from the developing world.[7-9] Since then, there have been substantial improvements in sanitation that could alter the distribution of pathogens commonly associated with AGE. In addition, advances in laboratory diagnostics in the past decade have markedly improved the ability to detect viral pathogens that cause AGE.[10-15]

To provide an updated understanding of AGE etiology in the Navajo and White Mountain Apache infant populations and in other resource-poor settings, we have used state-of-the-art molecular methods to detect enteric viruses causing AGE in placebo recipients during an efficacy trial of oral pentavalent rotavirus vaccine (PRV).[16]

Methods

The oral PRV, RotaTeq (Merck & Co, Whitehouse Station, New Jersey), was licensed in the United States in 2006 after the phase-III, double-blind, placebo-controlled, randomized multicenter efficacy trial conducted in 11 countries.[16] Two US-based sites that participated in the trial were the Navajo and Fort Apache

AGE	Acute gastroenteritis
AI	American Indian
AN	Alaska native
PRV	Pentavalent rotavirus vaccine
RT-PCR	Reverse transcriptase-polymerase chain reaction

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Funded by Merck & Co. and Centers for Disease Control and Prevention. M.S. has served as a consultant for GlaxoSmithKline and Merck & Co. J.W., R.R., M.S., K.B., L.G., and R.W. received grant funding from Merck & Co for conduct of the original rotavirus vaccine trial. The findings and conclusions in this paper are those of the authors and do not necessarily reflect the views of the Indian Health Service or the Centers for Disease Control and Prevention. The other authors declare no conflicts of interest.

 10.1016/j.jpeds.2011.12.046

Reservations, which are home to the Navajo and White Mountain Apache tribes, respectively. Children were enrolled between March 2002 and October 2003. Follow-up concluded at the end of February 2004. The trial methodology has been described previously.[16,17]

We performed a case-control study of AGE etiology in Navajo and White Mountain Apache children that was retrospectively nested in the PRV trial. Both case and control subjects were drawn from the placebo group only. Case subjects were defined as children who experienced an AGE characterized by ≥3 watery or looser-than-normal stools within a 24-hour period, forceful vomiting, or both. For analytic purposes, an AGE was classified as having ended when a minimum set of symptomatology criteria were not met for 3 consecutive days. Control subjects were defined as healthy children who had a stool specimen obtained at one of 3 vaccination visits, which was part of the vaccine trial protocol. To be eligible for the case-control analysis, a child's clinical symptom data for determining AGE status had to be matched to a stool specimen and the AGE had to occur in a child ≤9 months of age.

Parents or caregivers of children who were enrolled in the vaccine trial provided written informed consent. Institutional review boards at Johns Hopkins Bloomberg School of Public Health, Centers for Disease Control and Prevention, the Navajo Nation, and the Phoenix Area Indian Health Service approved this study, as did the tribes.

Viral nucleic acid was extracted from 10% stool suspensions that were made by adding 0.1 grams or 100 μL of the fecal sample to 900 μL of sterile phosphate-buffered saline. Stool suspensions were homogenized with vortexing and clarified with centrifugation for 5 minutes at 5000 × g. Clarified supernatant was extracted according to the MagMAX-96 Viral RNA Isolation KIT protocol (Ambion, Austin, Texas) on the KingFisher instrument (Thermo Scientific, Vantaa, Finland).

Viral nucleic acid was tested for astrovirus, group F adenovirus, norovirus, group A rotavirus, and sapovirus with monoplex TaqMan real-time reverse-transcription polymerase chain reaction (RT-PCR; **Table I**; available at www.jpeds.com).[18-25] All assays were run on a 7500 real-time RT-PCR platform (Applied Biosystems, Foster City, California). A cycle threshold value <40 indicated the specimen was positive. The stool samples were not tested with culture or molecular laboratory assays for enteric bacteria or parasites.

