Endemic Non2013SARS-CoV-2 Human Coronaviruses in a Community-Based Australian Birth Cohort

Endemic non-SARS-CoV-2 human coronaviruses in a community-based Australian birth cohort

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Abbreviations: ARI: acute respiratory illness; CI: confidence interval; CoV: coronavirus; Ct: cycle threshold; ED: Emergency Department; ERV-3: Endogenous retrovirus-3; HCoV: human coronavirus; IQR: interquartile range: LRI: lower respiratory illness; MERS-CoV: Middle East respiratory syndrome coronavirus; ORChID: Observational Research in Childhood Infectious Diseases; PCR: polymerase chain reaction; SARS-CoV: severe acute respiratory syndrome coronavirus; URI: upper respiratory illness.

Table of contents summary: One or more of the four endemic (non-SARS-CoV-2) human coronaviruses are detected in most children by age 2-years. Infections can be recurrent and often asymptomatic.

What's known on this subject: Endemic (non-SARS-CoV-2) human coronaviruses are common causes of acute respiratory illness (ARIs). However, few pediatric community-based studies focused on all four species and viral testing was restricted to ARI episodes. Their actual incidence and disease burden may have been under-estimated.

What this study adds: In this community-based birth cohort, almost three-quarters of children had a endemic (non-SARS-CoV-2) human coronavirus infection by age 2-years. Incidence was 0.76 episodes per child-year. Recurrent infections involved the same or different species and half of all episodes were asymptomatic.

ABSTRACT

Background and objectives: The COVID-19 pandemic has drawn attention to the Coronavirus family of viruses. In community settings there is limited information on these viruses in healthy children. Our objective was to explore the epidemiology of the four endemic (non-SARS-CoV-2) human coronaviruses (HCoV) by species, including acute illness episodes, risk factors and healthcare burden in a cohort of Australian children in the first 2-years of life.

Methods: The Observational Research in Childhood Infectious Diseases (ORChID) community-based cohort was a prospective study describing acute respiratory illnesses in children from birth until their second birthday. Parents recorded daily symptoms, maintained an illness-burden diary and collected weekly nasal swabs, which were tested for 17 respiratory viruses, including HCoVs, by real-time polymerase chain reaction assays.

Results: 158 children participating in ORChID provided 11,126 weekly swabs, of which 168 were HCoV positive involving 130 incident episodes. HCoV-NL63 and HCoV-OC43 were most commonly detected and accounted for two-thirds of episodes. While 30 children had different HCoVs detected on different occasions, seven were reinfected with the same species 4–16 months later. The incidence of HCoV in the first 2-years of life was 0.76 episodes per child-year (95% confidence interval [CI] 0.63–0.91) with incidence greatest in the second year (1.06; 95%CI 0.84–1.33) and during Winter (1.32; 95%CI 1.02–1.71). Only 50% of HCoV episodes were symptomatic, of which 48.4% led to healthcare contact.

Conclusions: In children, HCoV infections are common, recurrent and frequently asymptomatic. Future studies should determine transmission pathways and immune mechanisms.

INTRODUCTION

Coronaviruses (CoV) are enveloped, single-stranded RNA viruses. Four endemic human coronaviruses (HCoV) distributed across two genogroups (*alphacoronaviruses* HCoV-229E and HCoV-NL63 and *betacoronaviruses* HCoV-OC43 and HCoV-HKU1) are known to circulate continuously worldwide causing mainly mild upper respiratory symptoms.[1] However, three highly-pathogenic *betacoronaviruses* (severe acute respiratory syndrome coronavirus [SARS-CoV],[2] Middle East respiratory syndrome coronavirus [MERS-CoV][3] and SARS-CoV-2[4]) have emerged this century, each of which caused outbreaks and illnesses ranging from mild or asymptomatic infections through to severe pneumonia with multi-organ failure and death.[2-4] Whilst causing severe disease, the SARS-CoV outbreak in 2002–2004 had limited scope with <9,000 confirmed cases globally.[1] MERS-CoV was first identified in Saudi Arabia in 2012 and has resulted in <3,000 cases globally to date, with <100 human cases annually, almost exclusively on the Arabian Peninsula.[1,3] SARS-CoV-2, which arose in China in late 2019 causes an illness termed COVID-19 and has resulted in a global pandemic.[5] However, with each of these new highly-pathogenic coronaviruses, children have milder symptoms than most adults.[3,6-8] The reasons for this observation are uncertain, but may relate in young children to angiotensin-converting enzyme-2 receptor levels, raised lymphocyte counts and activated innate immunity from frequent viral infections.[8,9] As SARS-CoV-2 is likely to remain circulating, at least until a safe and effective vaccine is implemented globally, insights into its future activity might be found from examining the four endemic (non-SARS-CoV-2) HCoVs in children.

Endemic HCoVs can be isolated from 2–9% of children presenting with an acute respiratory illness (ARI) to hospitals, Emergency Departments (ED) or clinics.[10-18] Such studies however, are limited by their cross-sectional design and lack of suitable controls. They are also likely to be biased towards more severe illness and to underestimate the burden of mild-to-moderate

disease and asymptomatic infections in the community. Seroprevalence studies indicate all four endemic HCoVs are usually encountered by 6-years of age.[19,20] However, outside of childcare centers,[21] there are few community-based studies describing early infections by all four HCoVs in young children.[1,14,22-28] Community studies employing sensitive polymerase chain reaction (PCR) assays have found that HCoVs rank between 2nd and 4th amongst the major respiratory viruses detected in children with ARI[21-24,27,28] and 7th with influenza-like illness presentations.[26] Nevertheless, only one study has collected respiratory samples between ARI episodes,[28] follow-up of infants was often for only 6-months to 1-year or a single respiratory season[22-28] and some studies included older aged cohorts and household occupants.[27,28] Thus, the incidence of HCoV infections in community-dwelling children is likely to have been under-estimated and seasonality and full spectrum of disease incompletely characterized.

An Australian prospective birth cohort, the Observational Research in Childhood Infectious Diseases (ORChID) study, attempted to address the limitations of these community-based studies.[29] It showed by age 2-years, 72% had experienced at least one HCoV infection and the median age at first detection was 18-months.[30,31] The objectives of the current study were to use ORChID data to further explore the epidemiology of endemic HCoVs by species, including ARI episodes, risk factors and healthcare burden for infection in the first 2-years of life.

METHODS

Study design

The ORChID study (clinicaltrials.gov: NCT01304914) progressively recruited healthy term newborn babies without underlying congenital abnormalities or chronic disorders from two metropolitan hospitals in Brisbane, Australia, between September 2010 and October 2012 and followed them until their second birthday.[29-31] Parents provided informed consent for their

child's participation shortly after birth. Children exited the study when we stopped receiving diaries and swabs, or when they had their second birthday. The Children's Health Queensland (HREC/10/QRCH/16), the Royal Brisbane and Women's Hospital (HREC/10/QRBW125) and The University of Queensland (2010000820) Human Research Ethics Committees approved the study.

Study procedures

At recruitment, parents provided their sociodemographic and health details, including information on the pregnancy and delivery. Telephone interviews were conducted every 3-months to learn about breast feeding and childcare attendance. Exclusive breast feeding was the period from birth to introducing solids or formula milk. Childcare was classified as formal, if outside the home by a regulated childcare service, or informal if non-regulated from friends or family. Parents completed a daily tick-box symptom diary, consisting of a set of pre-defined ARI symptoms they had been trained to recognize. Nasal discharge/congestion, dry cough or physician-diagnosed otitis media were categorized as an upper respiratory illness (URI) and lower respiratory illness (LRI) as rattly breathing, wet (moist) cough, shortness of breath, wheeze or physician-diagnosed pneumonia. Parents were also asked to complete an illness-burden diary, which documented healthcare-seeking behavior and antibiotic prescriptions, whenever the child had symptoms of an LRI or both nasal discharge/congestion and cough. To minimize parent inconvenience, we did not ask for illness-burden diary entries for either isolated nasal symptoms or a dry cough. We reasoned that under these circumstances, parents were unlikely to seek healthcare advice. Completed symptom and illness-burden diaries were mailed each month to the research team.

Parents were also taught to collect anterior nasal swabs (Virocult MW950, Medical Wire & Equipment, Wiltshire, England) from birth and then on the same day each week, irrespective of symptoms, until the child's second birthday. These were sent by mail, taking a median

3-days (interquartile range [IQR] 2–4) to reach the laboratory for processing and storage at -80°C. ED and hospital records of ORChID children were reviewed when the study ended.

