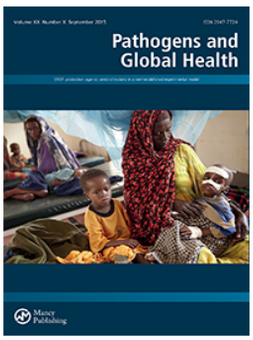

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Human coronavirus and severe acute respiratory infection in Southern Brazil

Hygor Trombetta[1], Heloisa Z. Faggion[1], Jaqueline Leotte[1], Meri B. Nogueira[2,3], Luine R.R. Vidal[2], Sonia M. Raboni[1,2]

[1]Division of Infectious Diseases, Universidade Federal do Paraná, Curitiba, Brazil, [2]Laboratory of Virology, Universidade Federal do Paraná, Curitiba, Brazil, [3]Department of Clinical Analysis, Universidade Federal do Paraná, Curitiba, Brazil

Human coronaviruses (HCoVs) are an important cause of respiratory tract infection and are responsible for causing the common cold in the general population. Thus, adequate surveillance of HCoV is essential. This study aimed to analyze the impact of HCoV infections and their relation to severe acute respiratory infection (SARI) in a hospitalized population in Southern Brazil. A cross-sectional study was conducted at a tertiary care hospital, and assessed inpatients under investigation for SARI by the hospital epidemiology department, and all patients who had nasopharyngeal aspirates collected from January 2012 to December 2013 to detect respiratory viruses (RVs). Viral infection was detected by multiplex reverse transcriptase polymerase chain reaction (RT-PCR), with primers specific to the subtypes HCoV-229E/NL63 and OC43/HKU1. The overall positivity rate was 58.8% (444/755), and HCoVs were detected in 7.6% (n = 34) of positive samples. Children below two years of age were most frequently affected (62%). Comorbidities were more likely to be associated with HCoVs than with other RVs. Immunosuppression was an independent risk factor for HCoV infection (OR = 3.5, 95% CI 1.6–7.6). Dyspnea was less frequently associated with HCoV infection ($p < 0.001$), and HCoV accounted for 6% of the SARI cases. Three patients infected with HCoV (9%) died from respiratory infection. HCoVs are important respiratory pathogens, especially in hospitalized children under 2 years of age and in immunosuppressed patients. They may account for a small proportion of SARI diagnoses, increased need for mechanical ventilation, intensive care unit admission, and death.

Keywords: Human Coronavirus, Epidemiology, Respiratory infections, Severe acute respiratory infection, RT-PCR multiplex

Background

Human coronaviruses (HCoVs) are enveloped, single-stranded, positive-sense RNA viruses that belong to the *Coronaviridae* family. HCoVs have been identified to be a frequent cause of respiratory tract infections.[1] Phylogenetic analysis has previously led to the identification of six strains. HCoV-229E, -OC43, -NL63, and -HKU1 are endemic worldwide and spread, mainly by the respiratory route.[2] More recently, severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome coronavirus (MERS-CoV) have been identified, both of which were introduced into the human population by zoonotic transmission, and are associated with severe disease.[3]

HCoVs are phylogenetically subdivided into alphacoronaviruses (HCoV-229E and HCoV-NL63) and betacoronaviruses (all other strains).[3] These viruses were first discovered in the mid-1960s by Hamre and Procknow, who described two strains: HCoV-OC43 and HCoV-229E.[4] Subsequently, HCoV-NL63 was identified in the Netherlands in 2004[5] and HCoV-HKU1 in Hong Kong in 2005.[6] Following studies demonstrated that these viruses were associated with the common cold, through the infection of healthy volunteers. Since then, HCoVs have been considered to be mild respiratory pathogens, although reports of lower respiratory tract infections caused by HCoVs have been published.[3,7]

The most common clinical presentations following HCoV infections are non-specific influenza symptoms with benign outcomes.[8] However, in select groups of patients, these viruses can cause severe and even fatal disease. Groups at high risk include young children, the elderly, immunocompromised patients, and those with underlying conditions such as cardiac or respiratory impairment.[1,2,9]

In Brazil, a national laboratory and clinical surveillance system was implemented following the influenza pandemic in 2009. This system aimed to monitor circulating respiratory viruses (RVs) in the country and identify novel viral pathogens. The system is based on the active investigation of hospitalized patients for influenza-like illness (ILI) and severe acute respiratory illness (SARI), along with outpatients who develop ILI. Flu-like symptoms include fever