Statistical Analyses

Only specimens from children ≤9 months of age were included in analyses because of the small number of control specimens collected from older children. Because the same child could experience multiple AGE throughout the follow-up period, a child could be classified as: (1) a "case" more than one time; (2) a "control" after first being a case; and (3) a "case" after first being a control. However, for a case to become a control, at least 14 days had to separate symptom onset of an AGE and collection of the control specimen. We defined 3 gastroenteritis seasons on the basis of periods that we expected would have different distributions of AGE etiology and incidence (September 2002-March 2003, April 2003-August 2003, and September 2003-February 2004).

Population-averaged logistic regression models were used to measure the association of AGE with detection of a virus, while adjusting for confounding variables and accounting for dependence in AGEs that occurred in the same child by assuming an equal-correlation structure. A regression model was built for each virus, in which the virus co-variate served as the primary exposure of interest in the model. The odds of AGE was modeled for infections when only one virus was detected in a stool specimen. Confounders and effect modifiers were identified in univariate and stratified analyses. ORs were adjusted for age and season of specimen collection, tribal affiliation, and ever-breastfed status. For the denominator used in the calculation of AGE incidence, each study participant contributed time between the date of the first vaccine dose and the date of censoring.

AGE severity was assessed with the 24-point Clark clinical scoring system, in which scores of 2 to 8, 9 to 16, and 17 to 24 were associated with mild, moderate, and severe disease, respectively.[26] Median values associated with each clinical characteristic of severity were compared with the Mann-Whitney *U* test for pair-wise comparisons. The proportions were compared with a two-sample test. Because only approximately 10% of children were a case more than one time, these analyses did not use methods to account for dependence in episodes occurring in the same child.

Statistical significance was achieved with a P value ≤.05, unless otherwise indicated, for adjustment of multiple pair-wise comparisons with the Bonferroni correction. Statistical analyses were completed with STATA software version 10.0 (StataCorp, College Station, Texas).[27]

Results

A total of 1008 Navajo and White Mountain Apache infants were enrolled in the PRV trial. Of these infants, 512 were randomized to receive the rotavirus vaccine and 496 a placebo. Of infants in the placebo group, 429 (86%) remained enrolled in the trial for an average of 11.9 months. The crude incidence of gastroenteritis in placebo recipients was 183 episodes per 100 child-years (95% CI, 170.9-196.1). A total of 832 AGE episodes occurred in placebo recipients during the 2-year trial period, 247 of which (30%) could be matched to a stool specimen for inclusion in the case-control study. In AGEs included (n = 247) and excluded (n = 585) from the case-control analysis on the basis of specimen availability, the distribution of age in months at AGE (mean = 5.6 months [included] versus 7.75 months [excluded]; $P < .001$), and ever breastfed status (60.2% [included] versus 49.4% [excluded]; $P = .0041$) were significantly different.

Clinical and Epidemiologic Results

Two hundred ninety-four infants ≤9 months of age contributed to the case-control analysis. Of these children, there were 247 AGEs with evaluable stool specimens ("cases") and 344 control specimens. Of the 66 control subjects who

previously were case subjects, 17 (26%) had the same virus detected during the earlier case episode. An average of 30 days separated detection of the virus in the case specimen and subsequently in the control specimen (IQR, 18-86 days). Characteristics of case and control subjects were compared for age, season, tribe, sex, and ever breastfed status (Table II). Overall, norovirus was detected most frequently (n = 150, 25%), followed by rotavirus (n = 115, 19%).

Seasonality was analyzed for each virus detected in an AGE stool specimen (Figure; available at www.jpeds.com). Across follow-up of cases, at least one virus was detected during each month. The proportion of cases with a virus detected during April 2003 to August 2003 was significantly lower compared with the proportion of cases with a virus detected during September 2002 to March 2003 ($P = .004$); however, this proportion did not significantly differ when comparing the September 2002 to March 2003 and September 2003 to February 2004 periods ($P > .15$). Rotavirus infections clustered around the colder months occurring between December 2002 to April 2003 and December 2003 to January 2004, with the highest incidence of rotavirus AGE in January 2003. Norovirus was detected throughout the year and displayed no seasonal trend or difference in detection by age group or tribe. Astrovirus, sapovirus, and enteric adenovirus infections were detected sporadically during the study period.

