Journal of Biosciences and Medicines, 2023, 11, 125-139
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Prevalence and Seroprevalence of Non-SARS Human Coronaviruses 229E and OC43 in East Tyrol/Austria

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How to cite this paper: Stefanie, S., Sissy, S., Hinterbichler, E. and Walder, G. (2023) Prevalence and Seroprevalence of Non-SARS Human Coronaviruses 229E and OC43 in East Tyrol/Austria. *Journal of Biosciences and Medicines*, **11**, 125-139.
https://doi.org/10.4236/jbm.2023.117011

Received: June 15, 2023
Accepted: July 17, 2023
Published: July 20, 2023

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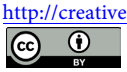

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Abstract

The recent SARS-CoV-2 pandemic renewed interest in other previously discovered human coronaviruses—the non-severe acute respiratory syndrome human coronavirus (non-SARS hCoVs) and this study is a starting point for a closer investigation of those. With the pandemic behind us we believe it to be important to also examine the current and past respiratory pathogen landscape in the patient population in our care. Therefore, 954 nasopharyngeal swabs of patients with respiratory diseases collected between October 2018 and March 2020 were tested against the pathogens *Mycoplasma pneumoniae*, *Bordetella pertussis*, Influenza A and virus, Human metapneumovirus, respiratory syncytial virus, Parainfluenza virus, human Adenovirus and Polyoma virus BK/JC. Swabs negative against these pathogens were further tested for OC43 and 229E by RT-qPCR. Human coronaviruses 229E and OC43 were proven as the causative agents in a considerable number of cases, confirmed by PCR. Overall, our results show that those two non-SARS hCoVs were responsible for 13.9% (11 of 79) of infections with flu-like symptoms of unknown etiology in the study area. In the subsequent seroprevalence study, it was shown that the seroprevalence rate of IgG antibodies against 229E and OC43 was over 50%, indicating that a big part of the population in our study area has been in contact with these non-SARS-CoVs and has built the specific humoral immune response accordingly.

Keywords

NonSARS Human Coronavirus, hCoV, hCoV OC43, hCoV 229E, Respiratory Pathogens

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1. Introduction

Coronaviridae are a family of monogeneric viruses, encompassing eleven virus species infecting vertebrates [1]. They are among the RNA-viruses with the largest genome and were named Coronaviruses (CoVs) because of their crown-like appearance under an electron microscope [2]. CoVs have been studied for almost 100 years and have first been reported and isolated in domesticated animals (chicken, turkey, cow, pig, cat and dog) and mice [3]. In the mid 1960s the first CoVs infecting humans (hCoV) were identified [3]. Hamre and Procknow isolated hCoV-229E while doing a study of medical students with respiratory illness at the University of Chicago [3] [4] [5], naming the novel alphacoronavirus hCoV229E. HCoV-OC43 (organ culture 43) was found in individuals with common cold and belongs to the betacoronavirus family [6]. In the meantime, four human coronaviruses have been identified that can be distinguished from the severely human pathogens (severe acute respiratory syndrome CoV (SARS) −1 and −2 and Middle East respiratory syndrome (MERS) CoV) on the basis of their lower human virulence and could be grouped together under the simplified term common cold hCoVs [7]. Since symptoms of infection with either one of them were relatively mild they were treated more as a nuisance rather than a global emergency. Nonetheless it has long been suspected that 10% - 30% of respiratory illnesses each year are caused by nonSARS human coronaviruses [8].

Research into nonSARS human coronaviruses and their prevalence in respiratory infections allows us to better understand the extent of the diseases caused. This allows us to develop appropriate prevention and treatment measures. In addition, they contribute to accurate differential diagnosis by helping us to distinguish the symptoms of these viruses from other respiratory infections. Furthermore, we gain insights into viral dynamics, including distribution and seasonal variations, which is useful in developing infection control strategies. Last but not least, they allow us to better understand the potential of future outbreaks of other human coronaviruses and to take appropriate preparatory measures. In summary, this study contributes to a comprehensive understanding of respiratory viruses and helps us develop appropriate respiratory infection control measures. Therefore, we investigated 954 nasopharyngeal swabs against the pathogens *Mycoplasma* (M.) *pneumoniae*, *Bordetella* (B.) *pertussis*, Influenza A and B virus (FLUAV, FLUABV), human metapneumovirus (hMPV), respiratory syncytial virus (RSV), Parainfluenza virus (PIV), human Adenovirus (HadV) and Polyoma virus BK/JC. 79 of the 954 swap samples (8.3%) collected between October 2018 and March 2020 with flu-like symptoms were tested negative in qPCR against the common respiratory pathogens and were therefore investigated for the presence of hCoVs OC43 and 229E via RT-qPCR. Human coronaviruses 229E and OC43 were proven as the causative agents in a considerable number of cases, confirmed by PCR.

