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Temporal and age distributions of SARS-CoV-2 and other coronaviruses, Southeastern France

Short title (for the running head): Temporal and age distribution of coronaviruses

Author list: Philippe COLSON[1,2,3], Vera ESTEVES-VIEIRA[1,2], Audrey GIRAUD-GATINEAU[1,2,3,4], Christine ZANDOTTI[1,2], Véronique FILOSA[1,2], Hervé CHAUDET[1,2,3,4], Jean-Christophe LAGIER[1,2,3], Didier RAOULT[1,2,3]*

Affiliations: [1] IHU Méditerranée Infection, Marseille, France; [2] Assistance Publique-Hôpitaux de Marseille (AP-HM), Marseille, France; [3] Aix-Marseille Univ., Marseille, France; [4] Service de Santé des Armées (SSA), Marseille, France

*** Corresponding author:** Didier Raoult, IHU - Méditerranée Infection, 19-21 boulevard Jean Moulin, 13005 Marseille, France. Tel.: +33 413 732 401, Fax: +33 413 732 402; email: didier.raoult@gmail.com

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ABSTRACT

Objectives. The SARS-CoV-2 epidemic presents a poorly understood epidemiological cycle. We aimed to compare the age and weekly distribution of the five human coronaviruses, including SARS-CoV-2 that circulated in southeastern France.

Methods. We analyzed all available diagnoses of respiratory viruses including SARS-CoV-2 performed between 09/2013 and 05/2020 at University Hospital Institute Méditerranée Infection in Marseille, Southeastern France.

Results. For SARS-CoV-2, positive children <15 years of age represented 3.4% (228/6,735) of all positive cases, which is significantly less than for endemic coronaviruses (46.1%; 533/1,156; p< 0.001). Among 10,026 patients tested for SARS-CoV-2 and endemic coronaviruses in 2020, children <15 years represented a significantly lower proportion of all positive cases for SARS-CoV-2 than for endemic coronaviruses [2.2% (24/1,067) vs 33.5% (149/445), respectively; p<0.001]. Epidemic curves for endemic coronaviruses and SARS-CoV-2 in 91,722 patients showed comparable bell-shaped distributions with a slight time lag. In contrast, age distribution of endemic coronaviruses and 14 other respiratory viruses differed very significantly compared to that of SARS-CoV2, which was the only virus to spare children.

Conclusions. Thus, we observed for SARS-CoV-2 a temporal distribution resembling that of endemic coronaviruses and an age distribution that spares the youngest subjects who are those the most exposed to endemic coronaviruses.

TEXT

INTRODUCTION

The SARS-CoV-2 epidemic, which apparently started in December in China (Wu and McGoogan, 2020), currently presents a poorly understood epidemiological cycle. It seems to have had in China, Korea and now in Europe a bell-shaped distribution (https://coronavirus.jhu.edu/data/new-cases; https://www.mediterranee-infection.com/covid-19/) as is common for viral respiratory infections. Furthermore, we and others have shown that detection of SARS-CoV-2 in children is rare, as are clinical cases (Colson et al., 2020; Gudbjartsson et al., 2020; Jones et al., 2020; Wu and McGoogan, 2020). Thus, in three large studies, children under 10 years of age accounted for <1%, 0% and 1.3% of SARS-CoV-2 cases in China (Wu and McGoogan, 2020), Iceland (Gudbjartsson et al., 2020) and Germany (Jones et al., 2020), respectively. The fate of this epidemic remains unknown, but we found it interesting to compare the age and weekly distribution of the five human coronaviruses, including SARS-CoV-2 that circulated in south-eastern France in order to compare the temporal and age distribution of these different viruses.

