Infections with human coronaviruses NL63 and OC43 among hospitalised and outpatient individuals in São Paulo, Brazil

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The incidence and clinical features of human coronaviruses (HCoVs) among Brazilian patients with respiratory illness are not well known. We investigated the prevalence of HCoVs among Brazilian outpatients and hospitalised patients with respiratory illnesses during 2009 and 2010. To identify the HCoVs, pancoronavirus and species-specific reverse-transcriptase polymerase chain reaction assays were performed. Five of 394 samples were positive for HCoVs (1.2%): 1/182 (0.5%) outpatients and 4/212 (1.8%) hospitalised patients. The OC43 and NL63 HCoVs were identified. Two patients were admitted to the intensive care unit. Underlying chronic disease was reported in cases and one diabetic adult died. HCoVs can cause lower respiratory infections and hospitalisation. Patients with pre-existing conditions and respiratory infections should be evaluated for HCoV infections.

Key words: human coronavirus OC43 - human coronavirus NL63 - respiratory tract infections

Five human coronaviruses (HCoVs) have been identified to date. HCoV-OC43 and HCoV-229E were first identified in the mid-1960s (Hamre & Procknow 1966) and were subsequently found to cause the common cold. These viruses were the only coronaviruses identified in humans until 2003, when HCoVs received worldwide attention with the emergence of severe acute respiratory syndrome (SARS), which is caused by a novel coronavirus (SARS-CoV). The increased research on these viruses led to the discovery of two additional HCoVs: HCoV-NL63 in the Netherlands and HCoV-HKU1 in China (van der Hoek et al. 2004, Woo et al. 2005). HCoVs cause upper respiratory tract illness and occasionally cause lower respiratory tract disease in susceptible individuals. The incidence and clinical features of HCoV infections in Brazilian patients with respiratory illness is not well known, especially among adults. There are few studies that describe HCoV infections among adults (Bellei et al. 2008, Gaunt et al. 2010). The aim of the present study was to investigate the occurrence of HCoVs among Brazilian outpatients and hospitalised patients who received care at the Hospital of São Paulo Federal University, Brazil, during 2009 and 2010. A total of 394 samples were collected with nasopharyngeal swabs. The final pool of subjects included 182 outpatient children from the general community and 212 hospitalised patients; of these 212 patients, 136 were children and 76 were adults. Assays to identify HCoVs in patients were performed using a two-step process. First, an initial pancoronavirus reverse-transcription polymerase chain reaction (RT-PCR) screening assay was performed (Vijgen et al. 2008). Second, species-specific RT-PCR assays were used to identify the specific HCoV species (Dare et al. 2007). Amplicons were further characterised by sequencing. Epidemiological and clinical data from outpatients were obtained using a standardised questionnaire and data from hospitalised patients were obtained from medical charts. The pancoronavirus assay detected five/394 (1.2%) coronavirus-positive samples. Within the outpatient group, which was comprised only children, one/182 (0.5%) patients was positive. Within the hospitalised patient group, four/212 (1.8%) patients were positive: two/136 were children (1.4%) and two/76 were adults (2.6%). HCoV-OC43 was documented in two samples from hospitalised patients, one of which was a nosocomial infection; three HCoV-NL63 positive samples were identified, one of which was obtained from an outpatient and two of which were obtained from hospitalised patients. Sequencing confirmed the specificity of the species-specific RT-PCR assays. Coronavirus infections occurred during the spring, autumn and winter and more coronavirus-infected cases were documented in 2010 than in 2009. The median age of HCoV-infected patients was seven years (ranging from 11 months to 46 years). Eighty percent (4/5) of infected patients reported several underlying conditions, but no chronic lung disease was reported (Table). Fever and cough were the most common symptoms at presentation. Dyspnea was reported in two cases and bronchospasm was only reported in the three HCoV-NL63 cases. Three patients presented with lower respiratory tract infections. Two of the hospitalised patients were further admitted to the intensive care unit during their hospital stay. Eight days was the median length of the hospital stay for hospitalised coronavirus-infected patients (ranging from 5-30 days). HCoV-OC43-infected patients had longer hospital stays than HCoV-NL63-infected patients. Three of the four hospitalised patients had good outcomes, but the diabetic adult died of HCoV-NL63-associated pneumonia nine days after admission. The present study is the first Brazilian report of HCoV infections in outpatients

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TABLE
Demographic and clinical data of coronavirus-infected patients during 2009-2010

	Patients				
	1	2	3	4	5
Characteristics					
HCoV species	HCoV-OC43	HCoV-NL63	HCoV-OC43	HCoV-NL63	HCoV-NL63
Sample date	September/09	May/10	April/10	June/10	June/10
Age	2 years	5 years	43 years	46 years	11 months
Gender	Female	Female	Male	Female	Female
Underlying	Megacolon	None	HIV	Diabetes	Liver
Disease	Prematurity	-	-	Mellitus	Transplant
History of smoking	-	-	+	+	-
Clinical features					
Fever	+	+	+	+	+
Cough	+	+	+	+	+
Myalgia	-	+	+	+	-
Headache	-	-	+	-	-
Diarrhoea	-	-	-	+	-
Rhinorrhoea	-	+	-	+	-
Sore throat	-	-	-	+	-
Bronchospasm	-	-	-	+	-
Dyspnea	-	-	+	+	-
LRTI	+	-	+	+	-
Hospitalization	+	-	+	+	+
Outcome	Survived	Survived	Survived	Died	Survived

HCoV: human coronaviruses; LRTI: lower respiratory tract illness; HIV: human immunodeficiency virus infected; +: present; -: absent.

and hospitalised patients to describe NL63 infection in an adult patient. Because the subjects of our study included both outpatients and hospitalised patients, the majority of the patients included in this study were believed to have a common cold. The composition of our subject pool could be a limitation of our study and might explain the small number of coronavirus-positive cases. Gaunt et al. (2010) detected 0.3-0.85% coronavirus-positive samples across all ages and we hypothesised that the H1N1 2009 influenza pandemic wave might have influenced the dynamics of the other respiratory viruses, mainly in 2009. The coronavirus-positive rate found among adults revealed that HCoVs may be representative causal agents of respiratory disease among adults as well as children. This study is one of the few studies to record a fatal case of HCoV-NL63 infection (Bastien et al. 2005, Cabeça & Bellei 2012). In conclusion, our data demonstrate that HCoVs can cause more than just the common cold and mild respiratory tract disease. Therefore, at-risk patients presenting with severe respiratory infections should be evaluated for HCoV infections. According to the data presented herein, the NL63 HCoV represents an important pathogen that is involved in unexplained respiratory illnesses.

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