Laboratory testing

Swabs were batch-tested for 17 respiratory viruses, including the four endemic HCoVs (229E, NL63, OC43, HKU1) using previously validated real-time PCR assays (Supplemental Table 5).[29] Endogenous retrovirus-3 (ERV-3), a marker of human DNA, determined nasal swab quality. Swabs with ERV-3 cycle threshold (Ct) values >38 were deemed to be of lower-quality and excluded from incidence calculations to avoid under-estimating incidence rates.[31,32] The Ct values were used as a semi-quantitative measure of HCoV loads as they are indirectly proportional to the amplified nucleic acid present in the sample. A 3.3 cycle difference represents a 10-fold difference in load.[33] A new HCoV infection episode was defined as either detecting a HCoV species for the first time or the same virus detected ≥30-days from the last positive swab. This was deemed symptomatic when symptoms were recorded within 7-days either side of a new virus detection.[30,31]

Data analysis

The association between single new HCoV episodes and both ARI symptoms and healthcare seeking behavior were tabulated. Incidence rates of single new HCoV episodes, and associations between pre-defined risk factors and single new HCoV episodes, were calculated using mixed-effects Poisson regression models, with child included as a random effect and models offset by the natural logarithm of child-years at risk. The association between HCoV swabs and other respiratory virus detections was investigated using log-binomial regression. All multivariable models adjusted for age, season of detection, presence of older children in the household and childcare attendance. All analyses were conducted using Stata statistical software v16 (StataCorp, College Station, TX, USA).

RESULTS

One-hundred and fifty-eight children returned 11,126 swabs and 154 provided 87,547 symptom diary-days of observation (78% of expected observation days; Figure 1). This included 10,811 swabs (66% expected) matched to 82,036 diary-days from 154 children, and 8,101 higher-quality swabs from 157 children. Cohort participants were predominantly the first-born child of highly-educated parents living in non-smoking households (Table 1).

There were 168 HCoV positive swabs, of which 130 were incident episodes (Table 2). The two most commonly detected HCoVs were HCoV-NL63 (n=45 episodes) and HCoV-OC43 (n=44 episodes). Most children (89.3%) shed the virus for 1–2 weeks. While one child had a single dual infection episode with HCoV-229E and HCoVNL63, 30 children within the cohort had different HCoV species detected on separate occasions (26 had two episodes with different HCoVs; [most commonly HCoV-OC43 and HCoV-HKU1, n=11; and HCoV-NL63 and HCoV-OC43, n=9] while four had three separate episodes, each with different HCoV species [HCoV-NL63, HCoV-OC43 and HCoV-HKU1, n=3; and HCoV-229E, HCoV-NL63 and HCoV-OC43 in one child]. Furthermore, seven children infected originally between 3 and 12-months of age were re-infected with the same HCoV species a median 13-months (range 4–16) later; five with HCoV-OC43 and one each with HCoV-NL63 and HCoV-HKU1. Another virus was co-detected on 20% of episodes (Supplemental Table 6). While there was a negative association between HCoV and human rhinoviruses (adjusted relative risk [RR] 0.3, 95% confidence interval [CI] 0.2–0.5]; Supplemental Table 7), the presence of other viruses was not associated with an increased risk of ARI symptoms (RR 1.1, 95%CI 0.6–1.9).

The overall incidence of HCoV in the first 2-years of life was 0.76 episodes per child-year (95%CI 0.63–0.91). The incidence in the first and second year was 0.51 (95%CI 0.38-0.69) and 1.06 (95%CI 0.84–1.33) episodes per child-year respectively. The overall incidence rate in the first 2-years of life for symptomatic HCoV infections was 0.38 (95%CI 0.26–0.55) per

child-year; and 0.20 (95%CI 0.09–0.49) and 0.58 (95%CI 0.42–0.79) episodes per child-year in the first and second years of life, respectively (Supplemental Table 8). HCoV-NL63 and HCoV-OC43 had the highest incidence rates overall, including symptomatic episodes. Although incidence was greatest in the Winter season (adjusted incident rate ratio 2.5, 95%CI [1.4–4.2]), HCoVs were detected year-round with HCoV-NL63 peaking some years during the Summer and HCoV-HKU1 during alternate Winters (Supplemental Table 9 and Supplemental Figure 2). Other than increasing age, there was no significant association between HCoV incidence and the characteristics sex, season of birth, type of delivery, breast feeding duration, family history of asthma/atopy, tobacco smoke exposure, older children in household , maternal education or childcare.

There were 124 incident episodes linked to symptom diaries, and 62 (50%) of HCoV infections were associated with an ARI (Table 3). Of the 62 symptomatic ARI episodes, 48.4% led to a healthcare consultation, which included 37.1% being treated only by the family physician (Table 4 and Supplemental Table 10). There were six ED presentations, one of which resulted in hospitalization. Of the seven children re-infected with the same HCoV species, four had ARI symptoms with the original infection, but only one became symptomatic (nasal congestion) with their second episode. No significant differences in peak Ct values (reflecting viral loads) between symptomatic or asymptomatic HCoV detections were identified (mean [standard deviation] 29.2 [3.7] versus 30.1 [4.7]; mean difference 0.9 [95%CI -0.6–2.5] (Supplemental Table 11). In contrast, symptomatic children shed the virus for slightly longer periods than those without symptoms (mean 1.7 [1.1] versus 1.1 [0.6] weeks; mean difference 0.6 [0.3–0.9].

DISCUSSION

In agreement with seroprevalence studies,[19,20] Australian children were commonly exposed to endemic (non-SARS-CoV-2) HCoVs from a young age and 50% of episodes were asymptomatic. Of the 17 respiratory viruses tested, endemic HCoVs ranked third in frequency behind human rhinoviruses and human polyomaviruses WU/KI.[31] The overall community incidence of HCoV in the first 2-years of life was greater than reported in other community-based studies. However, in their first 6-months of life, Nepalese infants experienced 0.26 (95%CI 0.23–0.29) symptomatic HCoV episodes per child-year,[25] which is similar to our rate of symptomatic episodes per child-year from 0–12 months of age. In contrast, Queensland children had almost three times the incidence of HCoV-related ARIs in their second year of life than children of the same age attending fulltime childcare in Seattle (0.20 [95%CI 0.10–0.50] episodes per child-year).[21] A much lower incidence rate of 0.04 (95%CI 0.03–0.05) episodes per child-year was observed in a multi-country population-based study of children aged 6-months to <10-years presenting with an influenza-like illness.[26] In agreement with studies from temperate climates, the peak seasonal activity for HCoVs in subtropical South-East Queensland was in Winter with HCoV-NL63 showing a more variable pattern.[11-14,17,18,25,27,34,35]

In other reports from hospital[10,13-15,17,18] and most community-based studies,[14,24,25,27] HCoV-NL63 and HCoV-OC43 were also the two most prevalent species detected, followed by HCoV-HKU1 and HCoV-229E. In most cases, virus shedding was transient and restricted to 1–2 weeks. The literature on HCoV shedding in healthy children is limited, but our results are consistent with reports from children attending childcare in the United States.[36] These contrast with another community-based birth cohort study from Switzerland, where four of 12 infants with a HCoV infection in the 1st-year of life were still shedding the virus 3-weeks after an ARI involving fever with cough or wheeze.[22] The differences between the two studies might be

explained by small numbers and differing methodologies where nasal swabs were collected by study nurses and the Swiss children may have been sicker. While we have shown previously that when employing PCR assays, parent-collected nasal swabs have similar virus detection rates to those obtained by health personnel,[37] in the current study symptomatic children shed the virus for a small, but significantly longer period than those lacking symptoms.