We decided to use qPCR as a diagnostic tool for pathogen screening, as it offers high sensitivity in the detection of even small amounts of pathogens, enabl-

ing reliable diagnosis. It provides rapid results within a few hours, enables specific identification of the target pathogen and can quantify viral load [9].

The ViraChip microarray immunoblot (Viramed Biotech AG, Germany) with IgG and IgA detection capability offers a broad spectrum of targets and simultaneous discrimination between the four nonSARS hCoVs and SARS-CoV-2, making it highly specific and enabling a comprehensive assessment of the immune response, and was therefore the tool of choice for serological testing in this study.

Our results show that those two non-SARS hCoVs were responsible for 13.9% (11 of 79) of infections with flu-like symptoms of unknown etiology in the study area. In the subsequent seroprevalence study, it was shown that the seroprevalence rate of IgG antibodies against 229E and OC43 was over 50%, indicating that a big part of the population in our study area has been in contact with these non-SARS-CoVs and has built the specific humoral immune response accordingly.

2. Material & Methods

2.1. Study Design

As part of routine diagnostics for the local hospital, 954 nasopharyngeal swabs from patients with flu-like symptoms were sent in from October 2018 to March 2020 and tested by PCR for the following pathogens: *Mycoplasma* (M.) *pneumoniae*, *Bordetella* (B.) *pertussis*, Influenza A and B (FLUAV, FLUABV), Human metapneumovirus (hMPV), respiratory syncytial virus (RSV), Parainfluenza virus (PIV) and human Adenovirus (HadV) and Polyoma virus BK/JC. The swabs that were negative in the above-mentioned PCR detection were further tested for OC43 and 229E by PCR.

2.2. Extraction

RNA and DNA were extracted using the QiAamp Viral RNA Mini kit and the QiAmp DNA mini kit (Qiagen, Germany) according to the manufacturer's instruction.

2.3. qPCR

Commercially available test kits were used to screen for the following pathogens: *M. pneumoniae* (GeneProof, Czech Republic), *B. pertussis* (GeneProof, Czech Republic), FLUAV, FLUABV (Altona RealStar, Germany), hMPV (Altona RealStar, Germany), RSV (Altona RealStar, Germany), PIV (Altona RealStar, Germany) human Adenovirus (HadV) (GeneProof, Czech Republic) and Polyoma virus BK/JC (GeneProof, Czech Republic). All tests were administered according to the manufacturers' protocols.

For hCoV 229E an hCoV OC43, RT-qPCR was assessed using the iTaq Universal SYBR Green One-Step RT-qPCR Kit (BioRad, Germany) using 15 µl mastermix per 10 µl sample containing 12.5 µl 2× reaction Mix, 1 pmol/µl of forward and reverse primer and 4 U/µl reverse transcriptase. RT-qPCR protocol was ad-

justed for the BioRad CFX96 following the manufacturer's protocol for 30 amplification cycles. Denaturation was done at 95˚C for 10 seconds, annealing, extension, and plate read at 58.5˚C for 30 seconds, followed by melt curve analyses at 68˚C - 92˚C with 0.5˚C increments. PCR-positive supernatant of cell cultures infected with HCoV-229E or HCoV-OC43, respectively, were used as positive controls in the screening PCRs. RNA-extract of SARS-CoV-2 positive cell culture supernatant with a concentration of 100 PFU/mL was used as a negative control sample, as described elsewhere [10] [11]. The primers used for screening PCRs for hCoV OC43 and 229E were published in Vabret *et al.*, 2020 [12] and are shown in Table 1.

