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New Human Coronavirus, HCoV-NL63, Associated With Severe Lower Respiratory Tract Disease in Australia

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A new human coronavirus, HCoV-NL63, was associated recently with bronchiolitis. The current study aimed to examine retrospectively stored specimens for the presence of HCoV-NL63 using nested RT-PCR assays targeting the *1a* and *1b* genes. The study population was composed of patients with acute respiratory disease warranting presentation to Queensland hospitals. HCoV-NL63 was detected in the nasopharyngeal aspirates (NPA) of 16 of 840 specimens representing 766 patients (2%). HCoV-NL63 positive individuals were diagnosed most commonly with lower respiratory tract (LRT) disease (81%). The clinical diagnosis was commonly supported by an abnormal chest X-ray (56%) together with respiratory distress (50%), wheeze (44%), and râles (25%) on first presentation with HCoV-NL63 infection. All patients positive for HCoV-NL63 required admission to hospital. Among 38% of HCoV-NL63 positive specimens a second pathogen was detected. Sequencing of amplicon from gene *1b* revealed more than 99% nucleotide homology with the viral type strains while sequencing amplicon from gene *1a* permitted the grouping of viral strains. It was shown that HCoV-NL63 is associated with severe LRT disease in an Australian hospital setting during the cooler months of the year. We propose that HCoV-NL63 is a global and seasonal pathogen of both children and adults associated with severe LRT illness. ***J. Med. Virol. 75:455–462, 2005.***

KEY WORDS: human coronavirus; emerging; respiratory; pediatric; disease

INTRODUCTION

Among humans, RNA viruses are the most frequent agent to cause the common cold; usually a self-limiting upper respiratory tract (URT) illness [Heikkinen and Järvinen, 2003]. Viruses are also a common cause of lower respiratory tract infection (LRTI) and include the diseases bronchitis, bronchiolitis, laryngotracheobronchitis, and pneumonia, which may necessitate mechanical ventilation. Human rhinoviruses (HRV), respiratory syncytial virus (hRSV), and parainfluenza viruses (PIV) are the viruses isolated most commonly [Selwyn, 1990; Hall, 2001; Hayden, 2004]. Features of mild URT disease include fever, cough, and coryza, while LRT infections may also exhibit the more severe signs of dyspnoea, râles, respiratory distress, and wheeze. Most recently, the discoveries of human metapneumovirus (hMPV) and SARS coronavirus (SARS-CoV) have exemplified the benefits of a molecular approach to rapidly identify and characterize previously unknown microbial causes of serious respiratory illness and to determine their epidemiology. Nonetheless, up to 60% of respiratory tract infections continue to go undiagnosed among hospitals and clinics worldwide [Nicholson et al., 1997].

The human coronaviruses (HCoV) are enveloped viruses, which carry a plus-sense RNA genome approximately 30 kb in length. The prototype HCoV, 229E, and OC43, were identified in the 1960s [Hamre and Procknow, 1966] but until the discovery of the SARS-CoV, received relatively little attention as human pathogens beyond their causal role in the common cold

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and associated viral wheeze [Hendley et al., 1972; Vile et al., 1995]. It is entirely possible that the prevalence of these viruses has been underestimated because of the widespread use of serological assays to diagnose infection and the fastidious nature of viral growth in vitro [Simons et al., 1996; van Elden LJR et al., 2004]. However, recent reports have associated HCoV with gastrointestinal problems [Vabret et al., 2003], acute disseminated encephalomyelitis [Yeh et al., 2004], and more severe respiratory tract infection, particularly in the elderly and the immunocompromised [Falsey et al., 2002; Pene et al., 2003], pointing to a greater role for HCoVs in disease than previously thought. Apart from the laboratory adapted type strains, related variants are known to exist yet there are few reports detailing the extent of genetic variation within the three serogroups within the genus *Coronavirus*.