Co-detections in our cohort at 20% were lower than those reported for other studies, which ranged from 27–70%.[11-13,15,17,18,21,25] While the presence of more than one virus did not impact upon clinical symptoms, there was a negative association between HCoV and human rhinoviruses. Others have also observed that human rhinoviruses reduce the likelihood of other RNA viruses, including HCoV, being present.[25,38,39] The mechanism for this interference is uncertain, but it has been suggested that RNA viruses might have a greater capacity than DNA viruses to initiate early innate interferon responses.[39] Repeated infections from different HCoV species were common in our cohort , including involvement of viruses from within the same genogroup, suggesting limited cross-protective immunity exists in young children.[19] Importantly, re-infections with the same HCoV species also occurred at a median 13-months after the original infection. However, these re-infections were either mild or asymptomatic. This observation is consistent with HCoVs remaining prevalent across all age groups[28] and with challenge trials in adult volunteers where re-infection from either HCoV-229E or HCoV-OC43 was successful a year after the original inoculation when serum antibodies had declined to pre-challenge levels.[40] The reasons for re-infection are unknown, but possibilities include incomplete homotypic immunity and exposure to a new genotypic variant of the same HCoV species. The latter is especially relevant as endemic HCoVs continue to evolve with the potential to cause outbreaks and severe LRIs.[41,42]

Although hospital-based studies report most children with HCoV had LRIs, such as bronchiolitis, pneumonia and croup,[12-14] community-based cohort studies are more likely to

describe symptoms of an URI.[14,21,22] However, illnesses vary between cohort studies as different criteria were used for collecting respiratory samples. These ranged from mild nasal symptoms alone for infants to combinations of fever, cough, wheeze or breathing difficulties. By collecting weekly nasal swabs, we were able to identify the underlying HCoV infection rate amongst young children outside of a clinical setting. Our findings agree with those of the BIG-LoVE family study where children had relatively high rates of asymptomatic infection.[28] This suggests other community-based studies may have under-estimated the true rate of HCoV infections. Amongst those with symptoms, almost two-thirds had only URIs and almost all were managed in the community without hospital involvement. Despite a large hospital-based study observing no association between severe LRI and HCoV species,[17] some community-based studies,[28] including our own,[31] have reported HCoV-OC43 to be more strongly associated with LRI episodes.

The ORChID study has several strengths. Progressive recruitment of unselected healthy newborns and following them over multiple seasons allowed better estimates of infection and disease burden within the community, while allowing for both seasonal and annual fluctuations in virus circulation. Comprehensive surveillance by daily symptom diaries and weekly nasal swabs provided very good returns of 78% and 66% respectively considering the intense and prolonged nature of the study for participating families. Having parents collect nasal swabs avoids the need for research personnel to make home visits that might otherwise prove prohibitively resource intensive and costly in many settings. Taking weekly swabs also ensured sampling before, during and after each ARI episode, which with sensitive PCR assays should assist detection rates and assessment of viral shedding kinetics. The longitudinal design allowed asymptomatic infections to be detected.

There are however, important limitations. First, symptom recognition, other than physician-diagnosed otitis media and pneumonia, was not validated. Although parents were trained before

the study to recognize respiratory symptoms and entered these in a simple tick-box diary, it is possible some very mild symptoms were missed, or others misclassified. Nevertheless, our rates of ARI in ORChID are similar to other cohort studies of children in the 1st 1-2 years of life.[43,44] Second, despite showing previously that parents could reliably collect respiratory specimens from their children,[37] we excluded 3,025 (27.2%) lower-quality swabs when analysing incidence rates to avoid these rates being under-estimated if there were any false-negative results.[32] Third, we may have also under-estimated viral shedding duration because of missing swabs or these being collected only weekly. Fourth, our study was not designed to determine who introduced HCoVs into the household. Finally, as often occurs with such intense longitudinal studies, our cohort comprised more advantaged and smaller household sized urban families, and results may not generalize to other settings. However, the exposures within the cohort on the study outcomes remain valid.

CONCLUSIONS

Infection by one of the four endemic (non-SARS-CoV-2) HCoVs was common in young and otherwise healthy Australian children. Of these, HCoV-NL63 and HCoV-OC43 were the most prevalent. Repeat infections by different and the same HCoV species suggests protective immunity in children is species-specific, but incomplete. Incidence was higher in the 2nd-year of life and during Winter, while most infections were either asymptomatic or resulted in URIs managed within the community. Future studies are needed to determine the transmission pathways and immune mechanisms induced by these viruses. Currently, it is too early to predict whether SAR-CoV-2 will transition from a pandemic to an endemic HCoV species. Should this happen, the present study suggests most SARS-CoV-2 infections in children are likely to be subclinical or mild,[7,8,45,46] but repeated infections may also occur. The latter is supported by serum anti-SARS-CoV-2 IgG antibodies decaying rapidly following mild infections.[47]

Although the true nature of protective immunity is unknown, antibodies are considered a reasonable correlate of anti-viral immunity and their loss may have important implications for SARS-CoV-2 vaccine programs. Furthermore, SARS-CoV-2 in children should not be regarded simply as yet another respiratory virus since it can be associated with severe extra-pulmonary complications, including the SARS-CoV-2-related multi-inflammatory syndrome.[48]

REFERENCES

1. Ogimi C, Kim YJ, Martin ET, Huh HJ, Chiu CH, Englund JA. What's new with the old coronaviruses? *J Pediatr Infect Dis Soc.* 2020;9(2):210-217.

2. Poutanen SM, Low DE, Henry B, et al. Identification of severe acute respiratory syndrome in Canada. *N Engl J Med.* 2003;348(20):1995-2005.

3. Memish ZA, Perlman S, Van Kerkhove MD, Zumla A. Middle East respiratory syndrome. *Lancet*. 2020;395(10229):1063-1077.

4. Li Q, Guan X, Wu P, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N. Engl J Med.* 2020;382(13):1199-1207.

5. Bedford J, Enria D, Giesecke J, et al. COVID-19: Towards controlling of a pandemic. *Lancet.* 2020;395(10229):1015-1018.

6. Bitnun A, Read S, Tellier R, Petric M, Richardson SE. Severe acute respiratory syndrome-associated coronavirus infection in Toronto children: A second look. *Pediatrics.* 2009; 123(1):97-101.

7. Gudbjartsson DF, Helgason A, Jonsson H, et al. Spread of SARS-CoV-2 in the Icelandic population. *N Engl J Med.* 2020382(24):2302-2315.

8. Zimmermann P, Curtis N. COVID-19 in children, pregnancy and neonates: A review of epidemiologic and clinical features. *Pediatr Infect Dis J.* 2020;39(6):469-477.

9. Cristiani L, Mancino E, Matera L, et al. Will children reveal their secret? The coronavirus dilemma. *Eur Respir J.* 2020; 55(4):2000749.

10. Leung TF, Li CY, Lam WY, et al. Epidemiology and clinical presentations of human coronavirus NL63 infections in Hong Kong children. *J Clin Microbiol.* 2009;47(11):3486-3492.

11. Mackay IM, Arden KE, Speicher DJ, et al. Co-circulation of four human coronaviruses (HCoVs) in Queensland children with acute respiratory tract illnesses in 2004. *Viruses.* 2012;4(4):637-653.

12. Jevsnik M, Ursic T, Zigon N, Lusa L, Krivec U, Petrovec M. Coronavirus infections in hospitalized pediatric patients with acute respiratory tract disease. *BMC Infect Dis.* 2012;12:365.

13. Prill MM, Iwane MK, Edwards KM, et al. Human coronavirus in young children hospitalized for acute respiratory illness and asymptomatic controls. *Pediatr Infect Dis J.* 2012;31(3):235-240.

14. Varghese L, Zachariah P, Vargas C, et al. Epidemiology and clinical features of human coronaviruses in the pediatric population. *J Pediatr Infect Dis Soc.* 2018;7(2):151-158.

15. Zeng ZQ, Chen DH, Tan WP, et al. Epidemiology and clinical characteristics of human coronaviruses OC43, 229E, NL63 and HKU1: A study of hospitalized children with acute respiratory tract infection in Guangzhou, China. *Eur J Clin Microbiol Infect Dis.* 2018;37(2):363-369.

16. Elfving K, Shakely D, Andersson M, et al. Pathogen clearance and new respiratory tract infections among febrile children in Zanzibar investigated with multitargeting real-time polymerase chain reaction on paired nasopharyngeal swab samples. *Pediatr Infect Dis J.* 2018;37(7):643-648.

17. Ogimi C, Englund JA, Bradford MC, Qin X, Boeckh M, Waghmare A. Characteristics and outcomes of coronavirus infection in children: The role of viral factors and an immunocompromised state. *J Pediatr Infect Dis Soc.* 2019;8(1):21-28.

18. Heimdal I, Moe N, Krokstad S, et al. Human coronavirus in hospitalized children with respiratory tract infections: A 9-year population-based study from Norway. *J Infect Dis.* 2019;219(8):1198-1206.

19. Dijkman R, Jebbink MF, El Idrissi NB, et al. Human coronavirus NL63 and 229E seroconversion in children. *J Clin Microbiol.* 2008;46(7):2368-2373.

20. Zhou W, Wang W, Wang H, Lu R, Tan W. First infection by all four non-severe acute respiratory syndrome human coronaviruses takes place in childhood. *BMC Infect Dis.* 2013;13:433.