2.4. Local Seroprevalence of HCoV-229E and HCoV-OC43

In addition to nasopharyngeal swap samples of patients with flu-like symptoms, blood samples were collected in the relevant time period of December 2020 to January 2021 in healthy donors to determine the local specific seroprevalence against hCoVs OC43 and 229E. Full blood was collected (Vacuette, Greiner Bio One, Austria), separation of the serum was performed by centrifugation for 10 minutes at 1500 g. The sample size required for the seroprevalence study was calculated according to the following formula:

$$n = (Z^2 * P * Q)/e^2;$$

whereas n is the required sample size, Z is the critical numerical value, depending on the confidence level, P is the estimate of the seroprevalence rate, $Q = 1 - P$ and e is the maximum permissible deviation in percent. Therefore, we considered a sample size of 285 at a confidence level of 99% to be sufficient to achieve the seroprevalence rate estimate with a maximum deviation of ±5%.

2.5. Microarray Immunoblots

The ViraChip assay (Viramed Biotech AG, Germany) detects temporal antibody profiles of different immunoglobulin classes against S1, S2, and nucleocapsid (N) as well as against N of the four non-SARS human coronaviruses in a commercial, miniaturized 96 wells protein microarray. The ViraChip assay is a useful

Table 1. The primers used in this study, amplifying the membrane protein gene (M) of hCoVs 229E as well as OC43 [12].

Virus	F/R	Gene	Positions	Sequence 5' - 3'
HCoV-229E	F	M	78 - 98	TGGCCCATTAAAAATGTGT
HCoV-229E	R	M	631 - 651	CCTGAACACCTGAAGCCAAT
HCoV-OC43	F	M	215 - 235	GGCTTATGTGGCCCTTACT
HCoV-OC43	R	M	530 - 549	GGCAATCTGCCAAGAATA

tool to identify the epitope-specificity of IgG and IgA in serum samples and can distinguish between the four different nonSARS-hCoVs. The quantitative antibody measurement was performed on a ViraChip Scanner using ViraChip Software.

2.6. Statistics

Chi square test was used to determine the significance level for normally distributed values. Mann-Whitney U test for not normally distributed independent variables was applied after testing for distribution (Kolmogorov-Smirnov-test) with SPSS (Version 24, Chicago, IL). Wilcoxon Rank test was used for not normally distributed dependent variables, Kruskall Wallis test was used for multiple not normally distributed variables. Bonferroni's correction was performed to avoid bias by multiple testing. A p-value < 0.05 was considered statistically significant.

2.7. Ethical Approval and Enrollment Criteria

Swabs were taken and sent to the laboratory in a standardized way at the regional hospital in the context of medical clarification. Every swab that was sent for clarification of a respiratory infection during the study period was used for the study. All clinical swab samples and data were collected for routine patient care and for public health interventions. The study was performed according to the principles of the declaration of Helsinki 2013. Serum samples were obtained from healthy volunteers from the general population, who were submitted to the laboratory after an information interview and informed consent for the assessment of humoral immune status and neutralization ability against SARS-CoV-2 and nonSARS hCoVs during the SARS-CoV-2 pandemic. The serum samples are a representative cross-section of the population in the study area in terms of age and sex.

3. Results

3.1. Prevalence of Respiratory Pathogens during Flu Season

Of the 954 nasopharyngeal swap samples of patients with flu-like symptoms in the pre-pandemic era of October 2018 - March 2020 51.2% (268) were tested for INFV, 50.5% (264) for hMPV, 50.5% (263) for RSV, 2.0% (10) for *B. pertussis*, 6.5% (34) for PIV, 3.3% (17) for *M. pneumoniae*, 3.4% (18) for HAdV and 0.1% (1) for Polyoma BK/JC virus, as shown in **Figure 1**.

In 79 of 954 (8.3%) samples from patients with flu-like symptoms, no pathogen could be detected. These extracts were then RT-qPCR tested for hCoVs OC43 and 229E.

