BMJ Paediatrics Open

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Journal:	*BMJ Paediatrics Open*
Manuscript ID	bmjpo-2020-000887
Article Type:	Original research
Date Submitted by the Author:	23-Sep-2020
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Keywords:	Epidemiology, Virology

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Assessment of spread of SARS-CoV-2 by RT-PCR and concomitant serology in children in a region heavily affected by COVID-19 pandemic

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Abstract

Background. Several studies indicated that children seem to be less frequently infected with SARS-CoV-2 and potentially less contagious. To examine the spread of SARS-CoV-2 we combined both RT-PCR testing and serology in children in the most affected region in France, during the COVID-19 epidemic.

Methods. From April 14, 2020 to May 12, 2020, we conducted a cross-sectional prospective, multicenter study. Healthy controls and pauci-symptomatic children from birth to age 15 years were enrolled by 27 ambulatory pediatricians. A nasopharyngeal swab was taken for detection of SARS-CoV-2 by RT-PCR and a microsample of blood for micro-method serology.

Results. Among the 605 children, 322 (53.2%) were asymptomatic and 283 (46.8%) symptomatic. RT-PCR testing and serology were positive for 11 (1.8%) and 65 (10.7%) of all children, respectively. Only 3 children were RT-PCR–positive without any antibody response have been detected. The frequency of positivity on RT-PCR for SARS-CoV-2 was significantly higher in children with positive serology than those with a negative one (12.3% vs 0.6%, p<0.001). Contact with a person with proven COVID-19 increased the odds of positivity on RT-PCR (OR 7.8, 95% confidence interval [1.5; 40.7]) and serology (15.1 [6.6; 34.6]).

Conclusion. In area heavily affected by COVID-19, after the peak of the first epidemic wave and during the lockdown, the rate of children with positive SARS-CoV-2 RT-PCR was very low (1.8%), but the rate of positive on serology was higher (10.7%). Most of PCR positive children had at the same time positive serology.

What is already known on this topic?

- As compared with adults, children seem to be less frequently infected with SARS-CoV-2 and potentially less contagious according to several studies.

- Most of the studies were based on RT-PCR SARS-CoV-2 testing, without antibody assays.

What this study adds?

- This study combining RT-PCR and serologic testing, assessed the spread of SARS-CoV-2 infection in children in area heavily affected by COVID-19 pandemic.
- Among a large cohort of children (>600), 11 (1.8%) were positive on RT-PCR for SARS-CoV-2 and 65 (10.7%) were positive on serology.
- The only factor affecting positivity of RT-PCR for SARS-CoV-2 or serology was the household contact COVID-19.

Introduction

Since the beginning of the COVID-19 pandemic, reports from several countries indicated that the disease was less frequent and less severe in children than adults.[1-3] Worldwide, the number of confirmed pediatric cases seems relatively low, and they account for less than 1% of hospitalized cases and deaths.[1, 4] Although most COVID-19 cases in children are not severe, serious COVID-19 illness resulting in hospitalization can occur in this age group, and recently, hyperinflammatory shock, showing features similar to atypical Kawasaki disease were reported in several countries.[5-10]

However, concerns have been raised that children could play an important role in the spread of the disease because community testing has demonstrated a significant number of children with no or subclinical symptoms.[11] Indeed, if as for influenza, children could be the primary drivers of household SARS-CoV-2 transmission, then a silent spread from children who did not alert anyone to their infection could be a serious driver in the dynamics of the epidemic.[12] On the basis of this prevailing hypothesis, school closures were implemented almost ubiquitously around the world to try to halt the potential spread of COVID-19 despite early modelling suggesting that this would have less impact than most other non-pharmacological interventions.[13, 14]

However, several studies had already shown that when SARS-CoV-2 infection was suspected (compatible clinical signs, contact with a person with COVID-19), the rate of positivity on RT-PCR for SARS-CoV-2 was lower in children than adults.[14,15] In contrast, in RT-PCR SARS-CoV-2–positive children, the viral load was comparable between children and adults.[16] Furthermore, one study suggested that children shed

infectious SARS-CoV-2.[17] However, results from a systematic review of household clusters of COVID-19 revealed that only 3/31 clusters were due to a child index case, and a population-based school contact-tracing study found minimal transmission by child or teacher index cases.[18, 19] Finally, other studies suggested that children were potentially less contagious than adults.[16, 20-22]

Some countries such as South Korea and Iceland have implemented widespread community testing. Both countries found children significantly underrepresented in cases. In Iceland, this was true in targeted testing of high-risk groups as compared with adults (6.7% < 10 years vs 13.7% ≥ 10 years positive), and in (invited) population screening, no child under 10 years old was positive for SARS-CoV-2 as compared with 0.8% in the general population. [23]

Of note, all these studies were based on RT-PCR testing, but serology diagnosis is also an important tool to understand the spread and burden of COVID-19.[24] A serology survey tested adolescents in a high school in the north of France, the site of a cluster at the end of February. Of the 242 students tested, 2.7% of children ≤ 14 years old and 40% aged 15-17 years were positive on SARS-CoV-2 serology (IgG), which suggests a difference in susceptibility to SARS-CoV-2 among younger children.[25]

To best approach the spread and dynamics of transmission of SARS-CoV-2 in children at a population level, we combined both RT-PCR testing for SARS-CoV-2 and serology in asymptomatic or pauci-symptomatic children (with mild clinical symptoms) in the Paris area, the most affected region in France, during the COVID-19 epidemic.

Patients and Methods

Study population

This was a cross-sectional prospective, multicenter study conducted by the Association Clinique et Thérapeutique Infantile du Val de Marne (ACTIV) network, a research unit expert in epidemiological surveillance and clinical studies in ambulatory pediatric infectious diseases, and the University Intercommunal Créteil Hospital.[26] Primary care pediatricians (n = 27) took part in the study from April 14, 2020 to May 12, 2020. The strategy of closing schools and the lockdown decided by the French government for the whole country started on March 17 and finished on May 11, 2020.