Correspondence to: Sonia Mara Raboni, Hospital de Clínicas/Universidade Federal do Paraná, 180 General Carneiro Street, 3rd floor, Curitiba, Paraná, Brazil 80060-900. Email: sraboni@ufpr.br

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of recent onset, along with either a cough or sore throat. The presence of flu-like symptoms and other indications of disease severity, defined as oxygen saturation below 95%, dyspnea, or respiratory discomfort, are considered for the diagnosis of SARI. The SARI surveillance system has provided a large amount of data for in regards to the epidemiology of many viral pathogens, including HCoV.

The aims of this study were to analyze the epidemiological and clinical findings from patients hospitalized for ILI and/or SARI, whereby one or more HCoVs were detected in their respiratory secretions.

Material and methods

Patients

This was a cross-sectional study performed at Hospital de Clínicas/Universidade Federal do Paraná (HC/UFPR), a 640-bed tertiary care academic center, in Curitiba, Southern Brazil. Hospital databases (Hospital Epidemiology Division and Laboratory of Virology) and/or medical charts of patients hospitalized for acute respiratory infection during the years 2012 and 2013 were reviewed.

Inclusion criteria were defined as either hospitalization due to SARI, identified by active surveillance of the Hospital Epidemiology Division, or investigation for other RVs (despite presence of SARI). SARI was defined as the presence of ILI and signs of respiratory insufficiency (dyspnea, oxygen saturation <95%, or respiratory distress). Data were collected by completion of a form with relevant epidemiological, clinical, and laboratory questions. The Institutional Review Board of HC-UFPR approved the study (Approval number # 18714013.4.0000.0096).

Detection of respiratory viruses

RVs were detected using a commercial multiplex reverse transcriptase polymerase chain reaction (RT-PCR) kit. Respiratory samples were collected by nasal swab, nasopharyngeal aspirate, or bronchoalveolar lavage. Viral DNA and RNA were extracted using a High-Pure Viral Nucleic Acid Kit (Roche Inc., Mannheim, Germany), in accordance with the manufacturer instructions. First-strand cDNA synthesis was carried out using random primers and an ImProm-II Reverse Transcription System (Promega Inc., Madison, WI, USA). The resulting cDNA was then amplified by PCR using a Seeplex® RV15 ACE Detection Kit (Seegene Inc., Korea), in accordance with the manufacturer protocol. This multiplex PCR technology enables simultaneous detection of multiple viruses including human adenovirus (HAdV), human metapneumovirus (HMPV), parainfluenza virus types 1, 2, 3, and 4 (PIV-1, PIV-2, PIV-3, PIV-4), influenza A (FLUA), influenza B (FLUB), respiratory syncytial virus types A and B (RSV-A, RSV-B), human rhinovirus types A, B, and C (HRV A/B/C), human enterovirus (HEV), human bocavirus (HBoV), as well as HCoV types 229E/NL63 (alphacoronaviruses) and OC43/HKU1 (betacoronaviruses).

Meteorological data

Curitiba is located in Southern Brazil and has a temperate climate. Data on monthly measures of temperature and humidity were provided by the Meteorological System of Paraná (SIMEPAR).

Statistical analysis

Data were compiled using JMP software, version 5.2.1 (SAS Institute Inc., Cary, NC, USA) and were analyzed using GraphPad Prism version 5.03 (GraphPad Software Inc., La Jolla, CA, USA). Baseline demographic and clinical characteristics with normal and non-normal distributions were presented as means ± standard deviation and medians with interquartile ranges (IQR), respectively. A univariate analysis was performed separately for each variable. The Fisher's exact test, chi-squared test, or Wilcoxon–Mann–Whitney U test were used where appropriate. Spearman's tests were performed and the correlation coefficient reported to assess non-parametric meteorological data. Variables with a p-value <0.05 in the univariate analysis were included in a logistic regression model. The defined end point was a positive HCoV sample. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for the included variables. The level of significance was considered to be $p < 0.05$.