A fourth human coronavirus entitled NL63 (HCoV-NL63; [van der Hoek et al., 2004]), together with the SARS-CoV, also suggest the genus *Coronavirus* contains members capable of much more significant disease [Rota et al., 2003]. A second group from the Netherlands recently described the detection of HCoV-NL63 from four infants and a child suffering from respiratory tract disease [Fouchier et al., 2004]. Detections of HCoV-NL63 by van der Hoek and colleagues occurred in four children and three adults, with a peak detection rate of 7% among ill visitors to a Medical Center in Amsterdam during mid-winter. No virus was detected during the warmer months. Interestingly, the data indicated the possible existence of several viral lineages within the HCoV-NL63-like genomes. Because very little is known about this pathogen, an Australian patient population was investigated for the presence of HCoV-NL63. Once found, this study determined the clinical features of disease associated with infection and examined local strains for genetic variability from the type strain.

METHODS

The study population comprised 840 specimens from 766 ill patients who had presented to Queensland hospitals or general practitioners during November, 2001 to February, 2004 with acute respiratory tract disease suspected of having an infectious etiology. Specimens were predominantly nasopharyngeal aspirates (NPA; 93%), but also included bronchoalveolar lavages (3.4%), endotracheal aspirates (1.4%), and bronchial washings (2.0%) collected either at the time of visit or following hospital admission. The majority of patients visited the Royal Children's Hospital, Royal Brisbane, and Women's Hospitals in Brisbane, Queensland while others visited the Ipswich, Logan, Redlands, Redcliffe, and Cairns Hospitals in Queensland. Nonconsecutive specimens were selected by season, without prior knowledge of patient details or microbiological status. The subjects ranged in age from 3 days to 80 years (median = 1.3 years), with children 5 years of age or younger comprising 77.6% of the study population. Specimens from winter (n = 492), spring (n = 183), summer (n = 86), and autumn (n = 79) were tested. Prior to this retrospective study, all specimens had been tested for common microbial respiratory pathogens and stored at −70°C. The laboratory assays included culture-amplified direct fluorescent assay and subsequent RT-PCR [Syrmis et al., 2004] of negative specimens for hRSV, human adenoviruses (hAdV), PIV types I, II, and III, and influenza viruses A (FluA) and B [Syrmis et al., 2004]. An in-house RT-PCR was used to simultaneously detect all four subtypes of hMPV. PCR assays for the HCoV OC43, and 229E (Table II) and *Bordetella pertussis* [Kosters et al., 2002] were also performed. Selective culture media were used to grow bacterial pathogens including *Pseudomonas aeruginosa*, *Streptococcus species*, *Hemophilus influenzae*, *Neisseria species*, *Staphylococcus species*, *Legionella pneumophila*, and *Candida albicans*. No microbes were detected in 557 specimens taken from 506 patients (66%) while a suspected pathogen was detected in the remaining 283 specimens representing 260 patients (34%).

To detect HCoV-NL63, two previously described nested assays ([van der Hoek et al., 2004]; Table II) were used to examine 1 μl of purified RNA. Testing of RNA consisted of a single-tube RT-PCR amplification followed by a nested PCR using 1 μl of the first round product (HotStarTaq, QIAGEN, Australia). Positive results from assay 1 were confirmed using assay 2 (Fig. 1). The two assays were in agreement.

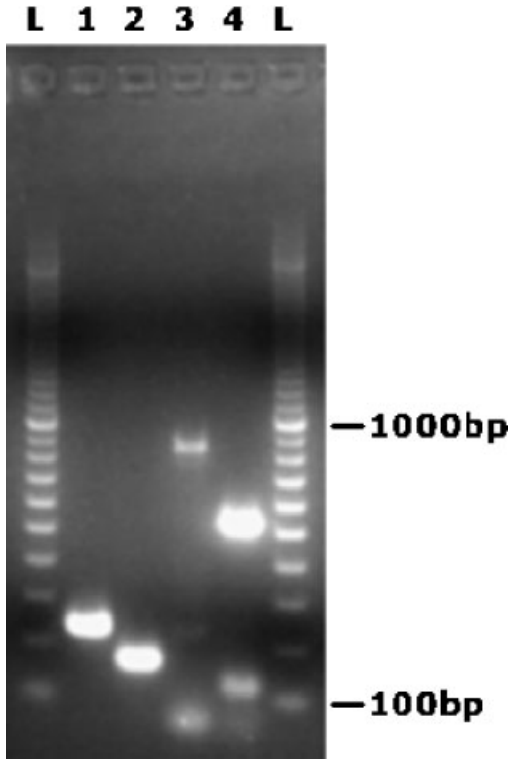

Fig. 1. Agarose gel electrophoresis of the amplicons from the two nested RT-PCR reactions. **Lane L**, 100 bp molecular weight DNA ladder. **Lane 1**, assay 1, round 1. **Lane 2**, assay 1, round 2. **Lane 3**, assay 2, round 1. **Lane 4**, assay 2, round 2.