METHODS

We analyzed all available diagnoses of respiratory viruses including SARS-CoV-2 performed between September 2013 and May 2020 at the clinical microbiology and virology laboratory of University Hospital Institute Méditerranée Infection (https://www.mediterranee-infection.com/) and University hospitals of Marseille, the second largest French city, Southeastern France. Testing of respiratory samples were performed using the FTD Respiratory pathogens 21 (Fast Track Diagnosis, Luxembourg), the Biofire FilmArray Respiratory panel 2 plus (Biomérieux, Marcy-l'Etoile, France), the Respiratory Multi Well

System r-gene (Argene, BioMérieux), or the GeneXpert Xpert Flu/RSV (Cepheid, Sunnyvale, CA) assays, or by one-step simplex real-time quantitative RT-PCR amplifications as previously reported (Hoang et al., 2019). Diagnosis by reverse transcription-PCR of SARS-CoV-2 infection was performed as previously described (Amrane et al., 2020). This study retrospectively analyzed patients' data issued from the hospital information system (RGPD/APHM 2019-73). Statistics were performed using OpenEpi version 3.01 software (https://www.openepi.com/Menu/OE_Menu.htm); a p-value< 0.05 was considered significant. Moreover, epidemic curves were analyzed by Markov Chain Monte Carlo fitting of five commonly used distributions with different skewnesses (Normal, Log-normal, Gamma, Weibull, Gompertz) using R-4.0.1 (https://www.r-project.org/). Distributions with the best goodness-of-fit criteria [Akaike's Information Criterion, (AIC)] were choosen and their parameters bootstrapped.

RESULTS

First we analyzed all available diagnoses of SARS-CoV-2 or other respiratory viruses for 141,227 patients. Between January and May 2020, we tested respiratory samples from 80,024 patients for SARS-CoV-2 and found 6,735 (8.4%) positive (Figure 1). In addition, between September 2013 and May 2020 we tested respiratory samples from 69,752 patients for respiratory viruses. Of them, 17,673 were tested for endemic coronaviruses (HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1) and 1,156 (6.5%) were positive. For SARS-CoV-2, positive children under 15 years represented 3.4% (228/6,735) of all positive patients. This proportion was significantly lower than for endemic coronaviruses (46.1%; 533/1,156; p< 0.001, Chi-square test). In fact, positive patients in each group 0-1 year, 1-5 years, 5-10 years and 10-15 years represented significantly lower proportions of all positive patients when considering SARS-CoV-2 than endemic coronavirus infections (Table 1). Compared to

SARS-CoV-2-positive patients, those infected with endemic coronaviruses or other respiratory viruses were significantly more likely to be <10 years of age (Figure 1). Therefore, this age group accounted for 1.8% of SARS-CoV-2 cases compared to 25.0% (for HCoV-229E) and 87.0% (for bocavirus) of infections with other respiratory viruses ($p< 0.05$ for all comparisons).

Second, we analyzed 10,026 patients tested for both SARS-CoV-2 and endemic coronaviruses between January 1st and May 25th, 2020. A total of 1,067 patients (10.6%) were SARS-CoV-2- positive and 445 (4.4%) were diagnosed with endemic coronaviruses. Children under 15 years of age accounted for a significantly lower proportion of all positive cases for SARS-CoV-2 than for endemic coronaviruses [2.2% (24/1,067) vs 33.5% (149/445), respectively; $p<0.001$] as was the case in each age group: 0-1 year, 1-5 years, 5-10 years and 10-15 years (Figure 2A, Table 2). Only 11 (0.11%) patients were infected with SARS-CoV-2 and an endemic coronavirus. They represented a significantly lower proportion than the proportion of SARS-CoV-2-positive patients among those negative for endemic coronaviruses [11/445 (2.5%) vs 1,056/9,581 (11.0%); $p< 0.001$]. None of these 11 patients was under 18 years of age.

Moreover, over a one-year period (from June 2019 to May 2020), we observed that epidemic curves were comparable for the four endemic coronaviruses and SARS-CoV-2 (Figure 2B). Cases of endemic coronavirus increased in December 2019, peaked in mid-March 2020 and ended in early April, while cases of SARS-CoV-2 increased in early March, peaked in late March and nearly ended in mid-May. The fitted distributions reflected three kinds of epidemic curves (Supplementary Figure 1). SARS-CoV-2 fitted with a left-skewed Gamma distribution (AIC=26345.6). HCoV-OC43 fitted with a quasi-symmetric curve and Normal distribution (AIC=971.4). Epidemic curves of HCoV-229E, HCoV-NL63 and HCoV-HKU1 were right skewed and fitted with a Gompertz distribution (AIC= 394.5, 1191.2, and

1861.2, respectively).