This study aimed to enroll children from birth to 15 years of age consulting an ambulatory pediatrician and distributed in two groups: asymptomatic and pauci-symptomatic. Asymptomatic children were defined as children without any symptoms or signs suggesting infectious disease during the last 7 days. In this group, we defined two subgroups of children: those who had history of symptoms (fever or respiratory or digestive) between 7 days and 2 months before enrollment, and those without any history if symptoms. Pauci-symptomatic children were defined as those with fever isolated or associated with respiratory signs such as cough, dysphagia, rhinorrhea, diarrhea, vomiting, cutaneous signs, taste loss and/or anosmia during the last 7 days. Children were excluded if the clinical condition at enrollment required transfer to pediatric emergency unit or hospitalization.

After informing the parents of the participating children and obtaining their signed consent, an electronic case report form (eCRF) was completed by the

pediatrician to collect socio-demographic data, history, contact with a person with confirmed COVID-19 by RT-PCR SARS-CoV-2, clinical symptoms and signs, and additional positive biological tests. We have also collected suspected COVID-19 contacts, because of the limited availability of testing. Indeed, during the lockdown, the diagnostic RT-PCR SARS-CoV-2 test was mainly available for patients with severe disease and/or healthcare workers, and all symptomatic individuals could not be tested. For all enrolled children, during the same visit, a nasopharyngeal (NP) swab was taken for RT-PCR detection of SARS-CoV-2 and a microsample of blood for micro-method serology.

Calculation of the number of patients

To have an appropriate proportion of confirmed RT-PCR SARS-CoV-2–positive patients among asymptomatic children and pauci-symptomatic patients, with a 95% confidence interval (CI) of +/-3%, assuming a positivity proportion < 10%, we needed to enroll 300 children per group (asymptomatic and pauci-symptomatic), for 600 patients in total.

Serological assays

Pediatricians collected fingerstick whole-blood specimens and used the Biosynex COVID-19 BSS test, a rapid chromatographic immunoassay, for qualitive detection of IgG and IgM antibodies to SARS-CoV-2 in blood. This test targeted the spike protein fragment receptor binding domain and was among those approved by the French national health authority.[27] According to the specifications of the manufacturer, the diagnostic accuracy of the test was sensitivity 91.8% [95% CI 83.8-96.6] and specificity

99.2% [95%CI 97.7-99.8] (https://www.biosynex.com/laboratoires-hopitaux-tests-covid-19/). However, the accuracy of the test was not stratified by age and for patients infected with other Coronavirus (OC43, 229E, NL63, HKU1) no cross-reaction was observed.

Furthermore, assessment by independent investigators confirmed the good diagnostic accuracy of this test among hospital staff with mild disease in eastern France [28].

SARS-CoV-2 RT-PCR methods

The NP specimens were obtained by using the collection system eSwab™ (Minitip size nylon flocked swab placed in 1 mL of modified liquid Amies transport medium, COPAN, Brescia, Italy). They were transported to the centralized microbiology laboratory (CHIC). Before extraction, each sample was inactivated by the addition of 750 µl / ml of STARmag lysis buffer solution (Seegene, South Korea). The RT-PCR for SARS-CoV-2 was performed on the automated Seegene STARlet system®, according to the manufacturer's instructions using the CE marked AllplexTM 2019-nCoV RT-PCR assay (Seegene, South Korea®) which targets N- (viral nucleocapsid protein) and RdRP-gene (RNA-dependent RNApolymerase), both SARS-CoV-2 specific genes, and the sarbecovirus specific E-gene.

The automated Hamilton STARlet system was used for automated viral RNA extraction using the STARMag 96 Universal Cartridge kit (Seegene, South Korea) and PCR set up. Subsequently, 8 µL of extracted nucleic acids was added to 17 µL of the PCR Master Mix, and amplification and detection were performed on the CFX96™ detection system

(Bio-Rad, France) as per manufacturer's instruction. Ct from FAM (E gene), Cal Red 610 (RDRP gene), Quasar 670 (N gene) and HEX (internal control) were acquired. Before extraction, internal control (10 µl) was added to verify extraction and determine PCR inhibition. Positive (plasmids encoding the three AllplexTM 2019-nCoV assay target sequences) and negative (RNase-free water added to the master mix prior to PCR) controls were included in each run. The cycle threshold values (Ct) were used as indicators of the copy number of SARS-CoV-2 RNA specimens with lower Ct values corresponding to higher viral copy numbers. NP samples were considered positive when a Ct less than 40 was obtained for any gene. Amplification of two or three targets indicated that SARS-CoV-2 RNA was detected, while amplification of only one target indicated a presumptive positive result. In addition, we defined as weakly positive any result with a Ct > 38 and < 40. A sample was considered negative if the internal control was amplified but not the viral target genes. A sample was considered invalid when no amplification was obtained for the internal control.

Statistical analysis

Data were entered by using the eCRF (PHP/MySQL) and analyzed by using Stata/SE v15 (StataCorp, College Station, TX, USA). Quantitative data were compared by Student *t* test and qualitative data by chi-square or Fisher exact test. We used a logistic regression model for analysis of factors associated with positivity on RT-PCR for SARS-CoV-2 and serology. Variables (age, clinical signs, contact, siblings and daycare attendance modalities) with $p < 0.20$ on univariate analysis were included in the model,

estimating odds ratios (ORs) and 95% CIs. Only significant variables ($p<0.05$) were kept in the final model. All tests were 2-sided and were considered significant at $p<0.05$.

Ethics

The study protocol was approved by an ethics committee (CPP IDF IX no. 08-022). Parents of all infants provided written informed consent. The study was registered at ClinicalTrials.gov NCT04318431.

Patient and Public Involvement

There were no patients or public involved in the research design, process and research findings dissemination.