Results

Prevalence of respiratory viruses

A total of 997 cases were identified in both databases (Hospital Epidemiology Division and Virology Laboratory), and 755 had been tested for RVs during the period from January 2012 to December 2013. The overall positivity rate was 58.8% (444/755). Table 1 shows the frequencies of identified viruses. HCoVs were detected in 7.6% of RV positive samples (34/444). The most frequently detected viruses were HRV and RSV (162 and 161 cases, respectively). HCoV was the eighth most frequently identified viral pathogen in this study. Two or more viruses were co-detected in 127 cases (29% of positive samples). Among the HCoVs, 9 cases exhibited co-infection, mainly with RSV[3] and HRV.[2]

Comparison of HCoV infections with other RV infections

Table 2 shows data comparing patients infected with HCoV and those infected with other RVs. Both groups were predominately composed of children under the age of 2. The median age was 0.5 (IQR 0.1–20.9) years for patients with HCoV infection and 0.8 (IQR 0.2–3.2) years for those with other RV infections. Nosocomial infection was significantly more frequent in the HCoV group (10/34, 29%) compared with the other RV group (43/410, 10%) ($p = 0.0035$). The difference in length of hospitalization was also significant; patients with HCoV infections and those with other RV infections had a median hospitalization

Table 1 Viruses detected in 444 positive respiratory samples at a tertiary hospital in Southern Brazil, from 2012 to 2013

Virus	Single virus *N* = 317	Viral co-detection *N* = 127	Total number of positive samples *N* = 444 (%)	% Co-infection
HRV A/B	74	88	162 (36.4)	54.3
RSV	103	58	161 (36.3)	36.0
HEV	15	45	60 (13.5)	75.0
FLU	38	8	46 (10.4)	17.4
PIV	18	18	36 (8.1)	50.0
HADV	11	25	36 (8.1)	69.4
HMPV	25	11	36 (8.1)	30.6
HCoV	25	9	34 (7.6)	26.5
HBoV	7	17	24 (5.4)	71

Note: HRV = human rhinovirus; RSV = respiratory syncytial virus; HEV = human enterovirus; FLU = influenza virus; PIV = parainfluenza virus; HAdV = human adenovirus; HMPV = human metapneumovirus; HCoV = human coronavirus; HBoV = human bocavirus.

Table 2 Comparison of the clinical and epidemiological presentation between hospitalized patients with human coronavirus and those with other respiratory virus infections, from 2012 to 2013

			p value	
	HCoV *N* = 34 (%)	Other RVs *N* = 410 (%)	Unadjusted analysis	Adjusted analysis (OR, 95%CI)
Virus co-detection	9/34 (26%)	118/410 (29%)	0.840	–
Year of occurrence				
2012	20/34 (59%)	201/410 (49%)	0.150	–
2013	14/34 (41%)	243/410 (51%)		–
Sex				
Male	18/34 (53%)	211/410 (51%)	1.000	-
Age				
<2 years	21/34 (62%)	285/410 (70%)		-
2–5 years	1/34 (3%)	40/410 (10%)		–
5–14 years	1/34 (3%)	24/410 (6%)		–
14–50 years	8/34 (23%)	37/410 (8%)		–
>50 years	3/34 (9%)	24/410 (6%)		–
Median	0.5	0.8	0.749	NS
(IQR 25–75)	(0.1–20.9)	(0.2–3.2)		
Nosocomial infection	10/34 (29%)	43/410 (10%)	**0.003**	NS
Length of hospitalization				
Median, days	12	7	**0.003**	NS
(IQR 25–75)	(5.7–36)	(4–15)		
Clinical characteristics				
Fever	27/34 (79%)	332/410 (81%)	0.813	–
Cough	28/34 (82%)	371/410 (90%)	0.130	–
Dyspnea	21/34 (61%)	369/410 (90%)	**<0.0001**	**<0.0001 0.17 (0.08–0.38)**
Radiological findings				
Missed	17	184		
Normal	5/17 (30%)	34/226 (15%)	0.160	–
Interstitial infiltrate	5/17 (30%)	75/226 (33%)	1.000	–
Pulmonary consolidation	3/17 (18%)	63/226 (28%)	0.570	–
Mixed	1/17 (6%)	20/226 (9%)	1.000	–
Other findings[a]	3/17 (18%)	34/226 (15%)	0.720	–
Comorbidities				
None	10/34 (29%)	281/410 (69%)	**<0.0001**	–
Immunosuppression	15/34 (44%)	57/410 (14%)	**<0.0001**	**0.001 3.5 (1.61–7.64)**
Chronic lung disease	5/34 (15%)	60/410 (15%)	1.000	–
Chronic heart disease	4/34 (12%)	26/410 (6%)	0.270	–
Mechanical ventilation	8/34 (24%)	87/410 (21%)	0.820	–-
ICU	13/34 (38%)	134/410 (33%)	0.570	–
Death	3/34 (9%)	23/410 (6%)	0.430	–
Severe disease	16/34 (47%)	141/410 (34%)	0.140	NS
Diagnosis of SARI	20/34 (59%)	314/410 (76%)	**0.021**	NS