3.2. Demographic Data of the Patients with Flu-Like Symptoms of Unknown Etiology

A distribution of the 79 patients with unknown etiology of flu-like symptoms is shown in **Table 2**, listed according to the hospital ward that had requested the

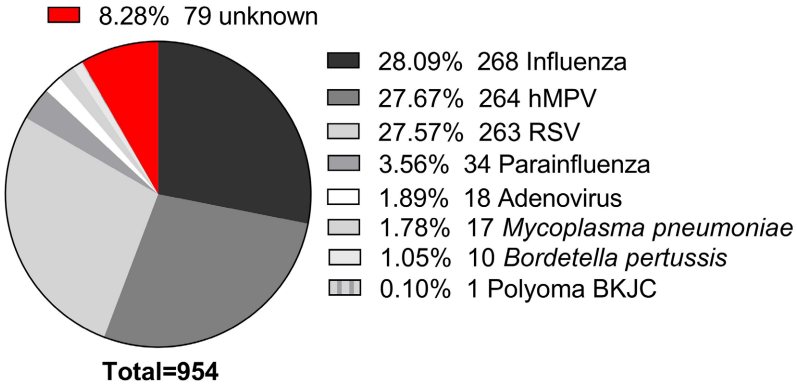

Figure 1. Distribution of pathogens detected by RT-qPCR in 954 nasopharyngeal swabs from patients with flu-like symptoms in the months October 2018 to March 2020.

Table 2. Demographic data of the patients with flu-like symptoms of unknown etiology in qPCR and the hospital wards which requested the PCR-testing.

(a)

Age group [years]	n male	Requesting hospital ward [%]			
		Internal	Paediatrics	Walk-in clinics	Others
0 - 30	6	0	2	2	2
31 - 60	11	3	0	7	1
61 - 100	22	17	1	2	2
Total male	39 [100.0]	20 [51.3]	3 [7.7]	11 [28.2]	5 [12.8]

(b)

Age group [years]	n female	Requesting hospital ward [%]			
		Internal	Paediatrics	Walk-in clinics	Others
0 - 30	10	1	3	2	4
31 - 60	9	0	0	5	4
61 - 100	21	9	0	6	6
Total female	40 [100.0]	10 [25]	3 [7.5]	13 [32.5]	14 [35]
Total	79 [100.0]	30 [38.0]	6 [7.6]	24 [30.4]	29 [36.7]

Internal—section for internal medicine, paediatrics—section for paediatrics, walk-in clinics—diverse walk-in clinics and emergency rooms, others—other hospital wards.

diagnosis of the flu-like symptoms via qPCR. 39 (49%) of these patients were male, 40 (51%) were female. In 2020 the gender distribution in Austria was 50.8% women and 49.2% men [1]. Interestingly, within the male group 51.3% of requests came from the ward for internal medicine. All patients' mean age was 57.8 years of age (sd = 27.6). The youngest patient was three years old, the oldest 97.

Overall, 38.0% and 36.7% of the requests were ordered by the internal or other hospital wards.

3.3. Prevalence of hCoVs OC43 and 229E in Patients with Flu-Like Symptoms of Unknown Etiology

Within the 79 samples with flu-like symptoms of unknown etiology, 11 samples (13.9%) were tested positive for either 229E or OC43 (7 for 229E (5.5%) and 4 for OC43 (3.2%)). Statistical analysis shows a comparable distribution among men and women as well as among age groups. As shown in Table 3 the eleven patients positive for hCoVs OC43 or 229E include five patients below the age of 61 as well as six above that age line. The same is true for the gender distribution—five women and six men.

3.4. Age of Patients with Proven Infections with OC43 or 229E

The average age of all 79 patients tested was 57.8 years old (sd = 27.6). Patients testing positive for 229E were on average 70.6 years old (sd = 18.7). Patients testing positive for OC43 on the other hand were on average 35.2 years old (sd = 22.9). The average age of the patients testing positive for 229E was substantially higher than that of the group testing positive for OC43 ($p > 0.05$). Nevertheless, further examination did not show any significance in this difference between age groups (chi square test; $p > 0.05$).

The distribution of male and female patients was comparable in our study group. Five of the eleven positive samples were female (45.5%) and 6 (54.5%) were male (chi square test; $p > 0.05$). Within the 229E positive group, three patients (42.9%) were female and four (57.1%) were male, whereas the OC43 positive group is split firmly down the middle—two female and two male patients.

Table 3. Prevalence of (a) 229E hCoV and (b) OC43 hCoV in the patients with flu-like symptoms of unknown etiology after testing against the seven common respiratory agents *M. pneumoniae*, *B. pertussis*, FLUAV, FLUBV, hMPV, RSV and PIV.