One hundred sixty-one of the 247 case samples tested positive for at least one of the 5 viruses. A single virus was detected in more than half the case samples (n = 135, 55%) and more than one virus was detected in 11% of case samples (n = 26). In mixed infections, there were two cases with 3 viruses identified, and the remaining were dual infections. Norovirus and either astrovirus or rotavirus were the most commonly detected mixed infections.

In cases with a single virus detected, more than half the AGEs were categorized as "mild" (Table III). An office or clinic visit was the most frequent type of setting in which care was sought, except for norovirus, for which the frequency of office or clinic visits and emergency department visits were the same. Case subjects with AGE caused by rotavirus were significantly more likely to seek care compared with case subjects with AGE caused by norovirus ($P = .004$) and other viruses ($P < .001$).

Table II. Comparison of characteristics for 247 case subjects with 344 control subjects

Characteristic	Cases, n (%)	Controls, n (%)	Percent difference	95% CI for percent difference	P value
Age group					
1-3 months	69 (28)	79 (23)	5	−2 to 12	.17
4-6 months	107 (43)	202 (59)	−16	−23 to −7	.002
7-9 months	71 (28)	63 (18)	10	3 to 17	.003
Season					
Sep 2002-Mar 2003	107 (43)	138 (40)	3	−5 to 11	.44
Apr 2003-Aug 2003	90 (36)	114 (33)	3	−4 to 11	.41
Sep 2003-Feb 2004	50 (20)	92 (27)	−7	−13 to 0.3	.068
Navajo	154 (62)	272 (79)	−17	−24 to −9	<.0001
Male	120 (49)	176 (51)	−2	−11 to 6	.54
Ever breastfed	122 (49)	205 (59)	−10	−18 to −2	.014

Case-Control Analysis

Adjusted ORs for the association between virus detection and AGE were calculated for any virus detection, for enteric adenovirus and sapovirus combined, and separately for the remaining viruses, with the virus co-variate as the primary exposure of interest (Table IV). Any virus, norovirus, and rotavirus detection was significantly associated with approximately a two to three times increased odds of AGE. Infants in the 4- to 6-month age group were one to two times less likely to experience an AGE compared with infants in the 7- to 9-month group; the odds of AGE did not significantly differ between infants 1 to 3 months of age and infants 7 to 9 months of age. Navajo infants had a significantly decreased odds of AGE when any of the viruses were detected compared with White Mountain Apache infants. For an "ever" breastfed infant, there was no significant reduction in odds of AGE compared with a never breastfed infant for any virus.

Discussion

At the time of earlier studies (before 1985) of etiology of AGE in Navajo and White Mountain Apache children, rotavirus, Shigella, enterotoxigenic *Escherichia coli*, *Camylobacter jejuni*, enteric adenovirus, and *Clostridium difficile* were most frequently associated with AGE in White Mountain Apache children, and Shigella was most frequently identified in Navajo children.[7-9] Many enteric pathogens were not adequately tested at that time because they were not known to be common causes of diarrhea (eg, norovirus) or detection methods used had low sensitivity (eg, electron microscopy).

Enhanced detection of enteric viruses has been a common finding in other diarrhea etiology studies that used molecular assay methods.[28] In our study, at least one virus was detected in 65% of case specimens and in 38% of control specimens. Overall, norovirus or rotavirus was associated with a significantly increased (2-3 fold) odds of AGE.