Results

From April 14, 2020 to May 12, 2020, 27 ambulatory pediatricians in the Paris area enrolled 605 children: 322 (53.2%) were asymptomatic and 283 (46.8%) pauci-symptomatic. Table 1 presents the characteristics of the enrolled children by group. In the pauci-symptomatic group, the main signs and symptoms were fever (187, 66.3%), cough (143, 50.7%), pharyngitis (143, 50.7%), rhinitis (137, 48.4%), diarrhea (81, 28.7%), cutaneous criteria (64, 23.0%), vomiting (52, 18.8%), taste loss (8, 3.0%) and anosmia (5, 3.3%).

Figure 1 presents the dynamics of the first epidemic wave in Paris area[29], the dates of the lockdown and the number of children enrolled by weeks.

RT-PCR SARS-CoV-2 tests were positive for 11 (1.8%) children, with no significant difference between the two groups (Table 2). The supplemental Table shows the details of the 11 positive RT-PCR for SARS-CoV-2. Only 3 children had positive RT-PCR SARS-CoV-2 result with Ct less than 31.

On multivariable analysis, contact with a person with proven COVID-19 was the only significant risk factor for RT-PCR–positive SARS-CoV-2 infection (OR 7.8, 95% CI [1.5; 40.7]).

Table 2 shows also the serology results by group. Serology was positive for 65 (10.7%) children, whatever the group, and among these, 87.3% had a confirmed or suspected contact. Children with history of symptoms during the preceding weeks, more frequently were positive on serology.

Table 3 presents the RT-PCR SARS-CoV-2 results by serology status. The frequency of positivity was significantly higher in children with positive serology than those with a negative one (12.3% vs 0.6%, p<0.001). Only 3 children were RT-PCR SARS-CoV-2–positive without any antibody response detected.

Table 4 shows RT-PCR SARS-CoV-2 and serology positivity by contact with a person with suspected or confirmed COVID-19. Only 2 of 275 (0.7%) children without any contact with a person with COVID-19 were positive on RT-PCR for SARS-CoV-2.

On multivariate analysis, positivity on serology was associated with contact with a person with proven or suspected COVID-19 (OR 15.1 [95% CI 6.6; 34.6] and 5.8 [95% CI 2.6; 13.2]).

Discussion

This study combines RT-PCR SARS-CoV-2 and serology results to assess the spread of SARS-CoV-2 infection in a large cohort of children in the community. In a region strongly affected by the epidemic (Paris area), but during the lockdown, very few children (1.8%) were positive on RT-PCR for SARS-CoV-2, but the rate of children positive on serology (10.7%) was higher. Despite the relatively large number of children included (>600), we did not find a significant difference in the rate of positive RT-PCR or serology results between asymptomatic and pauci-symptomatic children.

Among asymptomatic children, those with no history of symptoms during the preceding weeks accounted for two third of children with positive serology results (28/41), which supports that asymptomatic infections are frequent in children. By contrast, history of symptoms during the preceding weeks increased significantly the risk of positive serology. However, on multivariate analysis, the only factor influencing the positivity of RT-PCR or serology was the household contact who has previously presented symptoms suggestive of COVID-19. Of note, the number of siblings in the family did not significantly increase the probability of a positive RT-PCR or serology result. Several studies have shown that children were usually infected by an adult in the family.[18, 22, 30, 31] In our study, the importance of familial contagion in the modalities of SARS-Cov-2 transmission is suggested by a very low RT-PCR (0.7%) and serology positivity rate (3.6%) for children without an infected relative and in a period of lockdown.

Among the children positive on RT-PCR (n=11), only 3 had no antibody response, and 8 were positive for IgG with or without IgM positivity. This finding supports that for these 3 patients, contamination had occurred during the 2 weeks before enrollment.

We highlight that the frequency of positivity on RT-PCR for SARS-CoV-2 was significantly higher in children with positive serology than those with a negative one (12.3% vs 0.6%, p<0.001). This finding highlights the difficulties in interpreting the significance of a positive RT-PCR SARS-CoV-2 result without concomitant antibody testing after the epidemic wave. Indeed, children positive on RT-PCR for SARS-CoV-2 and positive for IgG probably had little or no infectivity.[32] In a study of 9 patients, attempts to isolate the virus in culture were not successful beyond day 8 of illness onset, which relates to the decreased infectivity beyond the first week.[33] In the study of Bullard *et al.*, SARS-CoV-2 Vero cell infectivity was only observed for RT-PCR Ct < 24 and symptom onset to test < 8 days.[34] It is likely that infectivity was low for the 8 of 11 RT-PCR SARS-CoV-2 positive children. Indeed, only 3 children had a Ct recorded under 31.

Our study has several limitations. First, the role of assumed household transmission probably has been over-estimated because of the well-followed lockdown in France.[35] Indeed, more than 86.5% of children with positive SARS-CoV-2 by RT-PCR or serology have had a confirmed or suspected COVID-19 household contact. However, our rate of positive serology for children in the Paris area was similar to the rate observed for hospitalized patients (11.7%) and at school children (8.8%).[22, 36]

Second, the ability to successfully collect nasopharyngeal swabs properly could be more difficult in young children and significantly affect the results and be a factor contributing to the low RT-PCR positivity prevalence observed in our population. However, the pediatricians who performed the study were all involved for many years in a pneumococcal nasopharyngeal carriage study (started in 2001 and currently ongoing) and were particularly well trained to collect appropriately nasopharyngeal samples.[37]

School closure or limitation (reduced number of students or days of attendance) has a major impact on children's development and access to learning.[38] Therefore, the usefulness of school closure or limitation needs evaluation in controlling the COVID-19 epidemic. [39] We plan to renew this study after the full re-opening of schools and day care centre in the Paris area to better assess the transmission of SARS-Cov-2 in children.

Funding. This work was supported by the French Ministry of Health "DGOS PHRC regional IDF 2020 no. AOR20095."