(a)

Age group [years]	229E RT-qPCR					
	Negative	[%]	Positive	[%]	Total	[%]
0 - 30	16	[20.3]	0	[0.0]	16	[20.3]
31 - 60	18	[22.8]	2	[2.5]	20	[25.3]
61 - 100	38	[48.1]	5	[6.3]	43	[54.4]
Total	72	[91.1]	7	[8.9]	79	[100.0]

(b)

Age group [years]	OC43 RT-qPCR					
	Negative	[%]	Positive	[%]	Total	[%]
0 - 30	15	[19.0]	1	[1.3]	16	[20.3]
31 - 60	18	[22.8]	2	[2.5]	20	[25.3]
61 - 100	42	[53.2]	1	[1.3]	43	[54.4]
Total	75	[94.9]	4	[5.1]	79	[100.0]

Nonetheless, none of these differences proved to be significant (chi square test; p = 0.485; p = 0.683).

3.5. Demographic Data of the Serum Samples

284 volunteers without acute flu-like were tested in a seroprevalence study screening of specific IgG antibodies against hCoV OC43 and 229E. 103 of the volunteers (36.3%) were male and 181 (63.4%) were female, as shown in Table 4. The serum samples were obtained from healthy volunteers who wanted to have their humoral immune response and neutralisation capacity against SARS-CoV-2 and nonSARS hCoVs tested. The samples were divided into three age groups (0 - 30, 31 - 60, 61 - 99 years of age). Within the male patient group, eleven (10.7%) were in the youngest age group, 65 (63.1%) in the middle and 27 (26.2%) in the oldest age group. For the female group 40 (22.1%) were in the youngest, 110 (60.8%) and 31 (17.1%) in the oldest age group. In total, 18.0% (51) were in the youngest, 32.4% (92) in the middle and 49.6% (141) were in the oldest age group. The age distribution was tested with a two-sample Kolmogorov-Smirnov-Test ($p > 0.05$) and could be shown to be normally distributed.

3.6. Seroprevalence and Cross Reactivity

In the IgG Blot against 229E, 148 were positive against 229E and 156 were positive against OC43. The seroprevalence rate was insignificantly higher for OC43 than for 229E (54.9% versus 52.3%) (Wilcoxon rank test; $p > 0.05$). Furthermore, 113 of the 285 sera tested were double positive and showed antibodies specific to both 229E and OC43 (39.6%).

A correlation between the IgG seroprevalence rates of OC43 and 229E in the study group was assessed to test for serological cross-reactivity. A Pearson correlation coefficient of $r = 0.483$ shows a moderate correlation and therefore gives hints for serological cross-reactivity of IgG antibodies against hCoVs OC43 and 229E.

That left us with the necessity to test for a possible correlation between SARS-CoV-2 and either 229E or OC43. Again, we calculated the Pearson correlation coefficient (r). r for 229E and SARS-CoV-2 was 0.424 and 0.089 for OC43 and SARS-CoV-2, giving hints for possible serological cross-reactivities of IgG antibodies against SARS-CoV-2 and 229E ($r = 0.424$). No correlation was shown between hCoV OC43 and SARS-CoV-2.

Table 4. Demographic data of the study group tested for IgG and IgA antibodies against 229E and OC43, divided into age groups (0 - 30, 31 - 60, 61 - 99 years of age).

	0 - 30	[%]	31 - 60	[%]	61 - 99	[%]	total	[%]
Male	11	10.7	65	63.1	27	26.2	103	36.6
Female	40	22.1	110	60.8	31	17.1	181	63.4
Total	51	18.0	92	32.4	141	49.6	284	100.0

3.7. Seroprevalence and Age Structure

We demonstrated a significant difference in seroprevalence against 229E amongst age groups. The older age group (61 - 99 years) showed specific antibodies against 229E significantly more frequently than both other age groups ($p < 0.05$ in ANOVA analysis). There was no significant difference between the young (0 - 30 years) and the middle age group (31 - 60 years) but between both, young and older as well as middle and older age groups ($p < 0.05$; ANOVA: $p = 0.02$), as shown in Figure 2.