21. Fairchok MP, Martin ET, Chambers S, et al. Epidemiology of viral respiratory tract infections in a prospective cohort of infants and toddlers attending daycare. *J Clin Virol.* 2010;49(1):16-20.

22. Regamey N, Kaiser L, Roiha HL, et al. Viral aetiology of acute respiratory infections with cough in infancy. A community-based birth cohort study. *Pediatr Infect Dis J.* 2008;28(2):100-105.

23. Anders KL, Nguyen HL, Nguyen NM, et al. Epidemiology and virology of acute respiratory infections during the first year of life. A birth cohort study in Vietnam. *Pediatr Infect Dis J.* 2015;34(4):361-370.

24. Kumar P, Medigeshi GR, Mishra VS, et al. Etiology of acute respiratory infections in infants. A prospective birth cohort study. *Pediatr Infect Dis J.* 2017;36(1):25-30.

25. Uddin SMI, Englund JA, Kuypers JY, et al. Burden and risk factors for coronavirus infections in infants in rural Nepal. *Clin Infect Dis.* 2018;67(10):1507-1514.

26. Taylor S, Lopez P, Weckx L, et al. Respiratory viruses and influenza-like illness: Epidemiology and outcomes in children aged 6 months to 10 years in a multi-country population sample. *J Infect.* 2017;74(1):29-41.

27. Monto AS, Malosh RE, Petrie JG, Thompson MG, Ohmit SE. Frequency of acute respiratory illnesses and circulation of respiratory viruses in households with children over 3 surveillance seasons. *J Infect Dis.* 2014;210(11):1792-1799.

28. Byington CL, Ampofo K, Stockmann C, et al. Community surveillance of respiratory viruses among families in the Utah Better Identification of Germs-Longitudinal Viral Epidemiology (BIG-LOVE) study. *Clin Infect Dis.* 2015;61(8):1217-1224.

29. Lambert SB, Ware RS, Cook AL, et al. Observational Research in Childhood Infectious Diseases (ORChID): A dynamic birth cohort study. *BMJ Open.* 2012;2(6):e002134.

30. Sarna M, Ware RS, Lambert SB, Sloots TP, Nissen MD, Grimwood K. Timing of respiratory virus detections in infants: A community-based birth cohort study. *J Infect Dis.* 2018;217(3):418-427.

31. Sarna M, Lambert SB, Sloots TP, et al. Viruses causing lower respiratory symptoms in young children: Findings from the ORChID birth cohort. *Thorax.* 2018;73(10):969-979.

32. Alsaleh AN, Whiley DM, Bialasiewicz S, et al. Nasal swab samples and real-time polymerase chain reaction assays in community-based, longitudinal studies of respiratory viruses: The importance of sample integrity and quality control. *BMC Infect Dis.* 2014;14:15.

33. O'Grady KF, Torzillo PJ, Rockett RJ, et al. Successful application of a simple specimen transport method for the conduct of respiratory virus surveillance in remote Indigenous communities in Australia. *Trop Med Int Health.* 2011;16(6):766-772.

34. Zimmermann P, Curtis N. Coronavirus infections in children including COVID-19. An overview of the epidemiology, clinical features, diagnosis, treatment and prevention options in children. *Pediatr Infect Dis J.* 2020;39(5):355-368.

35. Killerby ME, Biggs HM, Haynes A, et al. Human coronavirus circulation in the United States 2014-2017. *J Clin Virol.* 2018;101(1):52-56.

36. Martin ET, Fairchok MP, Stednick ZJ, Kuypers J, Englund JA. Epidemiology of multiple respiratory viruses in childcare attendees. *J Infect Dis.* 2013;207(6):982-989.

37. Zoch-Lesniak B, Ware RS, Grimwood K, Lambert SB. The Respiratory Specimen Collection Trial (ReSpeCT): a randomized controlled trial to compare quality and timeliness of respiratory sample collection in the home by parents and healthcare workers from children aged <2 years. *J Pediatr Infect Dis Soc.* 2020;9(2):134-141.

38. van den Bergh MR, Biesbroek G, Rossen JW, et al. Associations between pathogens in the upper respiratory tract of young children: Interplay between viruses and bacteria. *PLoS One.* 21012;7(10):e47711.

39. Mackay IM, Lambert SB, Faux CE, et al. Community-wide, contemporaneous circulation of a broad spectrum of human rhinoviruses in healthy Australian preschool-aged children during a 12-month period. *J Infect Dis.* 2013;207(9):1433-1441.

40. Callow KA, Parry HF, Sergeant M, Tyrell DA. The time course of the immune response to experimental coronavirus infection of man. *Epidemiol Infect.* 1990;105(2):435-446.

41. Zhu Y, Li C, Chen L, et al. A novel human coronavirus OC43 genotype detected in mainland China. *Emerg Microbes Infect.* 2018;7(1):173. doi: 10.1038/s41426-018-0171-5.

42. Wang Y, Li X, Liu W, et al. Discovery of a subgenotype of human coronavirus NL63 associated with severe lower respiratory tract infection in China, 2018. *Emerg Microbes Infect.* 2020;9(1):246-255.

43. Sarna M, Ware RS, Sloots TP, Nissen MD, Grimwood K, Lambert SB. The burden of community-managed acute respiratory infections in the first 2-years of life. *Pediatr Pulmonol.* 2016;51(12):1336-1346.

44. von Listow ML, Holst KK, Larsen K, Koch A, Andersen PK, Hogh B. Acute respiratory symptoms and general illness during the first year of life: A population-based birth cohort study. *Pediatr Pulmonol.* 2008;43(6):584-593.

45. Bi Q, Wu Y, Mei S, et al. Epidemiology and transmission of COVID-19 in 391 cases and 1286 of their close contacts in Shenzhen, China: A retrospective cohort study. *Lancet Infect Dis.* 2020; Apr 27 doi: 10.1016/S1473-3099(20)30287-5. Online ahead of print.

46. Dong Y, Mo X, Hu Y, et al. Epidemiology of COVID-19 among children in China. *Pediatrics.* 2020;145(6):e20200702.

47. Ibarrondo FJ, Fulcher JA, Goodman-Meza D, et al. Rapid decay of anti–SARS-CoV-2 antibodies in persons with mild Covid-19. *N Engl J Med.* 2020; Jul 21 doi: 10.1056/NEJMc2025179. Online ahead of print. Erratum Jul 23 doi: 10.1056/NEJMc2025179

48. Feldstein LR, Rose EB, Horwitz SM, et al. Multisystem inflammatory syndrome in U.S. children and adolescents. *N Engl J Med.* 2020;383(4):334-346.

Table 1. Sociodemographic characteristics of the ORChID cohort (n=158)

Characteristics	Total No. (%)
Gender (Male)	75 (47.5)
Season of birth	
Summer (December to February)	42 (26.6)
Fall (March to May)	30 (19.0)
Winter (June to August)	43 (27.2)
Spring (September to November)	43 (27.2)
Vaginal delivery	107 (67.7)
Gestational age at birth	
36-38 weeks[a]	36 (22.8)
39-41 weeks	122 (77.2)
First-born child	106 (67.1)
Family history	
Either parent has asthma/eczema	80 (50.6)
Mother smoked during pregnancy (n=156)	5 (3.2)
Household smoke exposure at birth (n=156)	19 (12.1)
Maternal education status (n=157)	
Tertiary	99 (63.1)
Diploma/certificate	38 (24.2)
Secondary school	15 (9.6)
Primary school	5 (3.2)
Mode of feeding (n=147)	
Exclusive breastfeeding beyond age 4-months	83 (56.5)
Childcare attendance at 6-months[b] (n=133)	
No childcare	102 (76.7)
Informal childcare only	14 (10.5)
Formal and/ or informal childcare	17 (12.8)
Childcare attendance at 12-months[b] (n=116)	
No childcare	44 (37.9)
Informal childcare only	21 (18.1)
Formal and/ or informal childcare	51 (44.0)
Childcare attendance at 18-months[b] (n=108)	
No childcare	16 (14.8)
Informal childcare only	23 (21.3)
Formal and/ or informal childcare	69 (63.9)
Childcare attendance at 24-months[b] (n=103)	
No childcare	17 (16.5)
Informal childcare only	18 (17.5)
Formal and/ or informal childcare	68 (66.0)

[a]Two participants were born between 36.0 and 36.6-days gestation.