Competing Interests Statement : All other authors declare no competing interests for this study. RC, CL, EV received personal fees and non-financial support from Pfizer. RC reports personal fees from Merck, GSK, Sanofi and AstraZeneca outside the submitted work. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Contributorship Statement: RC, CL, NO, CJ, OL and EV designed the study. RC, CL, NO, CJ, OL, AS, CB, AE, FC, FCS, AW, OR and EV analyzed and interpreted the data and drafted the article. SB&CL performed the statistical analysis. EV, SA, NS, CR, SLM performed the microbiological analysis. All authors revised and approved the manuscript.

Acknowledgments. We are grateful to the investigators of the COVILLE study Network:

Akou'ou M.H, Auvrignon A, Belaroussi N, Benani M, Cambier Nappo E, Chartier Albrech C, Coicadan L, Condor R, D'Acremont G, D'Ovidio N, De Brito B, Deberdt P, Delatour A, Gorde-Grosjean S, Louvel M, Michot-Cottias A-S, Ravilly S, Seror E, Turberg-Romain C,

We are grateful to Adjemian S, Auffroy O, Begard M, Harant J, Mouaouya M, Receveau F, and Sigere ML, for technical assistance ; Cuquemelle A for secretarial assistance.

We are grateful to the ACTIV team : Ramay I, Prieur C; Prieur A, Borg M, Meyet L, Levy J, and Zemmour E (Association Clinique et thérapeutique Infantile du Val de Marne);

We are grateful to the CRC team: Brussieux M and Hoffart C from the Clinical Reaserch Center of the CHI Créteil.

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Table 1. Characteristics of children enrolled in the study and by pauci-symptomatic and asymptomatic group

	Overall	Pauci-symptomatic children*	Asymptomatic children			p$
			History of symptoms **	No history of symptoms* **	Total	
	n=605	n=283	n=118	n=204	n=322	
Age (years)						
mean±SD	4.9±3.9	4.8±3.7	5.6±4.3	4.7±4.1	5.0±4.2	0.08
Median	3.8	4.0	4.4	3.4	3.7	
<3 mo.	8 (1.3)	6 (2.1)	0	2 (1.0)	2 (0.6)	
3-30 mo.	218 (36.0)	98 (34.6)	37 (31.4)	83 (40.7)	120	
31 mo. – 5 y.	184 (30.4)	96 (33.9)	34 (28.8)	54 (26.5)	(37.3)	0.1
6 y. – 10 y.	134 (22.2)	61 (21.6)	29 (24.6)	44 (21.6)	88 (27.3)	
≥11 y.	61 (10.1)	22 (7.8)	18 (15.2)	21 (10.3)	73 (22.7)	
					39 (12.1)	
Sex, male	322 (53.2)	152 (53.7)	65 (55.1)	105 (51.5)	170 (52.8)	0.8
Daycare attendance before lockdown						
Home	78 (13.8)	34 (13.0)	6 (5.4)	38 (19.7)	44 (14.5)	
Childminder	55 (9.7)	24 (9.2)	10 (9.0)	21 (10.9)	31 (10.2)	0.031
Daycare center	135 (23.9)	66 (25.2)	29 (26.1)	40 (20.7)	69 (22.7)	
School	298 (52.7)	138 (52.7)	66 (59.5)	94 (48.7)	160 (52.6)	
Comorbidities	93 (15.4)	45 (15.9)	28 (23.7)	20 (9.8)	48 (14.9)	0.004
Prematurity	35 (6.3)	15 (5.7)	7 (6.1)	13 (7.4)	20 (6.9)	0.8
Siblings						
0	115 (20.6)	57 (21.9)	18 (15.9)	40 (21.5)	58 (19.4)	
1	282 (50.5)	136 (52.3)	61 (54.0)	85 (45.7)	146	0.3
≥2	162 (29.0)	67 (25.8)	34 (30.1)	61 (32.8)	(48.8)	
					95 (31.8)	

Data are n (%) unless indicated.

$ p compares symptomatic children, asymptomatic children with history symptoms>7 days and asymptomatic children without history of symptoms

* Pauci-symptomatic children were defined as those with fever isolated or associated with respiratory signs such as cough, dysphagia, rhinorrhea, diarrhea, vomiting, cutaneous signs, taste loss and/or anosmia, during the last 7 days

** History of symptoms (fever or respiratory or digestive) between 7 days and 2 months before enrollment
*** No history of symptoms

Table 2. Results of RT-PCR SARS-Cov-2 testing and serology in children by pauci-symptomatic and asymptomatic group

	Overall	Pauci-symptomatic children*	Asymptomatic children		
			History of symptoms **	No history of symptoms***	Total
	n=605	n=283	n=118	n=204	n=322
RT-PCR					
Overall	11 (1.8) [0.9; 3.2]	7 (2.5) [1.0; 5.0]	1 (0.8) [0.0; 4.6]	3 (1.5) [0.3; 4.2]	4 (1.2) [0.3; 3.1]
Definite positive	5	3	0	2	2
Weakly positive	1	1	0	0	0
Probable	5	3	1	1	2
Serology					
IgM+ and/or IgG+	65 (10.7) [8.4; 13.5]	24 (8.5) [5.5; 12.4]	28 (23.7) $ [16.4; 32.4]	13 (6.4) $ [3.4; 10.7]	41 (12.7) [9.3; 16.9]
IgM+IgG-	7 (1.2) [0.5; 2.4]	4 (1.4) [0.4; 3.6]	2 (1.7) [0.2; 6.0]	1 (0.5) [0.0; 2.7]	3 (0.9) [0.2; 2.7]
IgM+IgG+	32 (5.3) [3.6; 7.4]	12 (4.2) [2.2; 7.3]	17 (14.4) $ [8.6; 22.1]	3 (1.5) $ [0.3; 4.2]	20 (6.2) [3.8; 9.4]
IgM-IgG+	26 (4.2) [2.8; 6.2]	8 (2.8) [1.2; 5.5]	9 (7.6) [3.5; 14.0]	9 (4.4) [2.0; 8.2]	18 (5.6) [3.3; 8.7]

Data are n (%) [95% confidence interval].

