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# The initial timing and burden of viral gastrointestinal infections in Australian infants: a birth cohort study

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**Authors' roles in the submitted work**:

GM: contributed to the design and conduct of the study, performed the analyses, prepared the draft version of the manuscript, and coordinated the preparation of the final manuscript.

KG: contributed to conception and design of the study, helped to interpret the data, assisted in critically revising the manuscript, and approved its final version.

SBL: contributed to conception and design of the study, helped to interpret the data, assisted in critically revising the manuscript, and approved its final version.

RSW: contributed to conception and design of the study, helped to interpret the data, assisted in critically revising the manuscript, and approved its final version.

All authors are accountable for all aspects of the work, including ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.


## ABSTRACT

The timing and nature of initial infections by potentially vaccine-preventable gastrointestinal viruses (group-F adenoviruses, classic human astrovirus, norovirus I/II, and sapovirus I/II/IV/V) was investigated in a community-based birth cohort. Weekly stool samples were collected from 158 children aged <2 years in an Australian subtropical city. Median age at initial infection was lowest for norovirus II (13.8 months) followed by sapovirus (14.3 months) and classic human astrovirus (17.6 months), and was >24 months for the remaining viruses. Norovirus II and sapovirus were most often associated with acute gastroenteritis symptoms (57% and 44%, respectively). Overall, healthcare was sought for 45% of symptomatic initial infections, which varied between 17% for norovirus I to 55% for norovirus II. Age at initial infection was lower when participants were exposed to other children. Norovirus II and sapovirus were the most important pathogens in this cohort, providing further evidence for them being priority targets for vaccine development.



**Key words**: acute gastroenteritis; infection; virus; children; ORChID birth cohort.


**What is known**

- Despite rotavirus vaccines, acute gastroenteritis remains common in high-income countries.
- Several new vaccines for enteric viruses and bacteria are currently under development.
- The timing of initial infections is an important factor in determining the age at which new vaccines are introduced.

**What is new**

- Most healthy children by their second birthday have had an infection by at least one of the following viruses (group-F adenoviruses, classic human astrovirus, norovirus I/II, and sapovirus I/II/IV/V), with infections most common after 6 months of age.
- Overall, 37% of initial infections were associated with symptoms of acute gastroenteritis and of these symptomatic episodes, 45% resulted in healthcare attendance.
- Norovirus II and sapovirus predominated in this age group, each affecting >75% of healthy children.

## INTRODUCTION

Acute gastroenteritis (AGE) is a common cause of childhood morbidity in high-income countries, including the United States, the United Kingdom, Western European nations, and Australia, despite rotavirus vaccines being part of their national immunization programs (1-3). The gastrointestinal viruses now replacing rotavirus as causing the greatest disease and healthcare burden in children from these countries are the human caliciviruses – noroviruses (NoV) and sapoviruses – and then classic human astroviruses (AstV) and group-F adenoviruses (AdV 40/41) (3, 4). These viruses affect all age groups, but are most frequent in the very young and are high-priority targets for public health-initiated preventive strategies, including vaccination (5).

The age at which new vaccines are introduced in children requires careful consideration of several factors, including the onset and peak age of natural disease caused by the pathogen, the potential for adverse events, the age at which protective immune responses can be induced, and local factors such as region-specific epidemiology and timing of routine childhood healthcare visits (6). Birth cohort studies provide an ideal opportunity to study the natural history of infection within the community, including the timing and severity of initial infection episodes. Here we describe the timing, healthcare burden, and risk factors associated with initial infections by the aforementioned potentially vaccine-preventable gastrointestinal viruses occurring before 2 years of age in a cohort of children born in an Australian subtropical city.

## METHODS

The Observational Research in Childhood Infectious Diseases (ORChID) study was a community-based birth cohort (7). Women attending antenatal clinics at two hospitals in Brisbane, Australia, between September 2010 and October 2012 were recruited, and their healthy, term infants enrolled at birth and followed until their second birthday. Socio-demographic and birth characteristics were recorded at enrolment. Telephone interviews were conducted 3 monthly to collect data on feeding and childcare attendance. Rotavirus vaccination details were collected from the Australian Childhood Immunisation Register. Parents provided their informed consent. The Children's Health Queensland, Royal Brisbane and Women's Hospital, and The University of Queensland Human Research Ethics Committees approved the study. The ORChID study was registered on clinicaltrials.gov (NCT01304914).

Parents collected stool swab samples from diapers weekly, which were mailed to the laboratory. Swabs were batch-tested employing validated real-time polymerase chain reaction (PCR) and reverse-transcriptase PCR assays for AdV type 40/41, AstV, NoV genogroups I and II, and SaV genogroups I/II/IV/V (SaV) (Supplementary Methods and Supplementary, http://links.lww.com/MPG/C532 and Supplementary Table 1, http://links.lww.com/MPG/C532) (7). Positive detection was a cycle threshold (Ct) value ≤40. Virus shedding continued until the

last positive swab for that virus, followed by 2 consecutive negative swabs, and was calculated as the number of weeks from the first to last positive swabs plus 1 week. Ct values from real-time PCR for virus-positive samples are inversely proportional to the amplified nucleic acid in the sample and were used as semi-quantitative markers of viral load (7).