Note: HCoV = Human coronavirus; RVs = Community respiratory viruses; IQR = interquartile range; ICU = intensive care unit; NS = not significant.
[a]Other findings: pleural effusion.

of 12 (IQR 5.75–36.0) days and 7 (IQR 4.0–15.0) days, respectively, ($p = 0.0036$). However, none of the parameters proved to be independently linked to HCoV infection, as a multivariate analysis showed no significant differences. Dyspnea was significantly less frequent during infection with HCoVs (21/34, 61%) compared with other RVs (369/410, 90%) in the adjusted analysis ($p < 0.001$).

Among the HCoV group, 24 patients had underlying medical conditions; 15 were immunosuppressed (mainly because of hematopoietic stem cell transplantation,

malignancy, corticosteroid treatment, and human immunodeficiency virus infection), 5 had a chronic respiratory disease, and 4 had cardiac illness. The HCoV group had a significantly lower number of patients without underlying conditions (10/34, 29% vs. 281/410, 69%, $p < 0.0001$). Immunosuppression was an independent risk factor associated with HCoV infection ($p < 0.0016$). The presence of underlying conditions affected the length of hospitalization; the median length of hospitalization for patients with no underlying condition, immunosuppression, cardiac illness, and chronic pulmonary disease was 5.5 days (IQR 3.75–7.25), 36 days (IQR 12–44), 27 days (IQR 11.25–33.0), and 8 days (IQR 5.0–45.5), respectively.

We defined severe disease as that requiring mechanical ventilation, admission to an ICU, or having a fatal outcome, and observed no statistical difference between the groups. SARI diagnosis was significantly less frequent in the HCoV group by univariate analysis (20/34, 59% vs. 314/410, 76%; $p = 0.021$), but was non-significant on the adjusted analysis. Three patients died following the respiratory infection; two were in an immunosuppressed state and one did not have an underlying disease but was co-infected with two HCoVs.

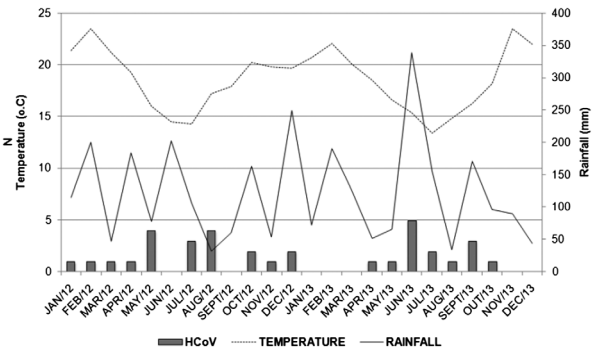

Figure 1 Human coronavirus detection and meteorological data from 2012 to 2013 in Curitiba, Brazil.

Table 3 Spearman's test correlations for monthly cases of human coronavirus and meteorological data

Parameter	*r*	*P*
Rainfall amount	0.14	0.50
Mean temperature	–0.54	0.0094

Seasonality and correlation with meteorological data

Figure 1 shows the temporal distribution of HCoV cases and meteorological data for Curitiba, Southern Brazil. As presented in Table 3, there was a negative correlation between the frequencies of HCoV positive samples and the mean temperature ($r = -0.54$; $p < 0.009$). No correlation was found between the number of positive samples and the amount of rainfall ($r = 0.14$; $p = 0.50$).