DISCUSSION

In this large study, two elements are particularly noteworthy. First, the temporal distributions in our geographical area of infections by all coronaviruses are comparable. Thus, all five viruses have a bell-shaped incidence curve and their circulation stopped in the spring, suggesting that this is the natural SARS-CoV-2 epidemic pattern. Hence, we can speculate for temperate countries including Europe that SARS-CoV-2 could reappear seasonally during winter and circulate epidemically until spring. Alternatively, SARS-CoV-2 might disappear in the absence of asymptomatic human chronic carriage, like SARS-CoV-1 (Raoult et al., 2020). Second, the age distribution of SARS-CoV-2 cases spares children considerably, which is radically different from other coronavirus and respiratory virus infections. Thus, SARS-CoV-2 is the only one we analyzed that does not significantly affect children. Therefore, its epidemiology could not be predicted based on previous knowledge of viral respiratory diseases. The simplest explanation for this difference is that a substantial proportion of children, and particularly those under 5 years of age, may have acquired immunity to endemic coronaviruses that infect young children with high frequencies (Raoult et al., 2020; Zhou et al., 2013). Indeed, there is evidence that part of the population was immunized against SARS-CoV-2 before the epidemic, supporting the hypothesis of cross-immunity between endemic coronaviruses and the new coronavirus. Thus, in the US, circulating SARS-CoV-2-specific CD4+ and CD8+ T cells were detected in ≈20-60% of unexposed individuals sampled in 2015-2018 (Grifoni et al., 2020). In the UK, IgG to SARS-CoV-2 were detected in 15% of SARS-CoV-2-uninfected patients with recent HCoV infection and in 10% of SARS-CoV-2-uninfected pregnant women (Ng et al., 2020). In addition, we detected IgM to SARS-CoV-2 at titers ≥1:100 in 9/50 patients with endemic coronaviruses (Edouard et al., 2020). It is also

worth noting that the coinfection rate observed here with SARS-CoV-2 and another coronavirus was very low (0.1%) and that SARS-CoV-2-positivity was significantly lower among patients positive than negative for an endemic coronavirus, which supports the hypothesis of a protective cross-immunity.

Overall, we believe that this work contributes to the understanding of the epidemiology of SARS-CoV-2, which has a temporal distribution resembling that of endemic coronaviruses and an age distribution that spares the youngest subjects who are precisely those the most frequently exposed to endemic coronaviruses and may have consequently acquired protective immunity. Susceptibility to SARS-CoV-2 in elderly perhaps reflects the loss of immunity acquired during childhood, or changes in social organization that occurred during recent decades. Indeed, a small proportion of people over the age of 50 lived in communities with very young children, whereas women's work development has led to a much earlier socialization of children. Finally, the fact that age distributions for infections by SARS-CoV-2 and other respiratory viruses differ underscores that real data collection and real-time analysis are critical in the event of an outbreak to decipher the epidemiology of emerging pathogens.

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Ethics. All data have been generated as part of the routine work at Assistance Publique-Hôpitaux de Marseille (Marseille university hospitals), and this study results from routine standard clinical management. Access to the patients' biological and registry data issued from the hospital information system was approved by the data protection committee of Assistance Publique-Hôpitaux de Marseille (APHM) and was recorded in the European General Data Protection Regulation registry under number RGPD/APHM 2019-73. This study has been approved by our institution's ethics committee. The authors have no conflicts of interest to declare. Funding sources had no role in the design and conduct of the study; collection, management, analysis and interpretation of the data; and preparation, review, or approval of the manuscript.

Author contributions. Conceived and designed the experiments: DR. Contributed materials/analysis tools: PC, VEV, AGG, CZ, VF, HC, JCL. Analyzed the data: PC, HC, DR. Wrote the paper: PC, DR.