All RT-PCR assays were performed using the OneStep RT-PCR kit (QIAGEN) incorporating 0.6 μM of each primer and subjecting the reaction mixes to a 20 min incubation at 50°C followed by 15 min at 95°C. PCR was performed for 45 cycles at 94°C for 30 sec; 55°C for 30 sec; and 72°C for 30 sec.

Infectious virus was isolated by inoculating 200 μl of NPA from positive patients onto monkey kidney cells (passage 5) in the presence of 5 μg/ml of trypsin (Gibco, USA). Cultures were maintained for 14 days and examined each day for cytopathic effects and for viral RNA by RT-PCR.

RESULTS

HCoV-NL63 was detected in the NPA specimens from 16 patients (Table I), comprising 13 males and 3 females. All patients positive for HCoV-NL63 required hospital admission and one ultimately died from possible complications. Ten positive patients (63%) had no other microbe identified nor any underlying medical condition, strongly suggesting that HCoV-NL63 was the sole cause of disease. Virus was isolated from Case 14 as determined by the production of pleomorphic cells in culture, cell detachment and a positive RT-PCR signal from the culture supernatant using assay 2.

Six patients infected with HCoV-NL63 (38%) were also infected with one other respiratory pathogen; either PIV III (Cases 1 and 2), hMPV (Case 16), hRSV (Case 11), *Bordetella pertussis* (Case 5), or *Legionella pneumophila* (Case 12). The age of infected patients ranged from 1 month to 62 years. The full medical records of all 16 subjects were examined. They all exhibited features of an acute respiratory tract infection at the time of detection including cough (81%), fever (69%), respiratory distress (50%), and coryza/vomiting/wheeze (44%). Minor clinical features of infection included; pharyngitis (38%), lethargy (31%), anorexia/râles (25%), irritability (19%), cervical lymphadenopathy/diarrhoea/dyspnoea/otitis media/rash/rhinorrhoea/rigors (13%), and conjunctivitis/cyanosis/headache/hematochezia/hepatitis/jaundice/poor sleep/stridor (6%). The duration of clinical symptoms ranged from 1 to 10 days prior to the collection of an NPA. The most common clinical diagnosis was LRTI (81%), in particular, bronchiolitis (38%). The same study population was also screened for the presence of other seasonal respiratory viruses including hRSV, hAdV, PIV I to III, and FluA. Rates of detection were 11% (85 patients), 4% (32 patients), 7% (51 patients), and 4% (33 patients), respectively. When available, a clinical diagnosis upon presentation indicated LRT disease in 46%, 25%, 35%, and 30% of patients, respectively.

Chest radiographs were obtained from 11 individuals (9 children and 2 adults) of which 9 (56%) were abnormal, demonstrating increased parahilar peribronchial markings consistent with a non-specific viral LRTI (Fig. 2).

Two individuals had pre-existing lung disease due to either chronic neonatal lung disease (Case 7) or chronic obstructive pulmonary disease (Case 12). One child had Down's syndrome (Case 6). One patient (Case 13) was severely immunosuppressed due to a bone marrow transplant and was acutely neutropaenic secondary to chemotherapy for relapsed chronic lymphocytic leukemia. He had been a long-term inpatient for 24 days prior to the detection of HCoV-NL63 and therefore was a probable nosocomial case. On two occasions induced sputum was negative for *Pneumocystis jiroveci* by direct fluorescent antigen and polymerase chain reaction. He died 25 days later from a secondary fungal septic illness due to *Scediosporum apiosperum*. While HCoV-NL63 was not the direct cause of death, a gradual decline in his respiratory status could be traced back to the time of first detection of the virus.

Three children (Cases 5, 7, and 16) had other family members who were unwell with upper respiratory tract infections (URTI) at the time of HCoV-NL63 detection. Another two children attended day care centers (Cases 2 and 8), identifying possible sites of exposure to infection.