Parents maintained a daily symptom diary, which included fever, the number of loose stools, and vomiting. Fever (defined as >37.5°C using an underarm digital thermometer) was recorded by the parent as yes/no as well as the maximum daily body temperature. Diarrhea was defined as ≥3 loose (or looser than normal) stools within 24 hours. Acute diarrheal/vomiting episodes consisted of ≥1 day of diarrhea/vomiting, separated by ≥3 days without loose stools/vomiting. Virus infections were categorized as symptomatic, and consequently associated with an AGE, if the positive sample was collected either within 7 days before or 7 days after the onset of a diarrheal or vomiting episode. The Modified Vesikari Score (MVS) of gastroenteritis severity was calculated for each symptomatic detection (Supplementary Table 2, http://links.lww.com/MPG/C532) (8). Parents recorded family physician consultations, emergency department presentations, and hospital admissions in an illness-burden diary. Healthcare use that occurred during the associated AGE illness was counted.

Children were included in analyses until their second birthday or until they failed to return stool samples for >30 days, whichever was sooner. Percentages of exclusive and mixed breastfeeding, childcare, and number infected at pre-specified ages were calculated from life tables. Ct values of asymptomatic and symptomatic first infections were compared using *t*-tests. Associations between personal/family characteristics and risk of initial infections were calculated using multivariable Cox regression models. Stata v16 (Stata Corporation, College Station, TX, USA) was used. Statistical significance was declared at $p<0.05$.

**RESULTS**

One-hundred and fifty-eight children (83 females) provided 11,124 stool samples prior to their second birthday, with 1,445 excluded as they were returned after a >30-day interval, leaving 9,679 samples for analysis. Symptom diaries returned from 153 children provided 71,385 child-days of observation (Supplementary Figure 1, http://links.lww.com/MPG/C532). Most participants were first-born children (103/158, 65%), with university-educated mothers (99/158, 63%), and from households in the top quartile for income (86/155, 55%). Food other than breastmilk was introduced to 55% of children by 3 months of age and breastfeeding ceased for 62% of children by 12 months of age (Supplementary Figure 2, http://links.lww.com/MPG/C532). Childcare attendance most often began between 12 and <18 months of age. Overall, 94% (123/131) of children remaining in the cohort had received 3 doses of the rotavirus vaccine, RotaTeq® (Merck and Co., Whitehouse Station, USA), by 32 weeks of age (Supplementary Table 3, http://links.lww.com/MPG/C532).

One-hundred and five children had at least one of the 5 viruses during the ORChID study: 79% for SaV, 78% for NoV II, 68% for AstV, 48% for AdV 40/41, and 36% for NoV I (Figure). Of the 293 initial infections observed, 287 (98%) were mono-infections. Median age at initial infection was lowest for NoV II (13.8 months; inter-quartile range [IQR] 9.8–23.5), followed by SaV (14.3 months; IQR 10.0–21.9), and AstV (17.6 months; IQR 10.5–>24), while it was >24 months for AdV 40/41 and NoV I (Supplementary Table 4, http://links.lww.com/MPG/C532). Virus shedding continued after the first detection for a median 1.9–2.7 weeks (Supplementary Table 5, http://links.lww.com/MPG/C532). Where symptom diary data were available (263/293 [90%] first infections), infections were associated with an AGE in 40/70 (57%) NoV II cases, 31/70 (44%) SaV cases, 6/25 (24%) NoV I cases, 13/60 (22%) AstV cases, and 8/43 (19%) AdV 40/41 cases (Supplementary Table 4, http://links.lww.com/MPG/C532). The Ct values of first detections were lower for symptomatic than asymptomatic first infections (mean differences of 1.9–12.2 cycles), reaching statistical significance for AdV 40/41, NoV II, and SaV (Supplementary Figure 3, http://links.lww.com/MPG/C532 and Supplementary Table 6, http://links.lww.com/MPG/C532). When the infection was symptomatic, the median MVS ranged from 2–7, indicating overall mild illness. Symptomatic illnesses lasted a median 1–4.5 days. Ct values were not associated with either the length of symptomatic illness or the MVS (Supplementary Figure 4, http://links.lww.com/MPG/C532). For symptomatic initial infections, healthcare was sought in 42/94 (45%) cases, specifically 22/40 (55%) for NoV II, 4/8 (50%) for AdV 40/41, 6/13 (46%) for AstV, 13/31 (42%) for SaV, and 1/6 (17%) for NoV I. No hospital admissions occurred with initial symptomatic viral infections (Supplementary Table 7, http://links.lww.com/MPG/C532).