REFERENCES

Amrane S, Tissot-Dupont H, Doudier B, Eldin C, Hocquart M, Mailhe M et al. Rapid viral diagnosis and ambulatory management of suspected COVID-19 cases presenting at the infectious diseases referral hospital in Marseille, France, - January 31st to March 1st, 2020: A respiratory virus snapshot. TravelMedInfect Dis 2020;101632.

Colson P, Tissot-Dupont H, Morand A, Boschi C, Ninove L, Esteves-Vieira V et al. Children account for a small proportion of diagnoses of SARS-CoV-2 infection and do not exhibit greater viral loads than adults. Eur J Infect Dis Clin Microbiol 2020; in press.

Edouard S, Colson P, Melenotte C, De Pinto F, Thomas L, La Scola B et al. Evaluating the serological status of COVID-19 patients using an indirect immunofluorescent assay, France. Preprint IHU 2020, URL; https://www.mediterranee-infection.com/children-account-for-a-small-proportion-of-diagnoses-of-sars-cov-2-infection-and-do-not-exhibit-greater-viral-loads-than-adults/

Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR et al. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. Cell 2020; S0092-8674(20)30610-3.

Gudbjartsson DF, Helgason A, Jonsson H, Magnusson OT, Melsted P, Norddahl GL et al. Spread of SARS-CoV-2 in the Icelandic Population. N.Engl.J Med 2020;382:2302-2315.

Hoang VT, Goumballa N, Dao TL, Ly TDA, Ninove L, Ranque S et al. Respiratory and gastrointestinal infections at the 2017 Grand Magal de Touba, Senegal: A prospective cohort survey. TravelMed InfectDis 2019;32:101410.

Jones TC, Muhlemann B, Veith T, Zuchowski M, Hofman J, Stein A et al. An analysis of SARS-CoV-2 viral load by patient age. medRxiv preprint 2020. doi: https://doi.org/10.1101/2020.06.08.20125484

Ng K, Faulkner N, Cornish G, Rosa A, Earl C, Wrobel A et al. Pre-existing and de novo humoral immunity to SARS-CoV-2 in humans. bioRxiv preprint 2020. doi: https://doi.org/10.1101/2020.05.14.095414

Raoult D, Zumla A, Locatelli F, Ippolito G, and Kroemer G. Coronavirus infections: Epidemiological, clinical and immunological features and hypotheses. Cell Stress 2020;4:66-75.

Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases From the Chinese Center for Disease Control and Prevention. JAMA 2020;2762130.

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

FIGURE LEGENDS

Figure 1. Age distribution of the proportions of patients diagnosed with coronaviruses and other respiratory viruses compared to the total population tested

(i) SARS-CoV-2; (ii) Coronavirus-229E; (iii) Coronavirus-NL63; (iv) Coronavirus-OC43; (v) Coronavirus-HKU1; (vi) Human parainfluenzavirus 1; (vii) Human parainfluenzavirus 2; (viii) Human parainfluenzavirus 3; (ix) Human parainfluenzavirus 4; (x) Metapneumovirus; (xi) Rhinovirus; (xii) Enterovirus; (xiii) Paraechovirus; (xiv) Adenovirus; (xv) Influenza A H3N2 virus; (xvi) Influenza A H1N1 virus; (xvii) Influenza B virus; (xviii) Respiratory syncytial virus; (xix) Bocavirus.

Figure 2. Number of diagnoses per age group for patients tested for all coronaviruses (A), and number of patients positive for coronaviruses over one year from June 2019 through May 2020 (B)

A. (i) SARS-CoV-2; (ii) Human coronavirus (HCoV)-229E; (iii) HCoV-NL63; (iv) HCoV-OC43; (v) HCoV-HKU1.

B: (i) SARS-CoV-2; (ii) HCoV-229E; (iii) HCoV-NL63; (iv) HCoV-OC43; (v) HCoV-HKU1.