This study demonstrates the contribution of norovirus as an important etiologic agent in a population at high risk for AGE in the United States. In etiology studies in children <5 years of age that have used molecular detection methods such as TaqMan RT-PCR, norovirus detection rates have ranged widely from 2% to 30%.[29] In our study, the percent of norovirus-positive cases exceeded the upper limit of this range (32%, n = 80), emphasizing the significant burden of norovirus in this age group and population. Rotavirus was detected as the second most frequent pathogen; however, after introduction of a highly effective rotavirus vaccine, the incidence of rotavirus-associated AGE has dropped dramatically in vaccinated children.[30]

Infants with norovirus and rotavirus gastroenteritis experienced mild AGE, with median severity scores of 7.0 and 8.5, respectively. Both viruses were associated with similar proportions of mild, moderate, and severe disease. Similar

Table III. Clinical and health care use characteristics associated with gastroenteritis with molecular detection of a single enteric virus from Navajo and White Mountain Apache infants

Clinical characteristics	Any virus (n = 135)		Norovirus (n = 62)		Rotavirus (n = 54)		Other viruses* (n = 19)		Mann-Whitney *U* test *P* value†		
	Median	Range	Median	Range	Median	Range	Median	Range	N/O	N/R	O/R
Duration of AGE, days‡	3	1-29	3	1-12	4	1-29	2	1-11	.30	.58	.10
Duration of diarrhea, days	5	1-30	5	1-15	5	1-30	4.5	2-12	.72	.90	.84
Max stools per 24 hours§	4	0-20	4	0-10	5	0-20	4	0-10	.65	.001	.03
Duration of vomiting, days	2	1-10	2	1-10	2	1-6	1.5	1-4	.45	.67	.62
Max vomiting episodes per 24 hours	1	0-10	2	0-10	1	0-10	0	0-4	.02	.64	.05
Duration of fever, days¶	1	1-3	1	1-3	1	1-3	1	1-1	.47	.96	.46
Highest temperature >100.4°F during episode	101.4	100.5-104.9	101.7	100.6-104.9	101.3	100.5-104.4	100.5	100.5-100.5	.10	.28	.11
Severity score (1-24)	7.5	1-19	7	1-17	8.5	2-19	6	2-13	.25	.08	.02

Severity category	Any virus, n (%)	Norovirus, n (%)	Rotavirus, n (%)	Other viruses, n (%)	z-test *P* value‖ N/O	N/R	O/R
Mild	83 (62)	40 (64)	27 (50)	16 (84)	.10	.11	.009
Moderate	50 (37)	21 (34)	26 (48)	3 (16)	.13	.12	.01
Severe	2 (1)	1 (2)	1 (2)	-	-	-	-

Type of health care visit	Any virus, n (%)	Norovirus, n (%)	Rotavirus, n (%)	Other viruses, n (%)	z-test *P* value** N/O¶	N/R	O/R
Office or clinic	37 (26)	13 (20)	22 (36)	2 (11)	.31	.021	.016
Emergency department	25 (17)	13 (20)	11 (18)	1 (5)	.11	.94	.13
Hospital admission	4 (3)	0	4 (7)	0	-	-	-
Any facility	66 (49)	26 (42)	37 (68)	3 (16)	.04	.004	<.001

N/O, norovirus versus other viruses; *N/R*, norovirus versus rotavirus; *O/R*, other viruses versus rotavirus.
*Other viruses are enteric adenovirus (n = 3), astrovirus (n = 12), and sapovirus (n = 4) combined.
†Pairwise comparison of medians for norovirus, rotavirus, and other viruses with the Mann-Whitney *U* test (Wilcoxon rank sum test). Statistical significance achieved with $P \leq .017$ to account for multiple pairwise comparisons.
‡The duration of an AGE is measured with a minimum set of symptom criteria for stool frequency/duration, vomiting frequency/duration, fever measurement/duration, and behavioral characteristics/duration.
§Max stools are watery and looser-than-normal combined.
¶Fever is >100.4°F, rectal temperature.
‖Comparison of the proportion of gastroenteritis cases with a single virus detected by severity category. Statistical significance achieved with $P \leq .017$ to account for multiple pairwise comparisons.
**Comparison of proportion of gastroenteritis cases with virus detected by type of health care facility accessed. Statistical significance achieved with $P \leq .017$ to account for multiple pairwise comparisons.

vaccine trial-based etiology studies have described norovirus and rotavirus AGE as non-severe (median score of 8 on the 20-point Vesikari severity scale).[11,31] More frequent health-seeking behavior for rotavirus gastroenteritis could reflect the tendency for disease associated with rotavirus to be of greater severity. Continued demonstration of norovirus as a significant contributor to AGE in many studies draws attention to the need for vaccine development.[32]