$ $p<0.001$

* Pauci-symptomatic children were defined as those with fever isolated or associated with respiratory signs such as cough, dysphagia, rhinorrhea, diarrhea, vomiting, cutaneous signs, taste loss and/or anosmia, during the last 7 days

** History of symptoms (fever or respiratory or digestive) between 7 days and 2 months before enrollment

*** No history of symptoms

Table 3. RT-PCR SARS-CoV-2–positive results by serology status

RT-PCR results	IgM- IgG- n=540	IgM+ and/or IgG+ n=65	IgM+ IgG- n = 7	IgM+ IgG+ n = 32	IgM - IgG+ n = 26
Overall positive*	3 (0.6)	8 (12.3)	0	6 (18.7)	2 (7.8)
Definite positive**	2 (0.4)	3 (0.6)	0	2 (6.2)	1 (3.9)
Weakly positive ***	0	1 (1.5)	0	1 (3.1)	0
Presumptive positive ****	1 (0.2)	4 (6.2)	0	3 (9.4)	1 (3.9)

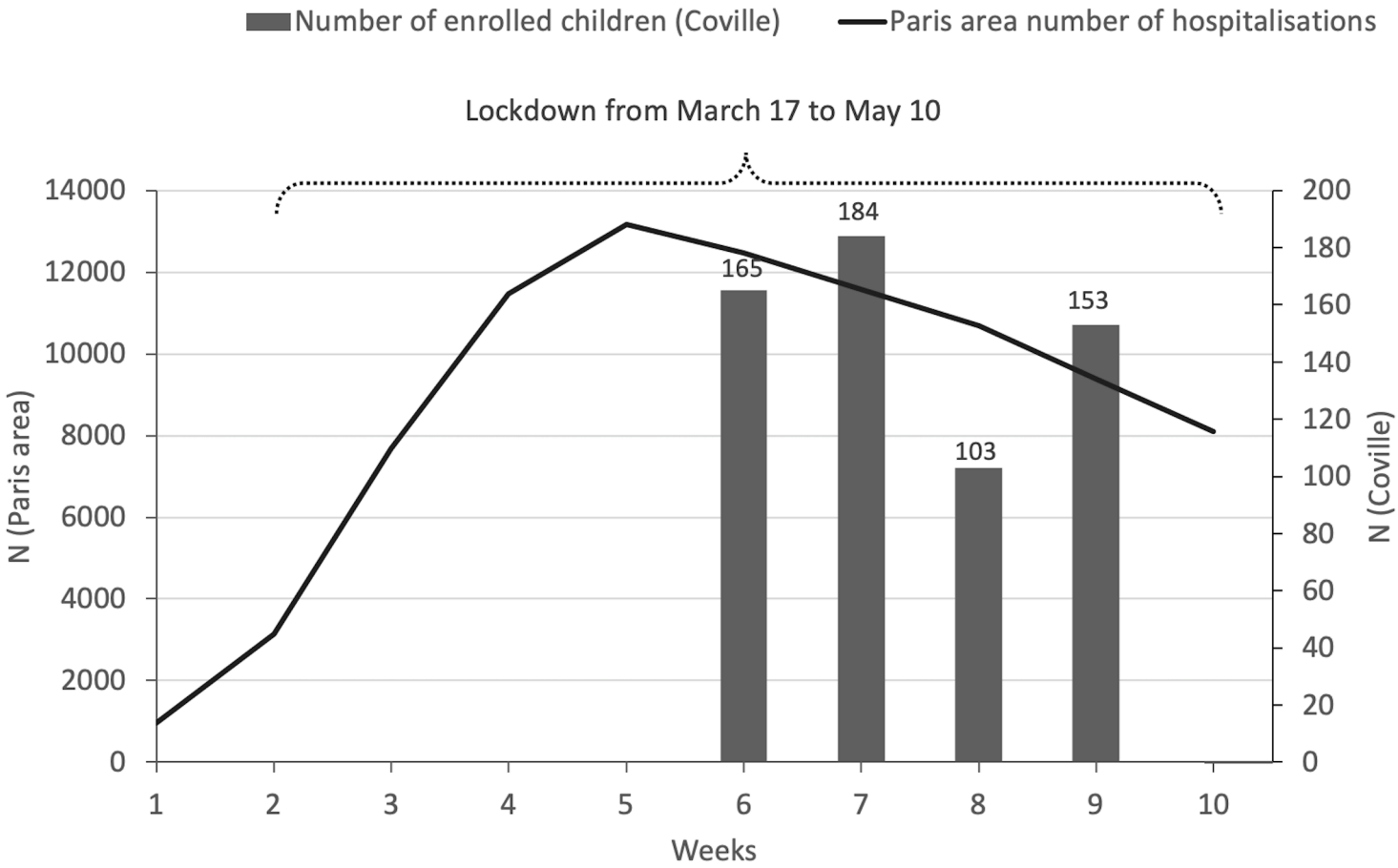
Data are n (%).
- p<0.001: comparison of overall RT-PCR with IgM-/IgG- vs IgM+ and/or IgG+
- p<0.001: comparison of definite or weakly or probable RT-PCR with IgM-/IgG- vs IgM+ and/or IgG+
* Overall positive: NP samples were considered positive when a cycle threshold value (Ct) less than 40 was obtained for any gene
** Definite positive: cycle threshold value (Ct) less than 38 obtained for 2 or 3 gene.
*** Weakly positive: any result with a Ct > 38 and < 40.
**** Presumptive: cycle threshold value (Ct) less than 38 obtained for only one target.