TABLES

Table 1. Number of cases per age group for all patients tested for SARS-CoV-2 or for endemic coronaviruses, and proportion of all tested patients per age group

Age group (years)	SARS-CoV-2				Endemic CoV				P *
	Tested		Positive		Tested		Positive		
	N	*% **	N	*% **	N	*% **	N	*% **	
0-1	796	*1.0*	32	*0.5*	2 412	*14.6*	207	*17.9*	<0.001
1-5	1 453	*1.8*	40	*0.6*	1 661	*11.3*	217	*18.8*	<0.001
5-10	1 231	*1.5*	50	*0.7*	628	*4.9*	65	*5.6*	<0.001
10-15	1 197	*1.5*	106	*1.6*	366	*3.0*	44	*3.8*	<0.001
15-18	1 090	*1.4*	118	*1.8*	202	*1.6*	12	*1*	0.051
18-25	6 680	*8.3*	594	*8.8*	409	*3.9*	43	*3.7*	<0.001
25-45	27 059	*33.6*	2 184	*32.4*	1 502	*14.9*	165	*14.3*	<0.001
45-65	24 487	*30.4*	2 257	*33.5*	2 250	*18.3*	176	*15.2*	<0.001
65-75	6 545	*8.1*	560	*8.3*	1 419	*10.3*	79	*6.8*	0.050
>75	9 986	*12.4*	794	*11.8*	2 528	*17.2*	148	*12.8*	0.175
Total	80 524	*100.0*	6 735	*100.0*	17 673	*100.0*	1 156	*100.0*	-

* Yates-corrected Chi-square test; ** Proportion of cases in the age group compared to the total number of cases

Table 2. Number of cases per age group for SARS-CoV-2 or endemic coronaviruses for patients tested for all five coronaviruses, and proportion of all tested patients per age group

Age group (years)	Tested		SARS-CoV-2-positive		Endemic CoV-positive		P *
	N	*% ***	N	*% ***	N	*% ***	
0-1	477	*4,8*	11	*1.0*	41	*9.2*	<0.001
1-5	715	*7,1*	5	*0.5*	68	*15.3*	<0.001
5-10	402	*4,0*	4	*0.4*	18	*4.0*	<0.001**
10-15	270	*2,7*	4	*0.4*	22	*4.9*	<0.001**
15-18	160	*1,6*	11	*1.0*	5	*1.1*	0.454
18-25	590	*5,9*	75	*7.0*	25	*5.6*	0.186
25-45	2 321	*23,1*	245	*23.0*	109	*24.5*	0.283
45-65	2 491	*24,8*	385	*36.1*	83	*18.7*	<0.001
65-75	1 002	*10,0*	128	*12.0*	26	*5.8*	<0.001
>75	1 598	*15,9*	199	*18.7*	48	*10.8*	<0.001
Total	10 026	*100,0*	1 067	*100.0*	445	*100.0*	-

* Yates-corrected Chi-square test; ** Fischer exact test; *** Proportion of cases in the age group compared to the total number of cases

Fig 1.