Table IV. Adjusted odds of AGE by virus detected comparing case subjects with control subjects

Variable	Any virus (N_A = 135, N_B = 104) OR_A†	95% CI OR_A	Norovirus (N_A = 62, N_B = 50) OR_A	95% CI OR_A	Rotavirus (N_A = 54, N_B = 30) OR_A	95% CI OR_A	Astrovirus (N_A = 12, N_B = 17) OR_A	95% CI OR_A	Other viruses* (N_A = 7, N_B = 7) OR_A	95% CI OR_A
Virus	3.22	2.11-4.91	2.00	1.22-3.26	2.69	1.52-4.79	1.07	0.49-2.35	1.99	0.64-6.18
Age group										
1-3 months	1.02	0.54-1.95	0.78	0.43-1.45	0.91	0.51-1.64	0.77	0.43-1.38	0.82	0.45-1.48
4-6 months	0.52	0.31-0.88	0.45	0.27-0.75	0.58	0.36-0.92	0.44	0.28-0.61	0.51	0.32-0.82
7-9 months	1.00‡		1.00		1.00		1.00		1.00	
Season										
Sep 2002-Mar 2003‡	1.00		1.00		1.00		1.00		1.00	
April 2003-Aug 2003	0.79	0.29-0.69	0.62	0.40-0.94	0.76	0.49-1.17	0.64	0.42-0.97	0.61	0.40-0.93
Sep 2003-Feb 2004	0.69	0.46-1.03	0.48	0.29-0.78	0.60	0.36-1.00	0.52	0.32-0.85	0.53	0.33-0.86
Tribe										
Apache‡	1.00		1.00		1.00		1.00		1.00	
Navajo	0.44	0.29-0.69	0.46	0.31-0.69	0.41	0.28-0.62	0.41	0.28-0.61	0.43	0.29-0.64
Ever breastfed	0.69	0.46-1.03	0.69	0.48-1.01	0.71	0.49-1.02	0.68	0.47-0.98	0.67	0.47-0.96

N_A, number of cases; N_B, number of controls with single infections.
*Sapovirus and enteric adenovirus combined.
†Adjusted for age at AGE represented by 3 age categories (1-3, 4-6, 7-9 months), season of infection, tribe and ever breastfed status.
‡Reference group in logistic regression model.

Although most studies on astrovirus, including ours, indicate that <10% of cases had astrovirus detected and that astrovirus-associated AGE is mild on average, other studies have shown higher detection frequencies and more severe disease.[25,33,34] In this study, astrovirus was detected in similar proportions in both case and control specimens and was not associated with an increased odds of AGE. Because nearly all astrovirus infections were co-infections, the clinical manifestations attributable to astrovirus gastroenteritis could not be well characterized.

Both enteric adenovirus and sapovirus were detected in 2% to 5% of gastroenteritis cases. A similar proportion of enteric adenovirus or sapovirus cases was detected in other studies, except in one study in which the prevalence of adenovirus was 25%.[11,12,24] Because enteric adenovirus and sapovirus were not significantly associated with AGE in our population, it is difficult to ascribe a clinical description of AGE to either virus.

Fourteen days were used to separate recurrent AGE and to identify control subjects who could have been case subjects previously. The decision to use this cutoff was based on a review of other studies in which repeated rotavirus episodes were analyzed. In the reviewed studies, the number of days used as the cutoff point ranged from 3 to 30 days or varied in study participants.[16,35-38] In our study, when longer intervals of 21 and 28 days were used to mark inclusion for a former case subject to become a control subject, there was no change in the number of eligible control subjects.

Our study has limitations. Episodes occurring in children between 10 and 12 months of age were not included in our case-control analysis because there were too few control specimens available from this age group.[16] Inclusion of children aged >9 months could increase the percentage of cases associated with any of the viruses described, because other studies have shown a substantial contribution in older children. In particular, rotavirus and norovirus remain important contributors to AGE etiology in children >1 year of age. For analyses that include older children, testing for bacteria such as Salmonella and enterotoxigenic *E coli* species would also be important becasue both have been detected frequently in older children.