Initial infections were 2–5 times more likely in children born during winter (AdV 40/41), for those with another child at home (NoV I), attending formal childcare (AstV, NoV I, NoV II, and SaV), or during the fall (SaV) or winter (AstV) seasons (Supplementary Table 8, http://links.lww.com/MPG/C532).

## DISCUSSION

Potentially vaccine-preventable viral gastrointestinal infections were detected in most ORChID study participants. Of the 5 viruses studied, NoV II appeared to be the most important. It was detected earliest at a median age of 13.8 months, affected 78% of children by their second birthday, with 57% of initial infections associated with AGE symptoms, and 55% of these resulting in a healthcare visit. SaV infected the highest number (79%) of children, was also detected early at 14.3 months, but fewer (44%) initial infections were associated with an AGE episode and required a healthcare visit (42%). These symptomatic infections were associated with lower Ct values (i.e., higher viral loads) than observed with asymptomatic episodes. The remaining 3 viruses were detected later and were less likely to be symptomatic. In contrast, and as reported previously, only 19% of children in the ORChID cohort had wild-type rotavirus

detected, all of whom except one aged 3 weeks had received the rotavirus vaccine, and just 26% developed AGE symptoms (9).

The viruses examined here caused fewer infections in the first 6 months of life, possibly due to a combination of factors, including maternally-derived transplacental and breastmilk antibodies, and relatively lower exposure to other people from outside the household (10, 11). In this cohort, however, the suspected protective effect of breastfeeding was not detectable with regression analyses. Beyond the first 6 months of life, the rate of initial infections by these viruses increased, and by 2 years of age approximately three-quarters of children had encountered NoV II and SaV, two-thirds had AstV, one-half had AdV 40/41, and one-third had their initial NoV I detections. Despite the generally held view that first exposures to infectious agents may result in more severe illness, the proportions of symptomatic infections ranged from just 19–57%. Healthcare-seeking behavior for these AGE episodes varied from 17–55%. Ct values are a proxy measure of viral load, and symptomatic infections had lower Ct values (higher viral load) than asymptomatic infections. Although these results can be influenced by individual assay performance characteristics and stool fluid composition (12), such large differences in Ct values are likely to be real. In line with previous evidence, our investigation of the risk factors that accelerated the initial acquisition of viruses revealed a positive association with exposure to other children, whether at home or at childcare (13).

Our study is the first to report the timing of initial infections for AdV 40/41, AstV, and SaV, and the first in a high-income setting. Our results benefited from good participant retention and protocol adherence, although the overall sample size was too low for more in-depth analyses, such as Ct values from samples collected before/after the onset of symptoms, risk factors of symptomatic initial infections, and healthcare use. Symptomatic co-detections were attributed to both viruses. The choice of viruses in this study was based upon their inclusion in multiplex real time PCR panels used by Diagnostic Laboratories and their potential as future candidate vaccine candidates, although only NoV vaccines are at an advanced development stage (5, 14). Our study was not designed to establish the source of an initial infection. Generalizability of our results may be limited to similar household income, educational, and climatic settings.

The timing of initial infections is a unique measure, can only be examined with birth cohorts, and not comparable to common measures used in epidemiology, such as prevalence or incidence. The paucity of information on this topic is masked by the ‘rate of infection’, ‘peak age of infection’, or ‘prevalence’, which are often reported in the literature (15-17), but they are materially different to the age at initial infection, as they include subsequent infections in the same child. Moreover, as most studies collect stool samples only from symptomatic children, estimates drawn from such data cannot be broadly generalizable due to the infections frequently being asymptomatic. This is important as those with subclinical infections can still transmit these viruses to others and could potentially act as silent reservoirs of infection. Also, due to differences in disease epidemiology and population characteristics between settings, the

introduction of public health initiatives is preferably informed by local evidence. Nevertheless, our results of the timing of initial NoV infections are comparable with the findings of two Peruvian birth cohorts (18, 19). To avoid these pitfalls, studies should be community-based, where most AGE is managed (2), and collect samples from both symptomatic and asymptomatic participants, in order to inform the prioritizing and planning of future public health interventions (20), such as reinforcing simple hygiene measures to the necessity and timing of vaccine-delivery. Here, in an urban setting within a high-income country, we provide prospective community-based evidence of young children frequently encountering 5 enteric viruses capable of causing AGE symptoms. Moreover, these viruses individually are now more common than rotaviruses in this highly vaccinated cohort. In particular we found that human caliciviruses, specifically NoV II, and to a lesser extent SaV, are important pathogens causing AGE in children during the first 2 years of life, further supporting these viruses as priority targets for future vaccine development (5, 11).

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**FIGURE LEGEND**

**Figure 1 Time to initial viral gastrointestinal infections in the Observational Research in Childhood Infectious Diseases birth cohort.**