[b]Formal childcare was defined as outside homecare from a regulated childcare service, while informal care comprised non-regulated care by relatives, friends or neighbors.

Table 2: Endemic (non-SARS-CoV-2) human coronavirus positive swabs and shedding duration (n=158 children, 11,126 swabs)

	HCoV overall	HCoV-229E	HCoV-NL63	HCoV-OC43	HCoV-HKU1
Positive swabs	168[a]	16	62	54	38
Episodes	130	11	45	44	31
Shedding duration, No. (%)					
1-week	99 (76.2)	7 (63.6)	31 (68.9)	36 (81.8)	25 (80.6)
2-weeks	17 (13.1)	2 (18.2)	8 (17.8)	5 (11.4)	3 (9.7)
3-weeks	8 (6.2)	1 (9.1)	4 (8.9)	2 (4.5)	1 (3.2)
4-weeks	2 (1.5)	1 (9.1)	0 (2.2)	0 (0.0)	1 (3.2)
5-weeks	4 (3.1)	0 (0.0)	2 (4.4)	1 (2.3)	1 (3.2)

Abbreviation: HCoV: human coronavirus. [a]One episode (two consecutive swabs) positive for both HCoV-229E and NL63.

Table 3: Association of endemic (non-SARS-CoV-2) human coronavirus species with acute respiratory illness (n=154 children, 10,811 swabs, 82,036 days).

	Total episodes No.	Asymptomatic episodes No. (%)	ARI No. (%)	URI No. (%)	LRI No. (%)
HCoV combined	124	62 (50.4)[a]	62 (50.0)	39 (31.5)	23 (18.5)
HCoV-229E	11	8 (72.7)	3 (27.3)	2 (18.2)	1 (9.1)
HCoV-NL63	43	21 (48.8)	22 (51.2)	14 (32.6)	8 (18.6)
HCoV-OC43	42	17 (40.5)	25 (59.5)	15 (35.7)	10 (23.8)
HCoV-HKU1	29	17 (58.6)	12 (41.4)	8 (27.6)	4 (13.8)

Abbreviations: ARI: acute respiratory illness; HCoV: human coronavirus; LRI: lower respiratory illness; URI: upper respiratory illness.

Of the total 130 HCoV detection episodes, 124 were linked to symptom diaries.

[a]One episode was positive for both HCoV-229E and HCoV-NL63 (no symptoms reported)

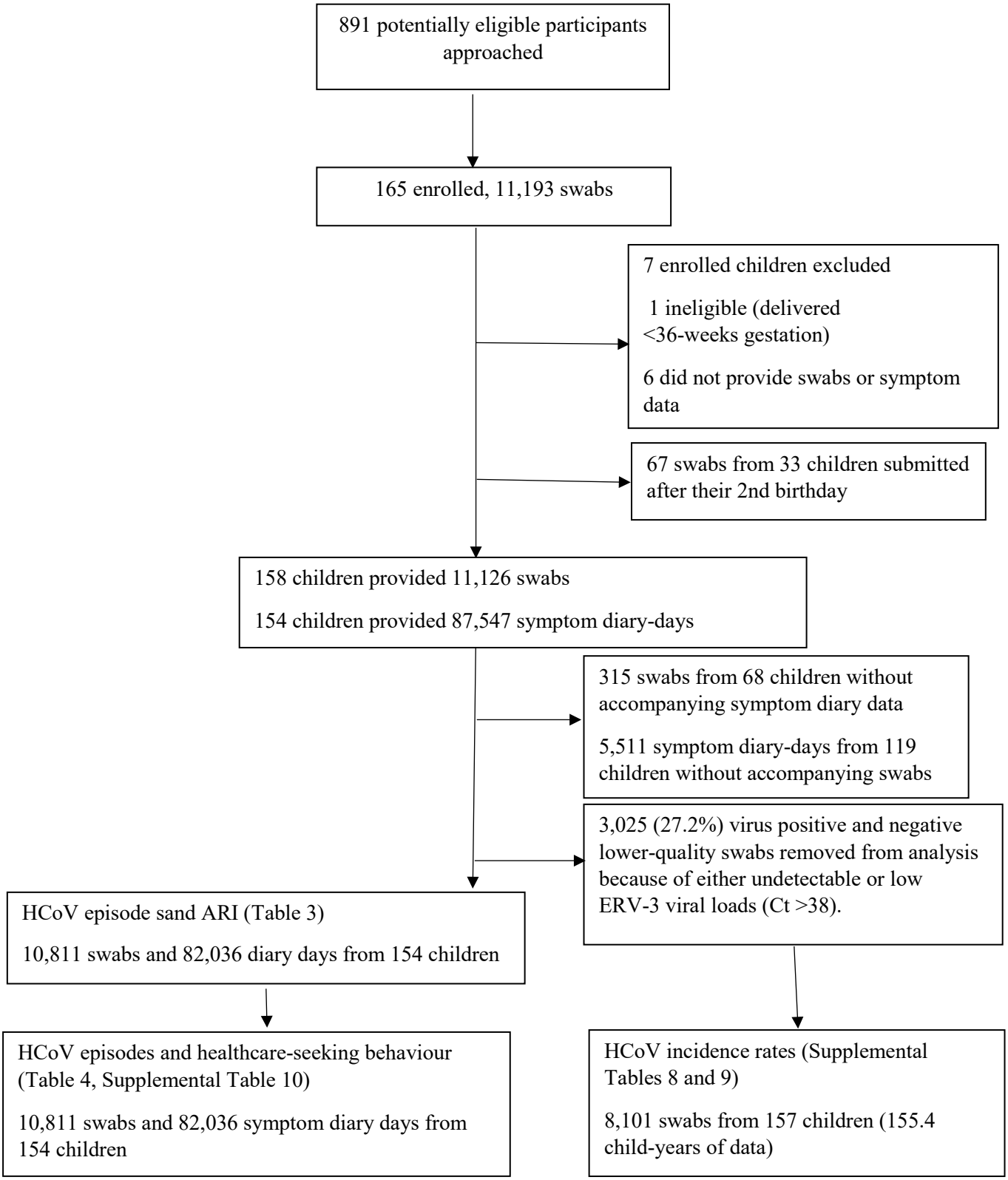
Table 4: Healthcare-seeking behavior by acute respiratory illness category (n=154 children, 10,811 swabs, 82,036 days)

	Illness-burden diaries completed/ not required[a]	Any healthcare contact	Any family physician visits	Family physician visit only	Other healthcare professional	ED presentation without admission	Hospital admission[b]	Antibiotics[c]
	No.	No. (%)	No. (%)	(No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
HCoV combined (n=124)[d]								
ARI (n=62)	47/15	30 (48.4)	26 (41.9)	23 (37.1)	2 (3.2)	5 (8.1)	1 (1.6)	11 (17.8)
URI (n=39)	24/15	14 (35.9)	13 (33.3)	13 (33.3)	1 (2.6)	0 (0.0)	0 (0.0)	7 (17.9)
LRI (n=23)	23/0	16 (69.6)	13 (56.5)	10 (43.5)	1 (4.3)	5 (25.0)	1 (4.3)	4 (17.4)

Abbreviations: ARI: acute respiratory illness; ED: emergency department; HCoV: human coronavirus; LRI: lower respiratory illness; URI: upper respiratory illness. [a]Illness-burden diary was when ARI symptomatology met a defined threshold (all LRI, acute otitis media and URI with a combination of dry cough and nasal symptoms). Of the 15 occasions when the diary was not required to be completed the single symptom was dry cough (n=3) or runny nose (n=12). [b]The presenting complaint for the hospitalized child was of fever and lower respiratory symptoms for which antibiotics were prescribed. [c]Medical visits and antibiotic information were derived from the illness-burden diary. [d]Of the total 130 HCoV detection episodes, 124 were linked to symptom diaries.

FIGURE LEGEND

Figure 1. Flow chart of nasal swabs and symptom diaries from children in the Observational Research in Childhood Infectious Diseases study. HCoV: endemic (non-SARS-CoV-2) human coronavirus.



SUPPLEMENTAL INFORMATION

Supplemental Table 5: Primer and probe sequences by virus.