Table 4. RT-PCR and serology results by contact with a person with confirmed and/or suspected COVID-19

Contact	Overall n=543*	Positive serology n=63	Negative serology n=480	Positive RT-PCR SARS-CoV-2 n=11	Negative RT-PCR SARS-CoV-2 n=532
Confirmed COVID-19**	93 (17.1) [14.1; 20.6]	29 (31.2) [22.0; 41.6]	64 (68.8) [58.4;78.0]	5 (5.4) [1.8; 12.1]	88 (94.6) [87.9; 98.2]
Suspected COVID-19***	175 (32.2) [28.3; 36.3]	26 (14.9) [9.9; 21.0]	149 (85.1) [79.0; 90.0]	4 (2.3) [0.6; 5.7]	171 (97.7) [94.3; 99.4]
Confirmed/ suspected COVID-19	268 (49.4) [45.1; 53.6]	55 (20.5) [15.9; 25.9]	213 (79.5) [74.1; 84.1]	9 (3.4) [1.5; 6.3]	259 (96.6) [93.7; 98.5]
No contact	275 (50.6) [46.4; 54.9]	8 (2.9) [1.3; 5.7]	267 (97.1) [94.3; 98.7]	2 (0.7) [0.1; 2.6]	273 (99.3) [97.4; 99.9]

Data are n (%) [95% confidence interval].
* 543 available data on 605 enrolled patients
** Confirmed by RT-PCR SARS-CoV-2

*** Suspected symptoms suggestive of COVID-19 because of the limited availability of testing


Number of enrolled children (Coville)
Paris area number of hospitalisations
Lockdown from March 17 to May 10
N (Paris area)
N (Coville)
Weeks
165
184
103
153

Supplemental table. Description of the 11 patients with positive RT-PCR for SARS-CoV-2

Patients	Target	Result	Ct
Patient 1 (9.4 years)	E gene	-	NA
	RdRP gene	-	NA
	N gene	+	38.45
	IC	+	25.52
Patient 2 (5.1 years)	rs	-	NA
	RdRP gene	-	NA
	N gene	+	34.85
	IC	+	25.04
Patient 3 (4.5 years)	E gene	-	NA
	RdRP gene	-	NA
	N gene	+	36.38
	IC	+	26.04
Patient 4 (5.6 years)	E gene	+	30.91
	RdRP gene	+	32.75
	N gene	+	32.83
	IC	+	26.05
Patient 5 (19 days)	E gene	+	27.20
	RdRP gene	+	28.66
	N gene	+	28.59
	IC	+	25.20
Patient 6 (2.1 years)	E gene	-	NA
	RdRP gene	+	38.91
	N gene	+	38.84
	IC	+	25.91
Patient 7 (4.8 years)	E gene	-	NA
	RdRP gene	+	34.60
	N gene	+	37.52
	IC	+	25.62
Patient 8 (9.5 years)	E gene	-	NA
	RdRP gene	-	NA
	N gene	+	38.63
	IC	+	25.97
Patient 9 (1.8 years)	E gene	-	NA
	RdRP gene	+	35.26
	N gene	+	35.51
	IC	+	25.95
Patient 10 (6.5 years)	E gene	-	NA
	RdRP gene	+	37.59
	N gene	-	NA
	IC	+	25.21
Patient 11 (9.3 years)	E gene	+	28.00
	RdRP gene	+	29.41
	N gene	+	30.14
	IC	+	25.69

Ct, Cycle threshold, IC, internal control, NA, not applicable

BMJ Paediatrics Open

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Journal:	*BMJ Paediatrics Open*
Manuscript ID	bmjpo-2020-000887.R1
Article Type:	Original research
Date Submitted by the Author:	21-Oct-2020
Complete List of Authors:	Cohen, Robert; Centre Hospitalier Intercommunal de Créteil, Paediatric Department Jung, Camille; Centre Hospitalier Intercommunal de Creteil Ouldali, Naim; ACTIV Sellam, Aurelie; Hôpital Jean Verdier, seine saint denis Batard, Christophe; ACTIV Cahn-Sellem, Fabienne; AFPA Elbez, Annie; ACTIV Wollner, Alain; ACTIV Romain, Olivier; South Paris University, Neonatal Intensive Care Unit Corrard, François; ACTIV Aberrane, Said; Centre Hospitalier Intercommunal de Créteil, Microbiology Soismier, Nathalie; Centre Hospitalier Intercommunal de Créteil, Microbiology Creidy, Rita; Centre Hospitalier Intercommunal de Créteil, Microbiology Smati-Lafarge, Mounira; Centre Hospitalier Intercommunal de Créteil, Microbiology Launay, Odile; Hôpital Cochin Béchet, Stéphane; ACTIV Varon, Emmanuelle; Centre Hospitalier Intercommunal de Créteil, Microbiology Levy, Corinne; ACTIV; Centre Hospitalier Intercommunal de Créteil
Keywords:	Epidemiology, Virology

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Assessment of SARS-CoV-2 infection by RT-PCR and serology in the Paris area: a cross-sectional study

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Abstract

Background. Several studies indicated that children seem to be less frequently infected with SARS-CoV-2 and potentially less contagious than adults. To examine the spread of SARS-CoV-2, we combined both RT-PCR testing and serology in children in the most affected region in France, Paris, during the COVID-19 epidemic.

Methods. From April 14, 2020 to May 12, 2020, we conducted a cross-sectional, prospective, multicenter study. Healthy controls and pauci-symptomatic children from birth to age 15 years were enrolled by 27 ambulatory pediatricians. A nasopharyngeal swab was taken for detection of SARS-CoV-2 by RT-PCR and a microsample of blood for micro-method serology.

Results. Among the 605 children, 322 (53.2%) were asymptomatic and 283 (46.8%) symptomatic. RT-PCR and serology results were positive for 11 (1.8%) and 65 (10.7%) children, respectively, with no significant difference between asymptomatic and pauci-symptomatic children. Only 3 children were RT-PCR–positive without any antibody response detected. The frequency of RT-PCR SARS-CoV-2 positivity was significantly higher for children with positive than negative serology results (12.3% vs 0.6%, $p<0.001$). Contact with a person with confirmed COVID-19 increased the odds of RT-PCR positivity (odds ratio 7.8, 95% confidence interval [1.5; 40.7]) and serology positivity (15.1 [6.6; 34.6]).