The new coronavirus was detected in 11 of 493 winter specimens (2.2%), which represents 5.6% of the total 171 microbial diagnoses made during this period. Additionally, HCoV-NL63 was detected in two specimens from autumn and three from spring. Other microbes detected in the specimens taken during winter included hRSV (25.4% of microbial detections), hAdV (14.7%), FluA (13.2%), hMPV (12.7%), PIV III (9.1%), and *Streptococcus* and *Staphylococcus* species (5.1%). The study found that the peak month for incidence of HCoV-NL63 was July (mid-winter), at 3.1% of specimens compared to 7.0% of specimens in the Netherlands. Overall, the prevalence of HCoV-NL63 in the study population was 1.9% (2.1% of total patients), compared to 1.3% and 2.9% in the Netherlands [Fouchier et al., 2004; van der Hoek et al., 2004]. In keeping with the expected incidence of HCoV, there were no examples of infection during the summer months despite testing a greater number of specimens from summer than autumn [Hendley et al., 1972].

The 237 bp amplicon resulting from RT-PCR of the HCoV-NL63 gene *1b* of five positive specimens was sequenced (GenBank accession numbers AY600442–AY600446). The Queensland strains shared 99.5%–100% nucleotide identity with the same region of the HCoV-NL63 type strain. All strains shared 100% predicted amino acid homology with the strains from both previous reports. Three Australian strains contained the same single synonymous nucleotide change compared to HCoV-NL63 and one other Australian strain contained a different synonymous change in a nearby location. We next sequenced the 525 bp amplicon resulting from amplification of the *1a* gene. Comparison of 425 bp of these sequences (GenBank accession numbers AY632576–AY632651) indicated that strains shared 98%–100% nucleotide identity and 96%–100% predicted amino acid homology (Fig. 3).

DISCUSSION

This study demonstrates for the first time that children and adults outside the Netherlands are subject to infection by HCoV-NL63. Additionally, given

TABLE I. Patients Positive for HCoV NL63

Case	Age	Sex	Date of collection[a]	Clinical features	Duration of symptoms (day(s))[b]	Other information	Clinical diagnosis[c]
1	1 year 4 months	F	September 2001	Anorexia, cervical lymphadenopathy, coryza, cough, fever, otitis media, pharyngitis, râles	3	Co-infection-PIV III, CXR-bilateral parahilar peribronchial infiltrates and hyperinflated	LRTI/pneumonia
2	10 months	M	September 2001	Cough, cyanosis, lethargy, respiratory distress, wheeze	1	Co-infection-PIV III, CXR-bilateral parahilar peribronchial infiltrates. Attends day care	LRTI/laryngotracheobronchitis
3	7 months	M	May 2003	Cough, fever, pharyngitis, râles, respiratory distress, stridor, vomiting	3	CXR-increased right upper lobe markings	LRTI/laryngotracheobronchitis
4	1 month	M	May 2003	Cough, fever, respiratory distress, vomiting	10	CXR-right lung opacity	Bronchiolitis
5	1 month	M	June 2003	Cough, pharyngitis, rhinorrhoea, vomiting	7	Co-infection- *B. pertussis*; CXR- bilateral parahilar peribronchial infiltrates, mother unwell with URTI	Pertussis
6	2 years 4 months	M	June 2003	Cervical lymphadenopathy, cough, fever, lethargy, pharyngitis, rhinorrhoea, wheeze	7	CXR- bilateral parahilar peribronchial infiltrates	LRTI
7	2 years 3 months	F	July 2003	Anorexia, conjunctivitis, coryza, fever, irritability, lethargy, pharyngitis	3	Sibling unwell with URTI	URTI/periorbital cellulitis
8	2 years	F	July 2003	Coryza, cough, vomiting	2	Attends day care	LRTI/asthma/"pertussis"
9	4 months	M	July 2003	Coryza, cough, respiratory distress, vomiting, wheeze	1	Down's syndrome	LRTI/bronchiolitis
10	9 months	M	July 2003	Cough, fever, irritability, poor sleep, respiratory distress, vomiting, wheeze	4	Chronic neonatal lung disease on supplemental oxygen	LRTI/bronchiolitis
11	9 months	M	July 2003	Anorexia, coryza, cough, fever, otitis media, râles, respiratory distress, wheeze	4	Co-infection-hRSV, CXR-bilateral parahilar peribronchial infiltrates	LRTI/bronchiolitis
12	55 years	M	July 2003	Anorexia, cough, dyspnoea, fever, lethargy, rigors, wheeze	6	COPD, co-infection-legionella pneumophila, CXR-R lobar pneumonia	LRTI/legionella pneumonia
13	62 years	M	July 2003	Cholestatic hepatitis & jaundice, diarrhoea, dyspnoea, fever, haematochezia, lethargy, petechial rash, rigors	6	CXR-bilateral pneumonic infiltrates, bone marrow transplant, CLL relapse, neutropaenic, deceased	LRTI
14	1 year 5 months	M	August 2003	Coryza, fever, headache, irritability, pharyngitis	1	CSF gram (+), cultures (-)	URTI/meningitis
15	5 months	M	August 2003	Anorexia, coryza, cough, respiratory distress, wheeze	7	CXR normal	LRTI/bronchiolitis
16	6 months	M	September 2003	Cough, diarrhoea, fever, râles, rash, respiratory distress, vomiting, wheeze	7	Co-infection-hMPV, CXR normal, family members unwell with URTIs	LRTI/bronchiolitis