Reaction Mix	Virus	Target Gene	Primer, Probe sequences (5'-3')	Source
1	Human coronavirus-HKU1	Polymerase	CCTTGCGAATGAATGTGCT TTGCATCACCACTGCTAGTACCAC FAM-TGTGTGGCGGTTGCTATTATGTTAAGCCTG-BHQ1	Dare et al (2007)[1]
2	Human coronavirus-OC43	Nucleocapsid	CGATGAGGCTATTCCGACTAGGT CCTTCCTGAGCCTTCAATATAGTAACC Q670-TCCGCCTGGCACGGTACTCCCT-BHQ2	Van Elden (2004)[2]
	Human coronavirus-NL63	Polyprotein 1a	ACGTACTTCTATTATGAAGCATGATATTAA AGCAGATCTAATGTTATACTTAAAACTACG YAK-ATTGCCAAGGCTCCTAAACGTACAGGTGTT-BBQ	Gunson et al. (2005)[3]
	Human coronavirus-229E	Nucleocapsid	CAGTCAAATGGGCTGATGCA AAAGGGCTATAAAGAGAATAAGGTATTCT FAM-CCCTGACGACCACGTTGTGGTTCA-BHQ1	Van Elden (2004)[2]
3	Human rhinovirus[a]	5' UTR	CY+AGCC+TGCGTGGY GAAACACGGACACCCAAAGTA FAM-TCCTCCGGCCCCTGAATGYGGC-BHQ1	Lu et al. (2008)[4] Arden & Mackay (2010)[5]
4	Influenza A	Matrix	CTTCTAACCGAGGTCGAAACGTA GGTGACAGGATTGGTCTTGTCTTTA Q670-TCAGGCCCCCTCAAAGCCGAG-BHQ2	Whiley et al. (2005)[6]
	Influenza B	Matrix	GCATCTTTTGTTTTTTATCCATTCC CACAATTGCCTACCTGCTTTCA FAM-TGCTAGTTCTGCTTTGCCTTCTCCATCTTCT-BHQ1	Lambert et al. (2008)[7]
5	Respiratory syncytial virus-A	Nucleocapsid	AGATCAACTTCTGTCATCCAGCAA TTCTGCACATCATAATTAGGAGTATCAAT FAM-CACCATCCAACGGAGCACAGGAGAT –BHQ1	Van Elden (2003)[8]
	Respiratory syncytial virus-B[b]	Nucleocapsid	AAGATGCAAATCATAAATTCACAGGA TGATATCCAGCATCTTTAAGTATCTTTATAGTG YAK-TATGTCC+AGG+TTAGGAAG+G+G+AA-BBQ	Van Elden (2003)[8]
6	Parainfluenza-1	Hemagglutinin-neuraminidase	TTTAAACCCGGTAATTTCTCATACCT CCCCTTGTTCCTGCAGCTATT FAM-TGACATCAACGACAACAGGAAATCATGTTCTG-BHQ1	Lambert et al (2008)[7]

	Parainfluenza-2	Nucleocapsid	AGAGTTCCAACATTCAATGAATCAGT CTCAAGAGAAATGTCATTCCCATCT YAK-CCTCTGTATTGCTCATGCATAGCACGGA-BBQ	Lambert et al. (2008)[7]
	Parainfluenza-3	Nucleocapsid	CGGTGACACAGTGGATCAGATT AGGTCATTTCTGCTAGTATTCATTGTTATT Q670-TCAATCATGCGGTCTCAACAGAGCTTG-BHQ2	Lambert et al (2008)[7]
7	Human metapneumovirus	Nucleocapsid	CATATAAGCATGCTATATTAAAAGAGTCTC CCTATTTCTGCAGCATATTTGTAATCAG FAM-TGYAATGATGAGGGTGTCACTGCGGTTG-BHQ1	Maertzdorf et al. (2004)[9]
8	Adenovirus	Hexon	GCCACGGTGGGGTTTCTAAACTT GCCCCAGTGGTCTTACATGCACATC FAM-TGCACCAGACCCGGGCTCAGGTACTCCGA-BHQ1	Heim et al. (2003)[10]
9	Human polyomavirus WU	NCCR	GCCGACAGCCGTTGGATATA TTTCAGGCACAGCAAGCAAT FAM-AGGGTCACCATTTTTATTTCAGATGGGCA-BHQ1	Antonsson et al (2012)[11]
	Human polyomavirus KI	NCCR	GAACTTCTACTGTCCTTGACACAGGTA GGATTAGAACTTACAGTCTTAGCATTTCAG Q670-ACCCTTTGTAGGCCAAAGGAGAGTGAAGG-BHQ2	Antonsson et al (2012)[11]
	Human polyomavirus KI	STAg	CACAGGTGGTTTTCTATAAATTTTGTACTT GAAGCAGTGGGATGTATGCATTC YAK-TGCATTGGCATTCGTGATTGTAGCCA-BBQ	Antonsson et al (2012)[11]
10	Human bocavirus-1	VP1	GGCAGAATTCAGCCATACTCAAA TCTGGGTTAGTGCAAACCATGA FAM-AGAGTAGGACCACAGTCATCAGACACTGCTCC-BHQ1	Tozer et al (2009)[12]
11	Endogenous retrovirus-3	ENV gene	CATGGGAAGCAAGGGAACTAATG CCCAGCGAGCAATACAGAATTT FAM-TCTTCCCTCGAACCTGCACCATCAAGTCA-BHQ1	Yuan et al. (2001)[13]
	Equine Herpesvirus	Glycoprotein B gene	GATGACACTAGCGACTTCGA CAGGGCAGAAACCATAGACA Q670-TTTCGCGTGCCTCCTCCAG-BHQ2	Bialasiewicz et al. (2009)[14]

+ indicates Locked Nucleic Acid (LNA) base (eg: +A is a LNA Adenine analogue) [a]Rhinovirus pan assay; [b]Modified probe from published version presented.

REFERENCES

1. Dare RK, Fry AM, Chittaganpitch M, Sawanpanyalert P, Olsen SJ, Erdman DD. Human coronavirus infections in Thailand: a comprehensive study using real-time reverse-transcription polymerase chain reaction assays. *J Infect Dis*. 2007;196(9):1321-1328.

2. van Elden LJ, van Loon AM, van Alphen F, et al. Frequent detection of human coronaviruses in clinical specimens from patients with respiratory tract infection by use of a novel real-time reverse-transcriptase polymerase chain reaction. *J Infect Dis.* 2004;189(4):652-657.

3. Gunson RN, Collins TC, Carman WF. Real-time RT-PCR detection of 12 respiratory viral infections in four triplex reactions. *J Clin Virol*. 2005;33(4):341-344.

4. Lu X, Holloway B, Dare RK, et al. Real-time reverse transcription-PCR assay for comprehensive detection of human rhinoviruses. *J Clin Microbiol.* 2008;46(2):533-539.

5. Arden KE, Mackay IM. Newly identified human rhinoviruses: Molecular methods heat up the cold viruses. *Rev Med Virol.* 2010;20(3):156-176.

6. Whiley DM, Sloots TP. A 5'-nuclease real-time reverse transcriptase-polymerase chain reaction assay for the detection of a broad range of influenza A subtypes, including H5N1. *Diagn Microbiol Infect Dis*. 2005;53(4):335-337.

7. Lambert SB, Whiley DM, O'Neill NT, et al. Comparing nose-throat swabs and nasopharyngeal aspirates collected from children with symptoms for respiratory virus identification using real-time polymerase chain reaction. *Pediatrics.* 2008;122(3):e615-620.

8. van Elden LJR, van Loon AM, van der Beek A, et al. Applicability of a real-time quantitative PCR assay for diagnosis of respiratory syncytial virus infection in immunocompromised adults. *J Clin Microbiol.* 2003;41(9):4378-4381.

9. Maertzdorf J, Wang CK, Brown JB, et al. Real-time reverse transcriptase PCR assay for detection of human metapneumoviruses from all known genetic lineages. *J Clin Microbiol.* 2004;42(3):981–986.

10. Heim A, Ebnet C, Harste G, Pring-Akerblom P. Rapid and quantitative detection of human adenovirus DNA by real-time PCR. *J Med Virol.* 2003;70(2):228-239.

11. Antonsson A, Bialasiewicz S, Rockett RJ, Jacob K, Bennett IC, Sloots TP. Exploring the prevalence of ten polyomaviruses and two herpes viruses in breast cancer. *PLoS One*. 2012;7(8):e39842.

12. Tozer SJ, Lambert SB, Whiley DM, et al. Detection of Human Bocavirus in respiratory, fecal, and blood samples by real-time PCR. *J Med Virol*. 2009;81(3):488-493.

13. Yuan CC, Miley W, Waters D. A quantification of human cells using an ERV-3 real time PCR assay. *J Virol Methods.* 2001;91(2):109-117.