In contrast, the increase in seroprevalence rate against OC43 was comparable between the young and the middle age group but significantly higher in the old age group ($p > 0.05$ between young and/or middle versus old age group in ANOVA analysis). The seroprevalence rate was lower against 229E with 37.3% in the young age group than against OC43 with an initial 52.9%, but increased more steeply and finally reached a higher rate in the old age group (70.7% versus 60.3%; Kruskal Wallis test; $p < 0.05$).

3.8. Seroprevalence and Gender

The seroprevalence rate against 229E and against OC43 was equally distributed over both genders. No gender specific differences were detectable in the seroprevalence of specific antibodies against either 229E or OC43 (Figure 3).

For 229E, 48.3% of the women and (87 of 180) and 59.2% of the men (61 of 103) tested positive. For OC43, the gender distribution was comparable with

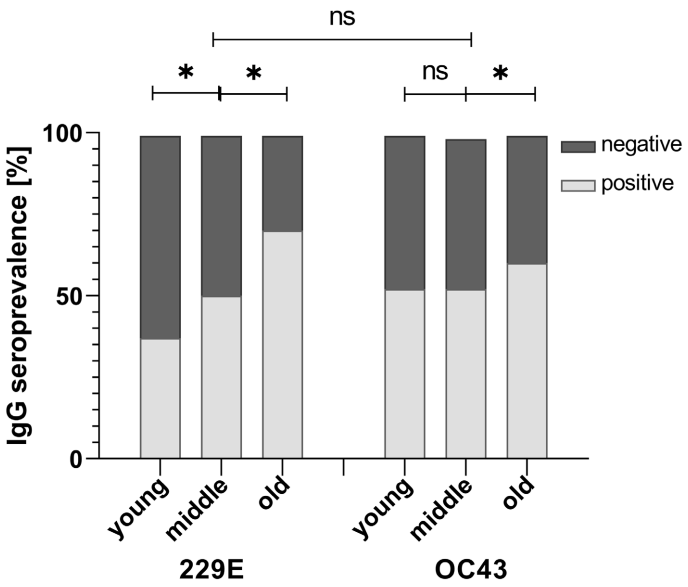

Figure 2. Stacked column charts showing the seroprevalence rate against hCoV 229E and OC43 in three age groups (young: 1 - 30 years; middle: 31 - 60 years; older 61 - 99 years). While the seroprevalence rate of 229E increased significantly in every age group, it remained comparable against OC43 in the young and the middle age group but increased significantly at the old age group (Kruskal Wallis test; $p < 0.05$). No significant difference was found in the seroprevalence rates of 229E and OC43 in the study group of 285 volunteers ($p < 0.05$; ANOVA: $p = 0.02$ versus $p > 0.05$ over all age groups in ANOVA analysis).

56.9% (103 of 180) and 56.6% of the men (53 of 103) tested positive.

3.9. Serological Indications for Acute Infection during Influenza Season

We also analyzed the study group of 284 volunteers for specific IgA antibodies to figure out the number of acute infections. The results of this examination are shown in Figure 4.

Overall, 3.2% of the 284 tested volunteers had a specific IgA antibody response against 229E (9 of 284) and 2.5% against OC43 (7 of 284; chi square test: $p > 0.05$).

Divided into age groups, the IgA rate against 229E increased from 0% in the young study group to 3.4% in the middle and 5.2% in the old age group (chi

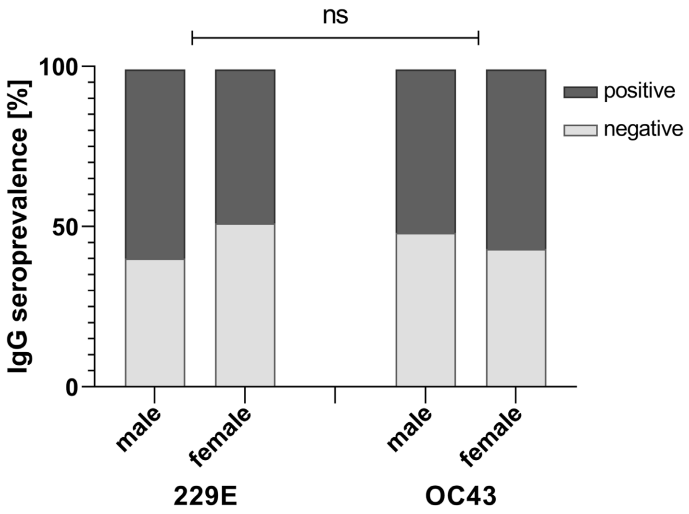

Figure 3. Stacked column charts showing the seroprevalence rates of specific IgG antibodies against 229E and OC43 divided by gender. There is no significant difference in seroprevalence rate between male and female patients ($p > 0.05$).