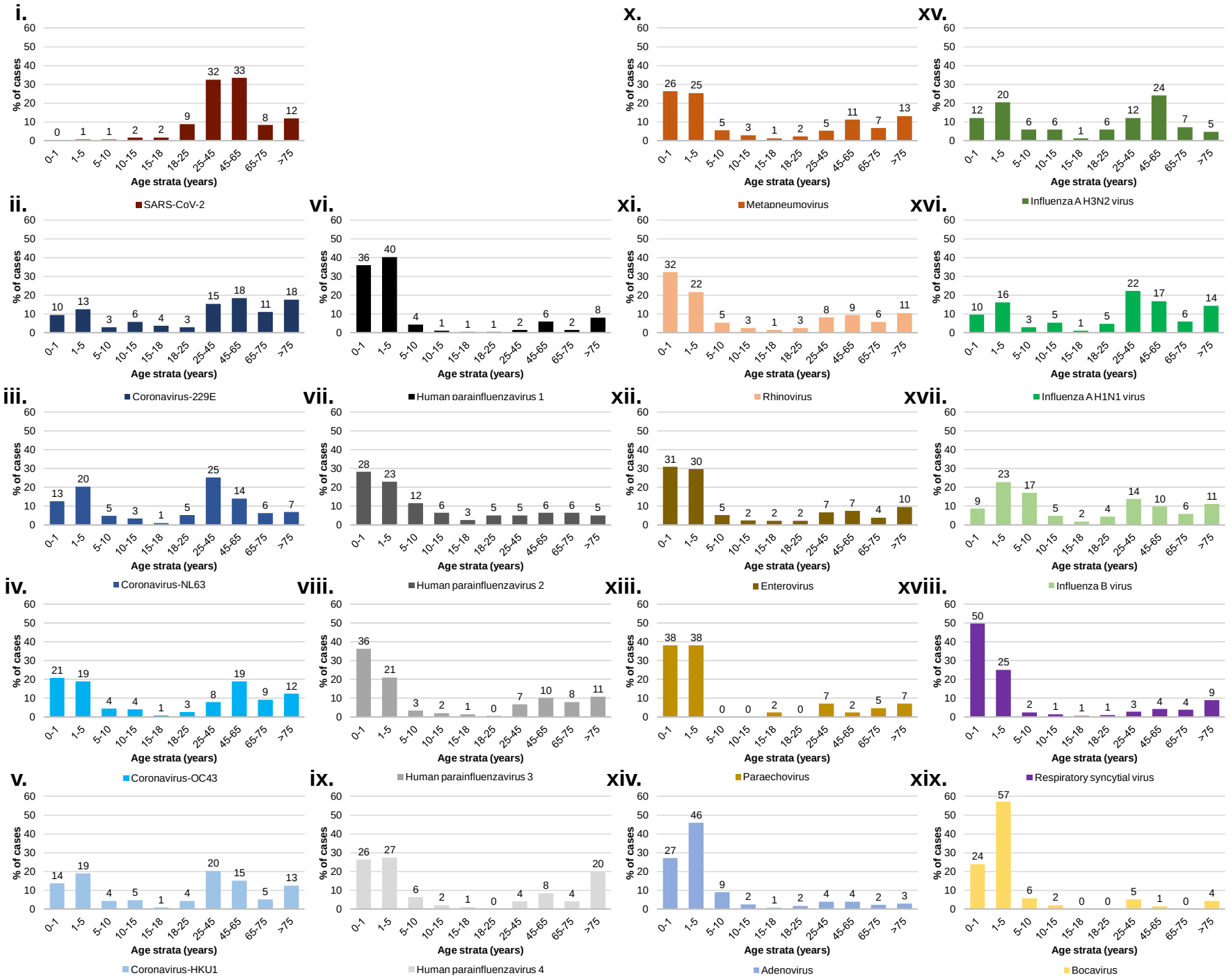
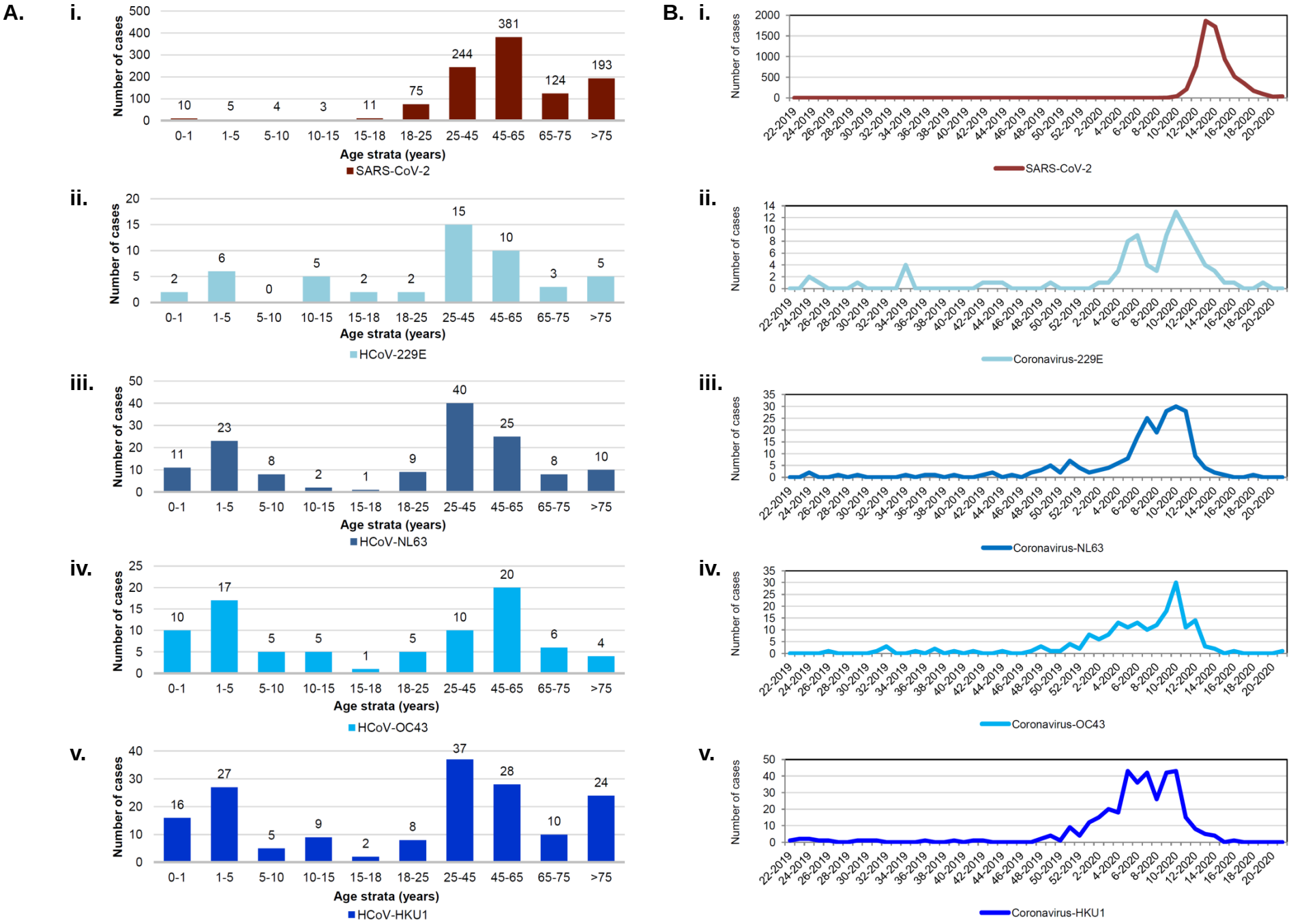