[a]Date of collection of nasopharyngeal aspirate.
[b]Duration of symptoms prior to medical presentation.
[c]LRTI, lower respiratory tract infection; URTI, upper respiratory tract infection; CXR, chest X-ray; COPD, chronic obstructive pulmonary disease; CLL, chronic lymphocytic leukaemic; CSF, cerebrospinal fluid.

TABLE II. Primers for Virus RT-PCR

Virus target	Primer	Sequence (5′–3′)	Location	Design	Nested PCR
NL63	s	GTGATGCATATGCTAATTTG	*1b* gene; 15974–15993[a]	[van der Hoek et al., 2004]	Assay 1, round 1
	as	CTCTTGCAGGTATAATCCTA	*1b* gene; 16191–16210		
	s	TTGGTAAACAAAAGATAACT	*1b* gene; 16013–16032		Assay 1, round 2
	as	TCAATGCTATAAACAGTCAT	*1b* gene; 16162–16181		
	s	CTTTTGATAACGGTCACTATG	*1a* gene; 5778–5798		Assay 2, round 1
	as	CTCATTACATAAAACATCAAACGG	*1a* gene; 6593–6616		
	s	GGTCACTATGTAGTTTATGATG	*1a* gene; 5789–5810		
	as	GGATTTTTCATAACCACTTAC	*1a* gene; 6293–6313		Assay 2, round 2
229E	s	CAGTCAAATGGGCTGATGCA	*N* gene; 25693–25712[b]	[van Elden LJR et al., 2004]	—
	as	CGCCTAACACCGTAACCTGT	*N* gene; 26022–26041	This study	—
OC43	s	CGATGAGGCTATTCCGACTAGGT	*N* gene; 29607–29629[c]	[van Elden LJR et al., 2004]	—
	as	CTTGCTGAGGTTTAGTGGCAT	*N* gene; 29843–29863	This study	—
hMPV	s	GGATCAGAGATGCAATGATTGG	*P* gene; 1864–1885[d]	This study	—
	as	GTCTACTAGGTAGGACTCCAT	*M* gene; 2165–2185	This study	—

s, sense primer; as, anti-sense primer.
[a,b,c,d]Primer positions on the relevant complete viral genome are provided according to GenBank accession numbers NC_005831, AF304460, AF39177 and NC_004148.

Australia's isolation, location in the southern hemisphere and distance from the Netherlands, we propose that HCoV-NL63 infection has a global distribution.