14. Bialasiewicz S, Whiley DM, Buhrer-Skinner M, et al. A novel gel-based method for self-collection and ambient temperature postal transport of urine for PCR detection of Chlamydia trachomatis. *Sex Transm Infect*. 2009;85(2):102-105.

Supplemental Table 6: Co-detection of endemic (non-SARS-CoV-2) human coronaviruses with at least one other virus (n=168 swabs)

			Acute respiratory illness[a]					
			No symptoms		Upper respiratory illness		Lower respiratory illness	
	N	%	N	%	N	%	N	%
No co-detection	134	79.8	64	47.8	36	26.9	26	19.4
Human polyomavirus WU/KI	13	7.7	6	46.2	1	7.7	6	46.2
Human rhinovirus	13	7.7	5	38.5	6	46.2	2	15.4
Human parainfluenza viruses	3	1.8	2	66.7	0	0.0	1	33.3
Adenovirus	2	1.2	2	100.0	0	0.0	0	0.0
Human bocavirus-1	2	1.2	0	0.0	2	100.0	0	0.0
Human metapneumovirus	1	0.6	1	100.0	0	0.0	0	0.0
Respiratory syncytial virus	1	0.6	1	100.0	0	0.0	0	0.0

[a]160 human coronavirus positive swabs matched with symptom diary data. Of the 8 swabs without symptom diary data, none had a virus co-detection. There were also no co-detections identified between human coronaviruses and influenza, although 17 influenza positive swabs were identified in the ORChID cohort. One swab was positive for human coronavirus, human rhinovirus and human polyomavirus KI; the child was asymptomatic when this swab was collected.

Supplemental Table 7: Association between endemic (non-SARS-CoV-2) human coronaviruses and other respiratory virus detections (n=11,216 swabs).

Virus	No HCoV No. (%)	HCoV No. (%)	Relative risk (95%CI) Crude	Relative risk (95%CI) Adjusted[a]
Total swabs	10958 (98.5)	168 (1.5%)		
No HRV	8650 (98.2)	155 (1.8)	1.0	1.0
HRV	2308 (99.4)	13 (0.6)	**0.4 (0.2–0.6)**	**0.3 (0.2–0.5)**
No RSV	10855 (95.5)	167 (1.5)	1.0	1.0
RSV	103 (99.0)	1 (1.0)	0.6 (0.1–4.5)	0.6 (0.1–4.3)
No HPIV	10880 (98.5)	165 (1.5)	1.0	1.0
HPIV	78 (86.3)	3 (3.7)	2.5 (0.8–7.9)	1.9 (0.6–6.0)
No Influenza	10941 (98.5)	168 (1.5)	1.0	1.0
Influenza	17 (100.0)	0 (0.0)	Not calculable	Not calculable
No HMPV	10932 (98.5)	167 (1.5)	1.0	1.0
HMPV	26 (96.3)	1 (3.7)	2.5 (0.3–18.4)	1.3 (0.2–9.9)
No AdV	10850 (98.5)	166 (1.5)	1.0	1.0
AdV	108 (98.2)	2 (1.8)	1.2 (0.3–4.9)	0.9 (0.2–3.7)
No HPyV WU/KI	10678 (98.6)	155 (1.4)	1.0	1.0
HPyV WU/KI	280 (95.6)	13 (4.4)	**3.0 (1.8–5.2)**	**2.2 (1.3–3.7)**
No HBoV-1	10803 (98.5)	166 (1.5)	1.0	1.0
HBoV-1	155 (98.7)	2 (1.3)	0.8 (0.2–3.4)	0.5 (0.1–2.1)

Abbreviations: AdV: adenovirus: HBoV-1: human bocavirus-1; HCoV: human coronavirus; HMPV: human metapneumovirus; HPIV: human parainfluenza viruses; HPyV: human polyomaviruses; HRV: human rhinovirus; RR: relative risk; RSV: respiratory syncytial virus.

[a]Multivariable models adjusted for age, season of detection, older children in the household and childcare attendance.

Supplemental Table 8: Annual incidence rates for overall and symptomatic endemic (non-SARS-CoV-2) human coronavirus infections in children in the Observational Research in Childhood Infectious Diseases cohort (n=157 children, 8,101 swabs).

	IR (95%CI) First 2-years	IR (95%CI) Months 1-12	IR (95%CI) Months 13-24
HCoV combined			
Overall	0.76 (0.63–0.91)	0.51 (0.38–0.69)	1.06 (0.84–1.33)
Symptomatic	0.38 (0.26–0.55)	0.20 (0.09–0.49)	0.58 (0.42–0.79)
HCoV-229E			
Overall	0.06 (0.03–0.12)	0.06 (0.02–0.14)	0.07 (0.03–0.17)
Symptomatic	0.01 (0.00–0.05)	0.00 (0.00–27.98)	0.01 (0.00–0.11)
HCoV-NL63			
Overall	0.26 (0.19–0.35)	0.19 (0.12–0.31)	0.35 (0.23–0.52)
Symptomatic	0.15 (0.10–0.22)	0.07 (0.03–0.16)	0.24 (0.15–0.39)
HCoV–OC43			
Overall	0.27 (0.20–0.36)	0.18 (0.11–0.29)	0.38 (0.26–0.55)
Symptomatic	0.15 (0.10–0.23)	0.12 (0.06–0.22)	0.20 (0.11–0.34)
HCoV-HKU1			
Overall	0.18 (0.13–0.26)	0.11 (0.05–0.20)	0.27 (0.18–0.43)
Symptomatic	0.07 (0.04–0.13)	0.02 (0.00–0.10)	0.14 (0.07–0.26)

Abbreviations: CI: confidence interval; HCoV: human coronavirus; IR: incidence rate per child-year.

Supplemental Table 9: Number of children, child-years, single new endemic (non-SARS-CoV-2) human coronavirus detection episodes, and incidence rates in children in the Observational Research in Childhood Infectious Diseases cohort (n=157 children, 8101 swabs, 118 episodes).

Risk factor	Number of children	Child-years observation	New HCoV episodes	Incidence rate per child-year (95% CI)	Incidence Rate Ratio (95% CI) Unadjusted	Incidence Rate Ratio (95% CI) Adjusted[a]
Age (months)						
0-<3	157	22.6	8	0.35 (0.18–0.71)	**0.3 (0.2–0.7)**	**0.3 (0.1–0.8)**
3-<6	144	21.7	6	0.28 (0.12–0.61)	**0.3 (0.1–0.6)**	**0.3 (0.1–0.6)**
6-<12	136	41.2	30	0.73 (0.51–1.04)	0.7 (0.4–1.0)	0.7 (0.4–1.1)
12-<24	120	69.8	74	1.06 (0.84–1.33)	Reference	Reference
Sex						
Male	75	69.3	50	0.72 (0.55–0.95)	Reference	Reference
Female	82	86.1	68	0.79 (0.62–1.00)	1.1 (0.8–1.6)	1.1 (0.7–1.5)
Season of birth						
Summer (December to February)	42	45.3	41	0.91 (0.67–1.23)	Reference	Reference
Fall (March to May)	30	29.5	12	0.41 (0.23–0.72)	**0.4 (0.2–0.9)**	0.5 (0.3–1.0)
Winter (June to August)	43	39.2	36	0.92 (0.66-1.27)	1.0 (0.6–1.6)	1.1 (0.7–1.7)
Spring (September to November)	42	41.5	29	0.70 (0.49-1.01)	0.8 (0.5–1.2)	0.8 (0.5–1.3)
Type of delivery						
Vaginal	107	103.5	77	0.74 (0.59–0.93)	Reference	Reference
Caesarean	50	51.8	41	0.79 (0.58–1.07)	1.1 (0.7–1.6)	1.1 (0.8–1.7)
Gestational age at birth						
36-38 weeks	35	32.3	18	0.56 (0.35–0.88)	0.7 (0.4–1.1)	0.7 (0.4–1.2)
39-41 weeks	122	123.1	100	0.81 (0.67–0.99)	Reference	Reference
Exclusive breast feeding (n=147)						
Exclusive BF beyond age 4-months	83	86.7	65	0.78 (0.59–1.02)	Reference	Reference
Non-exclusive BF by age ≤4-months	64	68.2	53	0.75 (0.59–0.96)	1.0 (0.7–1.4)	0.9 (0.6–1.3)
Season of detection						
Summer (December to February)	145	34.7	18	0.52 (0.33–0.82)	Reference	Reference
Fall (March to May)	146	38.1	11	0.29 (0.16–0.52)	0.6 (0.3–1.3)	0.5 (0.3–1.2)
Winter (June to August)	141	43.1	57	1.32 (1.02–1.71)	**2.6 (1.5–4.3)**	**2.5 (1.4–4.2)**
Spring (September to November)	141	39.4	32	0.81 (0.57–1.15)	1.6 (0.9–3.0)	1.5 (0.8–2.7)