Because control specimens were collected at designated times for dosing rather than at a similar time compared with when a case specimen was collected, case and control subjects could not be matched individually by age or season, which could result in residual confounding after adjustment. On the basis of an 80% cutoff point for statistical power, the study is underpowered to detect a significant difference in the proportion of case and control specimens testing positive for a virus for ORs <3.0. Only 30% of the specimens collected during the PRV trial were available for testing with molecular methods. The remaining specimens were not tested because the limited stool volume was completely used for protocol-specified rotavirus testing. Therefore, the tested samples may not be representative of the overall set of specimens collected during the trial.

Compared with studies using diagnostic methods such as electron microscopy or enzyme immunoassay, the proportion of cases with identified etiology can increase with use of molecular techniques. Although 4 to 6 years elapsed between specimen collection and nucleic acid extraction, the detection yield remained high, especially in cases. Multiple freeze-thaw cycles could have decreased viral nucleic acid detection, which could affect viruses differently, leading to an underestimation of some of or all the pathogens. Failure to detect viruses in some case and control specimens could bias the association of AGE and virus detection toward the null. Sensitive molecular methods may prove most valuable for epidemiologic study rather than for clinical diagnosis because these methods may detect virus for which detection is not correlated with AGE. Furthermore, limiting laboratory testing to include only rotavirus and bacterial enteric pathogens, particularly those studies conducted in the Navajo and White Mountain Apache populations, would have failed to establish the etiology for a substantial proportion of AGE cases. With the increased use of rotavirus vaccine in the United States, understanding the etiology of viral gastroenteritis caused by other enteric viruses, especially norovirus, becomes increasingly important. ■

The authors wish to express their deep gratitude to the children and their parents or caregivers from the Navajo and White Mountain Apache tribes who participated in the PRV vaccine trial. We thank the Center for American Indian Health nurses and research program assistants, especially Stevie Cosay for clinical specimen management, for their conduct of the rotavirus vaccine trial. We are indebted to Tara Kerin and Nicole Gregoricus from the Centers for Disease Control and Prevention for assistance and guidance in specimen processing. The authors also would like to thank the following Merck & Co employees for contribution to the understanding of the database, understanding how the analyses in the original PRV clinical trial were performed, and/or critically reviewing the manuscript: Michele L. Coia, Donna M. Hyatt, Michael J. Dallas, and Max Ciarlet.

Submitted for publication Jul 5, 2011; last revision received Nov 3, 2011; accepted Dec 28, 2011.

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Table I. Oligonucleotides used for detection of enteric viruses with real-time reverse transcriptase polymerase chain reaction