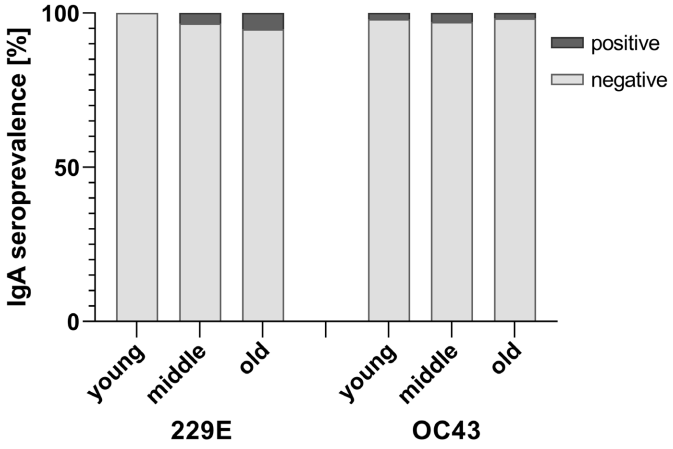

Figure 4. Bar diagram showing the age distribution regarding the seroprevalence rate of specific IgA antibodies against 229E and OC43 in 3 age groups (young: 1 - 30 years of age, middle: 31 - 60; older 61 - 99). The frequency of IgA antibodies is equally distributed for both pathogens over all 3 age groups ($p > 0.05$).

square test; $p > 0.05$). In contrast, we found comparable positivity rates of 2%, 2.9% and 1.7% in the IgA response against OC43.

4. Discussion

In this study we examined the significance and distribution of nonSARS human coronaviruses or cc hCoVs 229E and OC43 in the study area of East Tyrol/Austria. The study of nonSARS human coronaviruses and their frequency in respiratory infections allows us to better understand the extent of the diseases these viruses can cause and to develop appropriate prevention and treatment measures. Furthermore, it contributes to accurate differential diagnosis by helping us to distinguish the symptoms of these viruses from other respiratory infections. In addition, it provides insights into viral dynamics, including distribution and possible seasonal variations, which in turn helps in the development of infection control strategies. Finally, it allows us to better understand the potential of future outbreaks of other human coronaviruses and take appropriate preparatory measures. Overall, this research and studies contribute to a comprehensive understanding of respiratory viruses and the development of appropriate respiratory infection control measures to minimize the public health impact.

For this purpose, 954 nasopharyngeal swap samples were obtained from patients with flu-like symptoms and screened for nine common respiratory infections—*Mycoplasma pneumoniae*, *Bordetella pertussis*, Influenza A and B virus, hMPV, RSV, PIV, HAdV and Polyoma BKJC. If a sample was tested negative for either of the above it was subsequently tested for the presence of non-SARS hCoVs 229E and OC43. Additionally, the local IgG and IgA seroprevalence rates against hCoV 229E and OC43 were determined.

Our results showed that in 13.9% (11 of 79) of flu-like infections of unknown etiology in the study area, hCoV 229E or OC43, respectively, were the causative agents (8.9% 229E; 5% OC43). Overall, of 954 pharyngeal swabs of patients with flu-like symptoms, hCoV 229E or OC43 were the causative agents in 1.2% of the cases (0.7% 229E and 0.4% OC43). Our data match a similar study conducted in Hongkong on 4181 nasopharyngeal aspirates from patients with acute respiratory tract infections, in which the pathogen was identified as 229E in 0.1% of cases and OC43 in 1.3% [13]. A 2010 US study of a total of 1213 patients with flu-like symptoms found infection with hCoV 229E in 4.9% and OC43 in 8.3% [14], which is well above the prevalence rates in the underlying study.

The average age of those infected with 229E was quite high with 70.6 years, while infections with OC43 were more likely to be found in the younger age group with an average of 35.2 years. The significance of these values should be considered with caution due to the small sample size (11 positive for 229E and 7 for OC43). However, age-specific differences in the prevalence of infections with 229E or OC43 were also found in the literature. For example, Weinberg *et al.* found higher infection rates with 229E and OC43, respectively, in patients aged 18 to 49 years than in patients over 50 years [15].