The current data have confirmed and further expanded the epidemiological component of previous research by determining that HCoV-NL63 exists at a similar prevalence among ill patients in Australia and the Netherlands. Specimens were predominantly chosen to examine the prevalence of HCoV-NL63 during winter, however, specimens were also tested from spring, summer, and autumn. The peak monthly prevalence was on a par to that reported in the Netherlands, although this study detected a broader seasonal prevalence whereby a third of detections occurred outside of

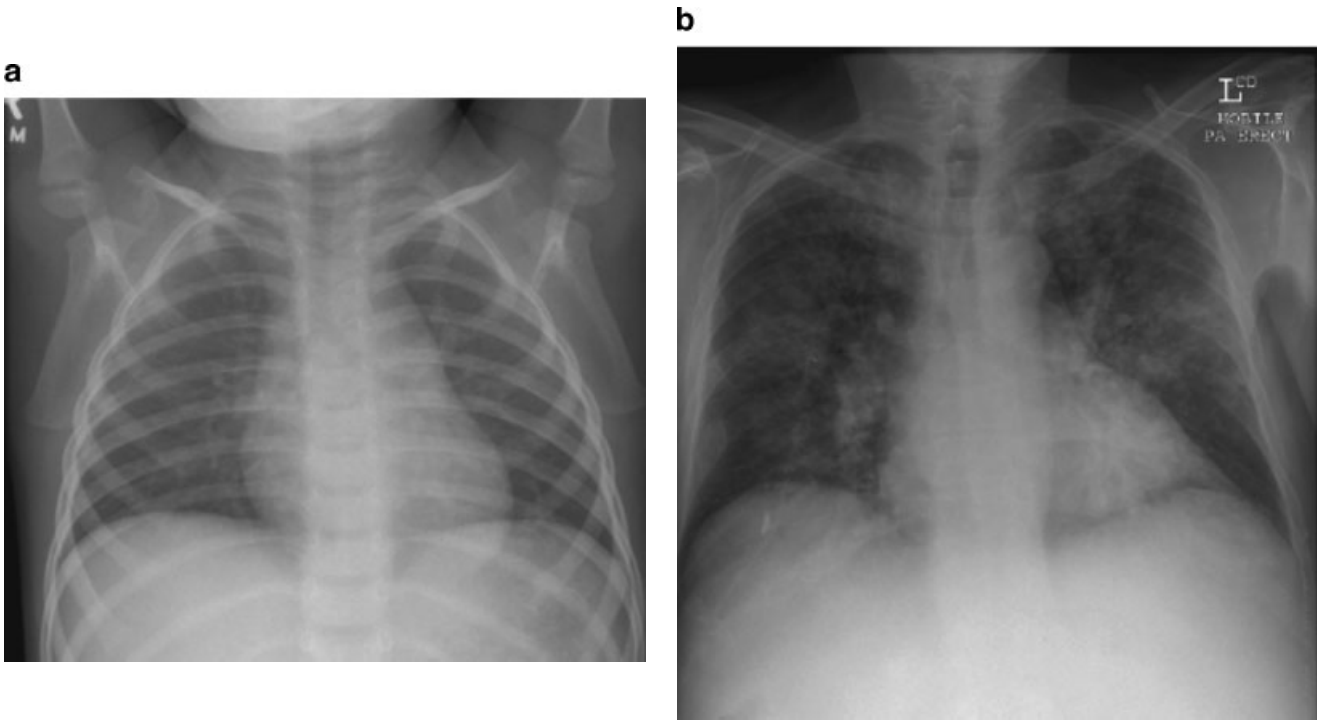

Fig. 2. Chest radiographs of patients infected with HCoV-NL63; (**a)** a child (Case 6) suffering from acute bronchiolitis that exhibits bilateral parahilar peribronchial infiltrates consistent with a lower respiratory tract (LRT) viral infection, and (**b)** an adult (Case 13) with bilateral pneumonitis.