Virus	Gene target	Primer/probe	Sequence (5′-3′)*	Polarity	Positions (nt)	Reference
Group F adenovirus	Fiber	JTVFF	AAC TTT CTC TCT TAA TAG ACG CC	(+)	619-641	18,19
		JTVFR	AGG GGG CTA GAA AAC AAA A	(−)	736-718	
		JTVFAP	FAM-CGA AGA GTG CCC GTG TCA GC-BHQ	(+)	671-652	
Astrovirus	ORF1b	AsFF	GGC CAG ACT CAC AGA AGA GCA	(+)	4269-4289	20
		AsFr	GTC CTG TGA CAC CTT GTT TCC TGA	(−)	4533-4556	
		AstZFb	HEX-CCA TCG CAT TTG GAG GGG AGG ACC AGC GA-BHQ	(+)	4296-4321	
Norovirus GI	ORF1-ORF2 junction	Cog1F	CGY TGG ATG CGI TTY CAT GA	(+)	5291†	21,22
		Cog1R	CTT AGA CGC CAT CAT CAT TYA C	(−)	5375†	
		Ring1C	FAM-AGA TYG CGI TCI CCT GTC CA-BHQ		(+)†	
Norovirus GII	ORF1-ORF2 junction	Cog2F	CAR GAR BCN ATG TTY AGR TGG ATG AG	(+)	5003‡	22,23
		Cog2R	TCG ACG CCA TCT TCA TTC ACA	(−)	5100‡	
		Ring2	FAM-TGG GAG GGC GAT CGC AAT CT-BHQ	(+)	5048‡	
Rotavirus	NSP3	NVP3-FDeg	ACC ATC TWaC ACR TRbA CCC TC	(+)	963-982§	24
		NVP3-R1	GGT CAC ATA ACG CCC CTA TA	(−)	1034-1053§	
		NVP3-Probe	HEX-ATG AGC ACA ATA GTT¶ AAA AGC TAA CAC TGT CAA	(+)	984-1026‡	
Sapovirus	ORF1	SaV124F	GAY CAS GCT CTC GCY ACC TAC	(+)	5078-5098**	
		SaV1F	TTG GCC CTC GCC ACC TAC	(+)	700-717††	
		SaV5F	TTT GAA CAA GCT GTG GCA TGC TAC	(+)	5112-5135††	
		SaV1245R	CCC TCC ATY TCA AAC ACT A	(−)	5163-5181**	
		SaV124TP	FAM-CCR CCT ATR AAC CA-[MGB-NQF]	(−)	5105-5118**	
		SaV5TP	FAM-TGC CAC CAA TGT ACC A-[MGB-NQF]	(−)	5142-5157‡‡	

FAM, 6-carboxyfluorenscein; *HEX*, hexachlorofluorenscein phsophramidite; *BHQ*, Black Hole Quencher; *MGB-NQF*, minor groove binding-nonfluorescent quencher.
*International Union of Pure and Applied Chemistry codes: W = A or T; R = A or G; Y = C or T; I = inosine; B = C or G or T; N = any base.
†Positions correspond to Norovirus GI Norwalk/68 strain (M87661).
‡Position corresponds to Norovirus GII strain Camberwell virus (AF145896).
§Position corresponds to Group A Rotavirus strain ST3 virus (X81436).
¶Nucleotide labeled with BHQ1 quenching dye.
**Positions correspond to Sapovirus GII Mc 10 strain (AY237420).
††Positions correspond to Sapovirus GI Parkville strain (U73124).
‡‡Positions correspond to Sapovirus GV NK24 strain (AY646856).

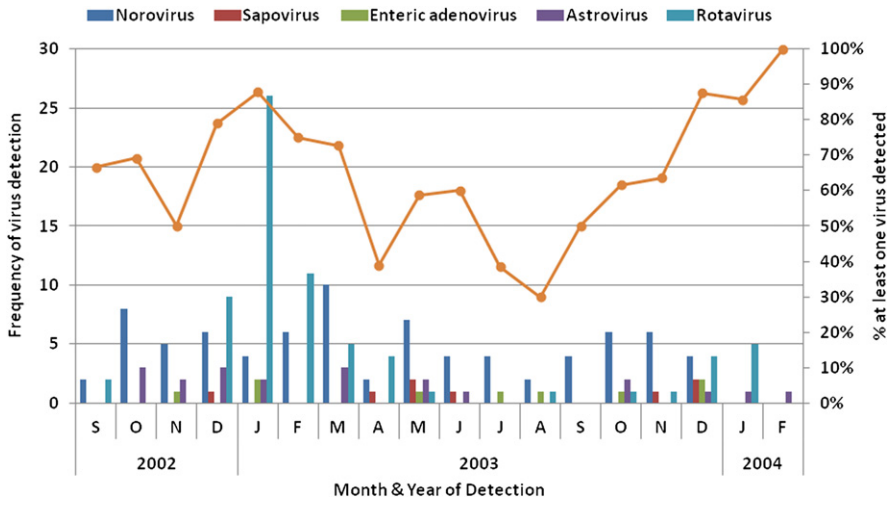

Figure. Seasonality of enteric viruses associated with AGE in the Southwest United States in Navajo and White Mountain Apache infants.