Conclusion. In an area heavily affected by COVID-19, after the peak of the first epidemic wave and during the lockdown, the rate of children with RT-PCR SARS-CoV-

2 positivity was very low (1.8%), but that of serology positivity was higher (10.7%). Most children with positive RT-PCR results also had positive serology results.

What is already known on this topic?

At this time, several studies suggested that children are less frequently infected with SARS-CoV-2 and potentially less contagious than adults.

- Most of the studies were based on RT-PCR SARS-CoV-2 testing, without antibody assays.

What does this study add?

- This study combining RT-PCR testing and serology assessed the prevalence of SARS-CoV-2 infection in children in an area heavily affected by the COVID-19 pandemic.
- Among a large cohort of children (>600), 11 (1.8%) had positive RT-PCR SARS-CoV-2 results and 65 (10.7%) had antibodies to SARS-CoV-2.
- The only factor associated with RT-PCR SARS-CoV-2 or serology positivity was the presence of a household contact with COVID-19.

Introduction

Since the beginning of the COVID-19 pandemic, reports from several countries indicated that the disease was less frequently reported and less severe in children than adults.[1-3] Worldwide, the number of confirmed pediatric cases seems relatively low, and they account for less than 1% of hospitalized cases and deaths.[1 4] Although most COVID-19 cases in children are not severe, serious COVID-19 illness resulting in hospitalization can occur in this age group, and recently, hyperinflammatory shock, with features similar to atypical Kawasaki disease, was reported in several countries.[5-10]

However, concerns have been raised that children could play an important role in the spread of the disease because community testing has demonstrated a significant number of children with no or subclinical symptoms.[11] Indeed, if as for influenza, children could be the primary drivers of household SARS-CoV-2 transmission, then a silent spread from children who did not alert anyone to their infection could be a serious driver in the dynamics of the epidemic.[12] On the basis of this prevailing hypothesis, school closures were implemented almost ubiquitously around the world to try to halt the potential spread of COVID-19.[13 14]

However, several studies had already shown that when SARS-CoV-2 infection was suspected (compatible clinical signs, contact with a person with COVID-19), the rate of positive RT-PCR SARS-CoV-2 results was lower in children than adults.[14,15] In contrast, in RT-PCR SARS-CoV-2–positive children, the viral load was comparable between children and adults.[16] Furthermore, one study suggested that children shed

infectious SARS-CoV-2.[17] However, results from a systematic review of household clusters of COVID-19 revealed that only 3/31 clusters were due to a child index case, and a population-based school contact-tracing study found minimal transmission by child or teacher index cases.[18 19] Finally, other studies suggested that children were potentially less contagious than adults but the design of these studies does not exclude the possibility of children being more contagious than adults.[16 20-22]

Some countries such as South Korea and Iceland have implemented widespread community testing. Both countries found children significantly underrepresented in cases. In Iceland, this was true in targeted testing of high-risk groups as compared with adults (6.7% < 10 years vs 13.7% ≥ 10 years positive cases), and in (invited) population screening, no child < 10 years old was positive for SARS-CoV-2 as compared with 0.8% in the general population. [23]

Of note, all these studies were based on RT-PCR testing, but serology diagnosis is also an important tool to understand the prevalence and burden of COVID-19.[24] A serology survey tested adolescents in a high school in the north of France, the site of a cluster at the end of February. Of the 242 students tested, 2.7% of children ≤ 14 years old and 40% aged 15-17 years had positive SARS-CoV-2 serology results (IgG), which suggests a difference in susceptibility to SARS-CoV-2 among younger children.[25]

To best approach the prevalence of SARS-CoV-2 in children at a population level, we combined both RT-PCR testing for SARS-CoV-2 and serology in asymptomatic or pauci-symptomatic children (with mild clinical symptoms) in the Paris area, the most affected region in France, during the COVID-19 epidemic.

Patients and Methods

Study population

This was a cross-sectional prospective, multicenter study conducted by the Association Clinique et Thérapeutique Infantile du Val de Marne (ACTIV) network, a research unit expert in epidemiological surveillance and clinical studies in ambulatory pediatric infectious diseases, and the University Intercommunal Créteil Hospital.[26] Primary care pediatricians (n = 27) took part in the study from April 14, 2020 to May 12, 2020. The strategy of closing schools and the lockdown decided by the French government for the whole country started on March 17 and finished on May 11, 2020.

This study aimed to enroll children from birth to 15 years old who were consulting an ambulatory pediatrician and distributed in two groups: asymptomatic and pauci-symptomatic. Asymptomatic children were defined as children without any symptoms or signs suggesting infectious disease during the previous 7 days. They usually came for vaccination visits. In this group, we defined two subgroups of children: those previously symptomatic (fever, respiratory or digestive symptoms) between 7 days and 2 months before enrollment, and those without any previous symptoms. Pauci-symptomatic children were defined as those with fever isolated or associated with respiratory signs such as cough, dysphagia, rhinorrhea, diarrhea, vomiting, cutaneous signs, taste loss and/or anosmia during the previous 7 days. Children were excluded if the clinical condition at enrollment required transfer to a pediatric emergency unit or hospitalization.

After informing the parents of the participating children and obtaining their signed consent, an electronic case report form (eCRF) was completed by the pediatrician to collect socio-demographic data, history, contact with a person with confirmed COVID-19 by RT-PCR for SARS-CoV-2, clinical symptoms and signs, and additional positive biological tests. We also collected suspected COVID-19 contacts because of the limited availability of testing. Indeed, during the lockdown, the diagnostic RT-PCR SARS-CoV-2 test was mainly available for patients with severe disease and/or healthcare workers, and all symptomatic individuals could not be tested. For all enrolled children, during the same visit, a nasopharyngeal (NP) swab was taken for RT-PCR detection of SARS-CoV-2 and a microsample of blood for micro-method serology.