i.
% of cases
Age strata (years)
0-1 1-5 5-10 10-15 15-18 18-25 25-45 45-65 65-75 >75
0 1 1 2 2 9 32 33 8 12
ii.
SARS-CoV-2
10 13 3 6 4 3 15 18 11 18
iii.
Coronavirus-229E
13 20 5 3 1 5 25 14 6 7
iv.
Coronavirus-NL63
21 19 4 4 1 3 8 19 9 12
v.
Coronavirus-OC43
14 19 4 5 1 4 20 15 5 13
vi.
36 40 4 1 1 1 2 6 2 8
vii.
Human parainfluenzavirus 1
28 23 12 6 3 5 5 6 6 5
viii.
Human parainfluenzavirus 2
36 21 3 2 1 0 7 10 8 11
ix.
Human parainfluenzavirus 3
26 27 6 2 1 0 4 8 4 20
x.
Metapneumovirus
26 25 5 3 1 2 5 11 7 13
xi.
32 22 5 3 1 3 8 9 6 11
xii.
Rhinovirus
31 30 5 2 2 2 7 7 4 10
xiii.
Enterovirus
38 38 0 0 2 0 7 2 5 7
xiv.
Paraechovirus
27 46 9 2 1 2 4 4 2 3
xv.
Influenza A H3N2 virus
12 20 6 6 1 6 12 24 7 5
xvi.
10 16 3 5 1 5 22 17 6 14
xvii.
Influenza A H1N1 virus
9 23 17 5 2 4 14 10 6 11
xviii.
Influenza B virus
50 25 2 1 1 1 3 4 4 9
xix.
Respiratory syncytial virus
24 57 6 2 0 0 5 1 0 4
Adenovirus
Bocavirus

Fig 2.