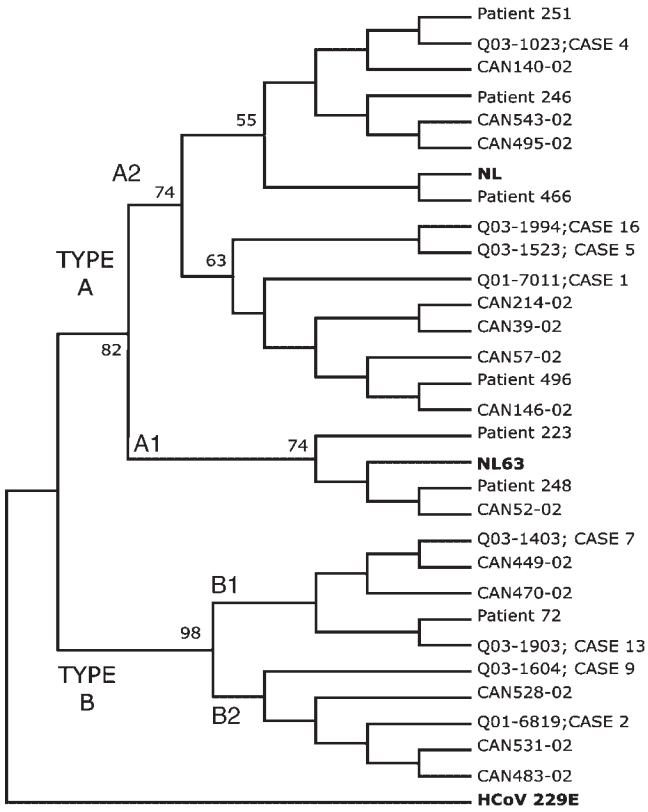

Fig. 3. Phylogenetic analysis of Queensland (Q), Dutch (Patient "x"), and Canadian (CAN) *1a* gene sequences from HCoV-NL63 strains presented on a topology tree prepared in MEGA 2.1. Branch lengths are irrelevant. The nucleotide distance matrix was generated using the p-distance estimation. Nodal confidence values indicate the results of boot strap resampling (n = 500). Two main sequence clusters are apparent (Type A and B) with subgroups indicated (A1, A2, B1, and B2).

winter; during late autumn or early spring. This may reflect the environmental impact of Queensland's tropical and sub-tropical climate.

Strains detected in Australia show a high degree of genetic homology to the known strains and to Canadian sequences submitted to GenBank during the preparation of this manuscript. The lack of variability in the predicted amino acid sequence in amplicon derived from the *1b* gene is in keeping with the essential nature of the coronavirus non-structural proteins including the replicase and proteases. However, the *1a* gene exhibited greater variability suggesting the existence of two main viral lineages. Further studies will be required to characterize the degree of genetic diversity among less conserved genes from viral strains worldwide. Until this can be defined and the applicability of current diagnostic molecular assays truly evaluated, the use of more than one PCR screening assay, as presented by both earlier reports is a sensible approach for studying the epidemiology of HCoV-NL63.

Within the study population, composed of patients with a range of respiratory illnesses, HCoV-NL63 was found to be more frequently associated with severe lower respiratory tract (LRT; 81%) disease than either of the two earlier studies (38% and 20%; [Fouchier et al., 2004; van der Hoek et al., 2004]. This assessment of disease severity is based on the fact that all patients positive for HCoV-NL63 required admission to hospital, the majority had an abnormal chest X-ray, many had clinical features of LRTI (dyspnoea, râles, respiratory distress, and wheeze) and one immunocompromised patient died. While the nature of the study population prevented a complete description of the annual incidence of HCoV-NL63 infection, the clinical features and diagnoses of patients infected with HCoV-NL63 were shown to be considerably more severe than those commonly attributed to infection of subjects by HCoV-229E and HCoV-OC43.

HCoV-NL63 was detected in the NPA of 10 patients (63% of detections) with no other known pathogen present, strongly supporting the role of this new virus in clinically diagnosed LRT disease. Nonetheless, the virus has been detected from tissue obtained directly from LRT in only one instance [van der Hoek et al., 2004]. Therefore severe URT infection with HCoV-NL63, which indirectly causes LRT disease cannot be excluded. Many diagnoses of viral LRT disease are based on the combined clinical data and laboratory detection of virus from URT tissue. A study using specimens collected from the LRT without possible contamination by tissue from the URT is essential to more accurately determine the etiology of HCoV-NL63 disease. Small animal and/or primate experiments are also necessary to determine the pathogenesis and incubation period for this new respiratory pathogen.