Table 4 Comparison of the clinical and epidemiological presentation between hospitalized patients with human betacoronavirus OC43/HKU1 and alphacoronavirus NL63/229E infection, from 2012 to 2013

			p value	
	OC43/HKU1 *n* = 15	NL63/229E *n* = 18	Unadjusted analysis	Adjusted analysis (OR, 95%CI)
Virus co-detection	7/15 (47%)	1/18 (6%)	**0.012**	**0.019 (15, 1.55–142.2)**
Year of occurrence				
2012	11/15 (73%)	9/18 (50%)	0.284	–
2013	4/15 (27%)	9/18 (50%)		
Sex				
Male	6/15 (40%)	11/18 (61%)	0.302	–
Age				
<2 years	11/15 (73%)	9/18 (50%)		
2–5 years	0/15 (0%)	1/18 (6%)		
5–14 years	0/15 (0%)	1/18 (6%)		
14–50 years	1/15 (7%)	7/18 (38%)		
>50 years	3/15 (20%)	0/18 (0%)		
Median, years	0.3	2.1	0.704	–
(IQR25 – 75)	(0.1–24.7)	(0.2–21.5)		
Nosocomial Infection	3/15 (20%)	11/18 (61%)	0.280	–
Length of hospitalization				
Median, days	11	21.5	0.663	–
(IQR25 – 75)	(6–33)	(5.7–36.2)		
Clinical characteristics				
Fever	11/15 (73%)	15/18 (83%)	0.674	–
Cough	14/15 (93%)	13/18 (72%)	0.186	–
Dyspnea	11/15 (73%)	9/18 (50%)	0.284	–
Comorbidities				
None	6/15 (40%)	3/18 (17%)	0.239	–
Immunosuppression	3/15 (20%)	12/18 (67%)	**0.013**	NS
Chronic lung disease	4/15 (27%)	1/18 (6%)	0.152	–
Chronic heart disease	2/15 (13%)	2/18 (10%)	1.000	–
Mechanical ventilation	1/15 (7%)	6/18 (33%)	0.095	–
ICU	6/15 (40%)	6/18 (33%)	0.730	–
Death	0/15 (0%)	2/18 (11%)	0.488	–
Severe disease	7/15 (47%)	8/18 (44%)	1.000	–
Diagnosis of SARI	10/15 (67%)	9/18 (50%)	0.482	NS

Note: NS = not significant; IQR = interquartile range; ICU = intensive care unit; SARI = severe acute respiratory infection.

Differences between HCoV subtypes

Clinical and epidemiological data from different HCoV subtypes are shown in Table 4. Co-detection of betacoronavirus OC43/HKU1 subtypes (7/15, 47%) was significantly more frequent than co-detection of alphacoronavirus NL63/229E subtypes (1/18, 6%) (p = 0.019). Immunosuppression was less frequent in the first group (3/15, 20%) compared with the second (12/18, 67%), and the occurrence of nosocomial infection was higher in the betacoronavirus group compared with the alphacoronavirus group, both without statistical significance. Table 4 includes only 33 cases, as one patient was identified to be co-infected with an alpha and betacoronavirus, and this case was excluded from the analysis.

Discussion

HCoVs were first identified more than 50 years ago. However, this pathogen and its impact on public health have only recently received attention and detailed investigation. Few reports on the prevalence of these viruses are available, especially in developing countries such as Brazil. This study is relevant, as it presents primary data regarding HCoV infection within the Brazilian southern region, and aids in the contribution of data to the surveillance programs that are being conducted worldwide.

The adoption of molecular methods for the investigation of RV in surveillance programs has contributed to increasing knowledge about the wide circulation of these pathogens, their seasonality, and impact on human health. Viral identification using multiplex RT-PCR has shown positivity rates that range from 31.2% in Southeast Brazil[10] to 61.4% in Gabon,[11] and 65% in Georgia, USA,[12] up to 82.3% in southern China.[13] This study found at least one RV in 58.8% of the samples analyzed, which is in accordance with current reports. HCoVs were detected in 7.6% of samples, which is similar to the values reported by Gaunt *et al.* (8.15%)[14] and Lee *et al.* (7%),[15] further demonstrating that it is a relevant pathogen of the respiratory tract. Other studies have shown even greater incidence of HCoV infection, up to 12%.[2,8,10] Co-infection with HCoV was observed in 26.5% of samples, and HCoV had the second lowest rate of co-infection within the group of viruses studied, behind only influenza.