Interestingly, there were significant differences in the seroprevalence rates of the two pathogens in the different age groups: While the seroprevalence rate against 229E increased steadily with age, it was already relatively high against OC43 in the young and middle age groups between 1 - 30 years and 31 - 60 years with over 50% and only increased significantly at the old age group. Generally, steadily increasing seroprevalence rates indicate that the pathogen generates a long-lasting immunity which is frequently boosted. This seems to be reflected in the seroprevalence rates of 229E in this study. On the other hand, the development of the IgG seroprevalence rate against OC43 gives the impression of a pathogen that increasingly affects young patients and that the seroprevalence rate is therefore already quite high at a young age. However, it could also be an indication that the infection with OC43 does not lead to a lasting immunity and that the higher age classes are boosted less frequently.

Similarly conducted studies found that IgG seroprevalence rates for both viruses increased with age [16], while other studies point out that OC43 and 229E circulate at intervals of 2 to 4 years, which argues against long-lasting immunity [7] [17].

The seroprevalence rate of IgA, which reflects acute infections, is therefore of particular interest in this context and could shed light on whether 229E and OC43 produce long-lasting immunity. IgA expression peaks for SARS-CoV-2 10 - 15 days after infection and wanes about one month after infection [18]. At about the 15 day mark IgG expression starts to rise significantly to start replacing IgA in terms of long-term humoral immune response [19]. The seroprevalence rate of IgA against 229E increased insignificantly with age in our study, which seems unusual as the old age group was also the one with the highest specific IgG immunity. On the contrary, re-infection with OC43 was comparable in all three age groups. This fits well with the results of the IgG seroprevalence study against OC43 and is further evidence that immunity to this pathogen is not sustained and re-infection is common.

In this study, we used correlation analyses to determine any cross-reactivity of specific IgG antibodies against 229E, OC43 and SARS-CoV-2 with each other. The results were clear and consistent with those of comparable studies: No correlation, and thus no significant serological cross-reactivity, was found between OC43 and 229E or SARS-CoV-2. On the contrary, we found moderate correlation and thus probably moderate cross-reactivity between 229E and SARS-CoV-2. A comparable study in Finland also found that there is no cross-reactivity between OC43 and 229E in adults, which is consistent with our findings [20]. Also congruent with other studies is our sero-reactivity of cc hCoVs with SARS-CoV-2 [21]. In this study, the authors explain the reasons for possible sero-reactivity between SARS-CoV-2 and cc hCoVs: while the antibodies to the S1 and RBD domains are highly subtype-specific, the S2 domain of spike protein and the nucleocapsid are highly conserved among coronaviruses. SARS-CoV-2 has also produced high seroprevalence rates in the local population we studied, which are

unlikely to have produced neutralizing but possibly diagnostically cross-reactive antibodies [22]. Our correlation analysis was able to show that 229E and OC43 can be differentiated in a serological analysis concerning their respective antibodies and that no correlation exists between these serological results concerning the two cc hCoVs.

5. Conclusion

In summary, this study was able to detect OC43 and 229E in the study area by PCR and to identify them as respiratory pathogens in patients of different ages with flu-like symptoms, and that a small percentage of infections can lead to serious symptoms similar to influenza requiring medical care. Furthermore, for the first time, we were able to provide meaningful seroprevalence rates for IgG and IgA for the study area. The high seroprevalence rates show that both pathogens are common to the study region. Over 50% of the population did come into direct serological contact and a few showed acute infection with one of the two pathogens. The question of whether immunity to one or both pathogens is long-lasting or lasts only a few seasons and the pathogen can recur in waves was not conclusively answered, although our study provided some evidence for the latter theory. In our view the SARS-CoV-2 pandemic is a reminder to keep an eye on all coronaviruses as well as the distribution of different pathogens in our study area.

Declarations

Data Availability Statement

Raw data were generated at Dr. Walder GmbH, Medical Laboratory, Department of Virology, 9931 Ausservillgraten 30, Austria. Derived data supporting the findings of this study are available from the corresponding author (Stefanie Sonnleitner) on request.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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