Family history						
Neither parent has asthma/eczema	79	71.6	60	0.84 (0.65–1.08)	Reference	Reference
Either parent has asthma/eczema	78	83.7	58	0.69 (0.54–0.90)	0.8 (0.6–1.2)	0.8 (0.6–1.2)
Tobacco smoke exposure (n=155)						
No exposure	136	137.8	102	0.74(0.61–0.90)	Reference	Reference
Household resident smokes	19	16.3	15	0.92 (0.55–1.53)	1.2 (0.7–2.1)	1.1 (0.6–1.9)
Household size at birth						
No older children in household	102	101.4	70	0.69 (0.55–0.87)	Reference	Reference
More than one child in household	55	54.0	48	0.89 (0.67–1.18)	1.3 (0.9–1.9)	1.3 (0.9–1.9)
Maternal education status (n=156)						
University/higher university degree	99	102.8	74	0.72 (0.57–0.90)	Reference	Reference
No university degree	57	52.5	44	0.84 (0.62–1.13)	1.2 (0.8–1.7)	1.2 (0.8–1.7)
Childcare attendance[b]						
No childcare	156	78.8	47	0.60 (0.45–0.79)	Reference	Reference
Informal childcare only	42	19.1	11	0.57 (0.32–1.04)	1.0 (0.5–1.9)	0.6 (0.3–1.2)
Formal +/- informal childcare	89	57.4	60	1.04 (0.81–1.35)	**1.8 (1.2–26)**	1.0 (0.6–1.6)

Abbreviations: BF: breast feeding; CI: confidence interval; HCoV: human coronavirus.
For incidence calculations there were 118 incident HCoV episodes after removal of lower-quality swabs.
[a]Multivariable regression adjusted for age, season of detection, older child in household and childcare attendance.
[b]Formal childcare was defined as outside homecare from a regulated childcare service, while informal care comprised non-regulated care by relatives, friends or neighbors

Supplemental Table 10: Healthcare-seeking behavior by acute respiratory illness category and endemic (non-SARS-CoV-2) human coronavirus species (n=154 children, 10,811 swabs, 82,036 days)

	Illness-burden diaries completed/ not required[a]	Any healthcare contact	Any family physician visits	Family physician visit only	Other healthcare professional	ED presentation without admission	Hospital admission[b]	Antibiotics[c]
	No.	No. (%)	No. (%)	(No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
HCoV combined (n=124)[d]								
ARI (n=62)	47/15	30 (48.4)	26 (41.9)	23 (37.1)	2 (3.2)	5 (8.1)	1 (1.6)	11 (17.8)
URI (n=39)	24/15	14 (35.9)	13 (33.3)	13 (33.3)	1 (2.6)	0 (0.0)	0 (0.0)	7 (17.9)
LRI (n=23)	23/0	16 (69.6)	13 (56.5)	10 (43.5)	1 (4.3)	5 (25.0)	1 (4.3)	4 (17.4)
HCoV-229E (n=11)								
ARI (n=3)	1/2	1 (33.3)	1 (33.3)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
URI (n=2)	0/2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
LRI (n=1)	1/0	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
HCoV-NL63 (n=43)								
ARI (n=22)	17/5	10 (45.5)	9 (40.9)	8 (36.4)	1 (4.5)	1 (4.5)	0 (0.0)	5 (22.7)
URI (n=14)	9/5	5 (35.7)	4 (28.6)	4 (28.6)	1 (7.1)	0 (0.0)	0 (0.0)	3 (21.4)
LRI (n=8)	8/0	5 (62.5)	5 (62.5)	4 (50.0)	0 (0.0)	1 (12.5)	0 (0.0)	2 (25.0)
HCoV-OC43 (n=42)								
ARI (n=25)	19/6	12 (48.0)	10 (40.0)	9 (36.0)	1 (4.0)	3 (15.4)	0 (0.0)	3 (12.0)
URI (n=15)	9/6	5 (33.3)	5 (33.3)	5 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (13.3)
LRI (n=10)	10/0	7 (70.0)	5 (50.0)	4 (40.0)	1 (10.0)	3 (30.0)	0 (0.0)	1 (10.0)
HCoV-HKU1 (n=29)								
ARI (n=12)	10/2	7 (58.3)	6 (50.0)	5 (41.7)	0 (0.0)	1 (8.3)	1 (8.3)	3 (25.0)
URI (n=8)	6/2	4 (50.0)	4 (50.0)	4 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (25.0)
LRI (n=4)	4/0	3 (75.0)	2 (50.0)	1 (25.0)	0 (0.0)	1 (25.0)	1 (25.0)	1 (25.0)

Abbreviations: ARI: acute respiratory illness; ED: emergency department; HCoV: human coronavirus; LRI: lower respiratory illness; URI: upper respiratory illness. [a]Illness-burden diary was when ARI symptomatology met a defined threshold (all LRI, acute otitis media, and URI with a combination of dry cough and nasal symptoms). Of the 15 occasions when the diary was not required to be completed the single symptom was dry cough (n=3) or runny nose (n=12). [b]The presenting complaint for the hospitalized child was of fever, cough and poor oral intake for which antibiotics were prescribed. [c]Medical visits and antibiotic information were derived from the illness-burden diary. [d]Of the total 130 HCoV detection episodes, 124 were linked to symptom diaries.

Supplemental Table 11: Association between endemic (non-SARS-CoV-2) human coronavirus peak virus loads (inverse cycle threshold value) and presence of symptoms for each episode in children in the Observational Research in Childhood Infectious Diseases cohort (n=124 episodes from 154 children, 10,811 swabs, 82,036 days).

	Asymptomatic Mean (SD)[a]	Symptomatic Mean (SD)	Mean Difference (95%CI)
HCoV combined	30.1 (4.6) [n=62]	29.2 (4.0) [n=62]	0.9 (-0.6, 2.5)
HCoV-229E	30.5 (4.9) [n=8]	28.0 (4.1) [n=3]	2.5 (-4.7, 9.8)
HCoV-NL63	29.5 (5.1) [n=21]	30.2 (3.6) [n=22]	-0.7 (-3.4, 2.0)
HCoV-OC43	28.2 (3.1) [n=17]	28.1 (3.2) [n=25]	0.1 (-1.9, 2.2)
HCoV-HKU1	32.5 (4.6) [n=17]	29.8 (4.7) [n=12]	2.7 (-0.9, 6.3)

Abbreviations: CI: confidence interval; HCoV: human coronavirus; SD: standard deviation.

One episode from an asymptomatic child was positive for both HCoV-229E and HCoV-NL63.

Supplemental Table 12: Association between symptom-status and endemic (non-SARS-CoV-2) human coronavirus shedding duration for each episode in children in the Observational Research in Childhood Infectious Diseases cohort (n=124 episodes from 154 children, 10,811 swabs, 82,036 days).

	Number of episodes	Duration (weeks) mean (SD)	Mean Difference (95%CI)
HCoV combined			
Asymptomatic	62	1.1 (0.6)	Ref.
Symptomatic	62	1.7 (1.1)	0.6 (0.3, 0.9)
HCoV-229E			
Asymptomatic	8	1.4 (0.7)	Ref.
Symptomatic	3	2.3 (1.5)	1.0 (-0.5, 2.5)
HCoV-NL63			
Asymptomatic	21	1.3 (0.9)	Ref.
Symptomatic	22	1.8 (1.0)	0.5 (-0.1, 1.1)
HCoV–OC43			
Asymptomatic	17	1.1 (0.2)	Ref.
Symptomatic	25	1.5 (1.0)	0.4 (-0.1, 0.9)
HCoV-HKU1			
Asymptomatic	17	1.0 (0.0)	Ref.
Symptomatic	12	1.9 (1.4)	0.9 (0.2, 1.6)

Abbreviations: CI: confidence interval; HCoV: human coronavirus.

Supplemental Figure 2. Seasonality for the total endemic (non-SARS-CoV-2) human coronavirus infections and for each of the four endemic (non-SARS-CoV-2) human coronavirus species plotted as the 3-month moving average of monthly incidence rates. Data collected from September 2010 to October 2014. HCoV: human coronavirus.