Children under two years of age were found to be the main population affected by HCoV, accounting for more than 60% of patients. No significant difference in age was observed between patients infected with HCoV and those infected with other RVs, similar to the results from an American tertiary hospital reported by Litwin & Bosley (2014).[12] In contrast, a Chinese study analyzed outpatients under 16 years of age and detected RV in children between 2 and 5 years at a lower frequency (43.6%).[13]

Patients infected with HCoV had a higher probability of having an underlying disease than those affected by other RVs, and these data are in agreement with previous reports by Cabeça[2] and Lee.[15] In this study, immunosuppression was significantly more frequent among HCoV-infected patients, and was found to be an independent risk factor for infection by this virus. Between the HCoV group, although betacoronaviruses predominated in immunosuppressed patients, their detection was not significant on adjusted analysis. Previous reports have attributed this finding to the presence of the 229E subtype.[14] A cohort study previously conducted with healthy adults, healthy seniors, high-risk seniors, and hospitalized patients showed that the probability of an asymptomatic infection caused by strain 229E was higher than that caused by stain OC43, and symptoms were milder when they did occur.[16] Nevertheless, in immunocompromised patients, infection with strain 229E may result in presentation with symptoms more frequently than in healthy patients, resulting in a higher detection rate. Since in this study, the HCoV detection was performed by virus groups only (OC43/HKU1 and NL63/229E), it was not possible to evaluate the relationship between HCoV subtype and associated diseases.

A recent study conducted in Brazil analyzing the impact of HCoV in patients with cystic fibrosis showed greater respiratory exacerbation rate in patients infected with NL63 strain. However, such result was not confirmed by adjusted analysis, and no significant difference in rates of respiratory exacerbation or hospitalization in patients infected by HCoV in general or a given HCoV (including NL63) was found.[17] Our study reports similar findings, as the prevalence of comorbid chronic lung disease was not different between the HCoV and other RV groups, or between alphacoronavirus and betacoronavirus groups.

Lepiller *et al*. showed no statistical difference in the frequency of strains 229E and HKU1 between immunosuppressed and non-immunosuppressed patients. However, strain NL63 was significantly more frequent in immunosuppressed patients, whereas strain OC43 was predominately detected in non-immunosuppressed patients.[18] Moreover, their findings suggest that lower respiratory tract infections are unlikely to be caused by HCoV infection alone, as nearly 50% of HCoV infections were detected in association with another virus. Our data do not corroborate this conclusion, as we observed severe lower respiratory tract diseases caused by coronaviruses alone, both in the presence and absence of comorbidities.

Zhang *et al*. studied the viral etiology of SARI and found that HCoVs were responsible for 6.7% of cases.[19] Our study had similar results; coronaviruses were detected in 6% of SARI cases. The frequency of severe disease was smaller in the HCoV (59%) than in the other RV group (76%) on unadjusted analysis, but this finding was not confirmed by the adjusted analysis.

Viral detection and monthly mean temperature in Curitiba City were found to be negatively associated. Du Prel *et al.* reported very similar results,[20] offering evidence to support the relationship between cold weather and HCoV-induced respiratory tract infection. Such variation may be attributable to changes in the survival and spread

of the pathogens, the susceptibility of the host, and the host behavior during cold weather.[20] No relationship between monthly rainfall and viral detection was observed.

This study has some limitations. Firstly, more than 50% of the patients were children no older than two years, compromising the extension of our conclusions to older populations. Secondly, data were collected retrospectively, which may have resulted in the loss of some information. Third, detection of HCoV in two groups (OC43/HKU1 and NL63/229E), rather than individual strains, may impact the discovery of findings in this study. Despite these limitations, we present relevant data on the impact of coronaviruses in southern Brazil, and particularly on the relationship between HCoV and SARI.

In conclusion, although infection by coronaviruses does not carry the same lethality and frequency as that of other RVs, the burden of this infection should not be underestimated, particularly in patients younger than two years. Moreover, the detection of coronaviruses during respiratory infection does not warrant underestimation of the disease severity, and the presence of underlying medical conditions, such as immunosuppression, must be taken into account. Finally, some of the data described herein, especially the rate of severe disease, requires further evaluation. Future studies should aim to better characterize the impact of each HCoV subtype separately.

Conflicts of interest

The authors declare no conflicts of interest.

ORCID

Sonia M. Raboni http://orcid.org/0000-0001-7907-7585

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