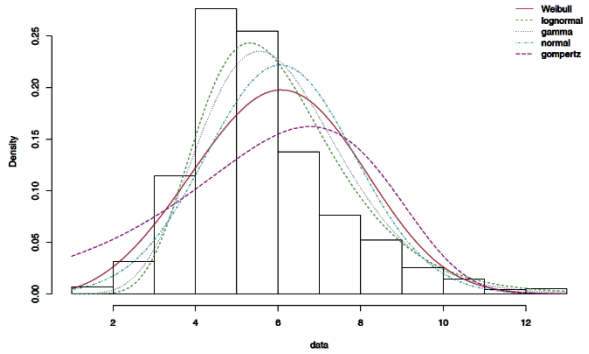

A.
i.
Number of cases
Age strata (years)
0-1 1-5 5-10 10-15 15-18 18-25 25-45 45-65 65-75 >75
10 5 4 3 11 75 244 381 124 193
SARS-CoV-2
ii.
2 6 0 5 2 2 15 10 3 5
HCoV-229E
iii.
11 23 8 2 1 9 40 25 8 10
HCoV-NL63
iv.
10 17 5 5 1 5 10 20 6 4
HCoV-OC43
v.
16 27 5 9 2 8 37 28 10 24
HCoV-HKU1
B.
i.
SARS-CoV-2
ii.
Coronavirus-229E
iii.
Coronavirus-NL63
iv.
Coronavirus-OC43
v.
Coronavirus-HKU1

LEGEND TO SUPPLEMENTARY FIGURE

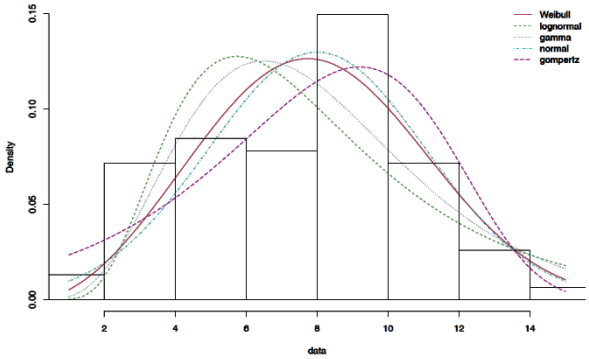
Supplementary Figure 1. Histograms and theoritical densities for distributions with different skewnesses (Normal, Log-normal, Gamma, Weibull, Gompertz) of epidemic curves of SARS-CoV-2 (a); HCoV-229E (b); HCoV-NL63 (c); HCoV-OC43 (d), and HCoV-HKU1 (e) over one year from June 2019 through May 2020.

Supplementary Fig. 1

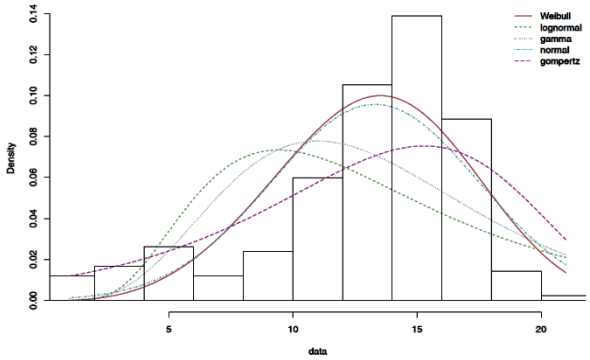
a. SARS-CoV-2



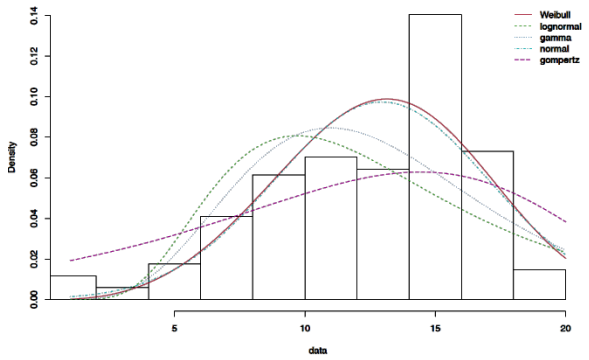
b. HCoV-229E



c. HCoV-NL63



d. HCoV-OC43



e. HCoV-HKU1