The new coronavirus was also co-detected with another pathogen in six subjects infected with either PIV III, hRSV, hMPV, *Bordetella pertussis*, or *Legionella pneumophila* [0.7% of all specimens, (0.8% of all patients); 38% of HCoV-NL63 infections]. Interestingly, no co-infections were seen with hAdV despite its high seasonal co-incidence. The previous studies detected HCoV-NL63 as a co-infection in 25% and 20% of the patients; comparable to this study's 38% co-infection rate [Fouchier et al., 2004; van der Hoek et al., 2004]. These data may indicate a significant characteristic of HCoV-NL63 epidemiology since such a high rate of co-infection is uncommon. Furthermore, fewer of the HCoV-NL63 infected patients in the study had underlying medical conditions than reported by Fouchier et al. [2004]. All studies to date have found HCoV-NL63 infections in patients with underlying disease, whether they are immunocompromised due to chemotherapy or HIV infection or have another medical condition such as chronic obstructive pulmonary disease. Further study will be required to determine whether patients with co-detected microbes have more severe disease or a worse long-term prognosis than patients with a single detected microbe using clinically validated severity scores. It is noteworthy, however, that HCoV-NL63 infection was more commonly associated with LRT disease than hRSV, HAdV, PIV, or FluA infection among our population. One of the dually infected patients was also a probable case of nosocomial infection (Case 13), highlighting the need for rapid detection of the virus,

as well as isolation or cohorting of HCoV-NL63 cases in health care institutions.

Two HCoV-NL63 positive patients (Cases 5 and 8) were clinically labeled as "pertussis" because of a paroxysmal cough, although the laboratory diagnosis was confirmed in only one of these (Case 5). Also, the association of HCoV-NL63 with acute laryngotracheobronchitis or "croup" (Cases 2 and 3) and PIV III infection (Cases 1 and 2) is of clinical interest. HCoV-NL63 should therefore be considered as a possible etiological agent when features of acute paroxysmal cough or croup are present.

Interestingly, two HCoV-NL63 positive patients (Cases 13 and 16) experienced gastrointestinal tract (GIT) symptoms, which are commonly attributed to animal coronaviruses and were reported recently in 57% of human cases during an HCoV-OC43 outbreak [Vabret et al., 2003]. Case 13 was intensively investigated for GIT pathogens including rotavirus, without detection. However, norovirus co-infection could not be excluded. Neither norovirus nor rotavirus could be excluded from Case 16 as these assays were not performed.

The new coronavirus accounted for 5% of all the microbes detected in the study population, however, 66% of the specimens remain undiagnosed. HRV were not tested here, however, there is an increasing body of literature linking HRV infection to severe lower respiratory disease suggesting that they may constitute a proportion of the pathogens present in the remaining undiagnosed group [Hayden, 2004].

While it remains to be proven, we believe that this virus is another example of a "new" microbe that has been infecting and causing disease among humans for many years and that the intensive application of molecular biology to specimens from patients with respiratory illness has succeeded in identifying a viral cause, where traditional methods of diagnostic culture and serology have failed. Taking into account the seasonal spread of infections over the study period, the recent detection of HCoV-NL63 in specimens dating back to 1988 by Fouchier et al. [2004] and the extent of genetic homology to HCoV-229E, it does not appear that HCoV-NL63 arose from a recent animal virus mutation as may have occurred for the SARS-CoV [Fouchier et al., 2004; Marra et al., 2004]. Therefore, the result of infection by HCoV-NL63 may more accurately be labeled a redefined infectious disease (RID) rather than an emerging infectious disease, which is defined as disease that has newly emerged in a population or significantly increased in incidence or geographic range [Morse, 1995]. While HIV, *Escherichia coli* O157:H7, and the SARS-CoV are examples that cause the former, hMPV exemplifies a cause of RID [van den Hoogen et al., 2001].

It is apparent from our study population that HCoV-NL63 may be a cause of severe LRT disease. It should be considered in the differential diagnosis of hospitalized children, the elderly and those with pre-existing immunocompromise or chronic respiratory disease during the cooler months of the year. Ongoing studies will address the transmission, incidence, and molecular epidemiology of HCoV-NL63 in the non-hospitalized community to determine whether the pathogen circulates among the community in a similar seasonal pattern causing less severe disease. The identification of this respiratory pathogen will help clinicians improve methods of patient management, reduce antibiotic use, and implement better infection control procedures within the hospital environment. The development of a single well-characterized and evaluated molecular assay to detect all HCoV-NL63-like viruses is essential before the full impact of this pathogen on community acquired respiratory disease and the significance of co-infection with characterized microbes on clinical outcome among infected patients can be elucidated.

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