Power calculation

To have an appropriate proportion of confirmed RT-PCR SARS-CoV-2–positive patients among asymptomatic and pauci-symptomatic children, with a 95% confidence interval (CI) of ± 3%, assuming a positivity proportion < 10%, we needed to enroll 300 children per group (asymptomatic and pauci-symptomatic), for 600 patients in total.

Serology

Pediatricians collected fingerstick whole-blood specimens and used the Biosynex COVID-19 BSS test, a rapid chromatographic immunoassay, for qualitive detection of IgG and IgM antibodies to SARS-CoV-2 in blood. This test targeted the spike protein fragment receptor binding domain and was among those approved by the French

national health authority.[27] According to the specifications of the manufacturer, the test's sensitivity was 91.8% [95% CI 83.8-96.6] and specificity 99.2% [95%CI 97.7-99.8] (https://www.biosynex.com/laboratoires-hopitaux-tests-covid-19/). However, the accuracy of the test was not stratified by age. For patients infected with another coronavirus (OC43, 229E, NL63, HKU1), no cross-reaction was observed. Furthermore, assessment by independent investigators confirmed the good diagnostic accuracy of this test among hospital staff with mild disease in eastern France [28]. A positive serology result meant that the patient had a previous SARS-CoV-2 infection: positive for IgG or IgM or both. A serology result was considered negative if results for both IgM and IgG were negative.

SARS-CoV-2 RT-PCR

The NP specimens were obtained by using the collection system eSwab™ (Minitip size nylon flocked swab placed in 1 mL modified liquid Amies transport medium, COPAN, Brescia, Italy). They were transported to the centralized microbiology laboratory (CHIC). Before extraction, each sample was inactivated by the addition of 750 µl/ml STARmag lysis buffer solution (Seegene, South Korea). RT-PCR for SARS-CoV-2 involved using the automated Seegene STARlet system, according to the manufacturer's instructions, with the CE marked Allplex 2019-nCoV RT-PCR assay (Seegene, South Korea), which has three targets: the viral nucleocapsid (N) protein and RNA-dependent RNApolymerase (RdRP), both SARS-CoV-2–specific genes, and the sarbecovirus-specific envelope (E) protein. The automated Hamilton STARlet system was used for

viral RNA extraction with the STARMag 96 Universal Cartridge kit (Seegene, South Korea) and PCR setup.Positive and negative controls were included in each run.

The cycle threshold value (Ct) was used as an indicator of the copy number of SARS-CoV-2 RNA specimens, with lower Ct values corresponding to higher viral copy numbers. NP samples were considered positive with a Ct value < 40 obtained for any gene. Amplification of two or three targets indicated that SARS-CoV-2 RNA was detected, and amplification of only one target with Ct value < 38 indicated a presumptive positive result. We defined as weakly positive any result with Ct value > 38 and < 40. A sample was considered negative if the internal control was amplified but not the viral target genes. A sample was considered invalid when no amplification was obtained for the internal control.

Statistical analysis

Data were entered by using the eCRF (PHP/MySQL) and analyzed by using Stata/SE v15 (StataCorp, College Station, TX, USA). Quantitative data were compared by Student *t* test and categorical data by chi-square or Fisher exact test. We used a logistic regression model for analysis of factors associated with RT-PCR SARS-CoV-2 and serology positivity. Variables (age, clinical signs, contact, siblings and daycare attendance modalities) with $p < 0.20$ on univariate analysis were included in the multivariable model, estimating odds ratios (ORs) and 95% CIs. Only significant variables ($p<0.05$) were kept in the final model. All tests were 2-sided and were considered significant at $p<0.05$.

Ethics

The study protocol was approved by an ethics committee (CPP IDF IX no. 08-022). Parents of all infants and children provided written informed consent. The study was registered at ClinicalTrials.gov NCT04318431.

Patient and Public Involvement

There were no patients or public involved in the research design, process and research findings dissemination.

Results

From April 14, 2020 to May 12, 2020, 27 ambulatory pediatricians in the Paris area enrolled 605 children: 322 (53.2%) children were asymptomatic and 283 (46.8%) pauci-symptomatic. Table 1 presents the characteristics of the enrolled children by group. In the pauci-symptomatic group, the main signs and symptoms were fever (187, 66.3%), cough (143, 50.7%), pharyngitis (143, 50.7%), rhinitis (137, 48.4%), diarrhea (81, 28.7%), rash (64, 23.0%), vomiting (52, 18.8%), dysgeusia (8, 3.0%) and anosmia (5, 3.3%).

Figure 1 presents the dates of the lockdown and the number of children enrolled, by week, during the first epidemic wave in Paris.[29]

RT-PCR SARS-CoV-2 results were positive for 11 (1.8%) children, with no significant difference between children with and without symptoms (Table 2). The supplemental

Table shows the details of the 11 positive RT-PCR SARS-CoV-2 cases. Only 3 children had RT-PCR SARS-CoV-2 positivity with Ct value < 31.

On multivariable analysis, contact with a person with proven COVID-19 was the only significant risk factor for RT-PCR SARS-CoV-2 positivity (OR 7.8, 95% CI [1.5; 40.7]).

Table 2 shows the serology results by group. The age distribution of children was similar whatever the serology results, negative or positive: < 3 months, 1.3% vs 1.5%; 3 to 30 months, 37.2% vs 26.2%; 31 months to 5 years, 29.6% vs 36.9%; 6 to 10 years, 21.7% vs 26.2%; ≥11 years, 10.2% vs 9.2%. Serology was positive for 65 of 605 (10.7%) children, and among these, 87.3% had a confirmed or suspected contact. Children previously symptomatic during the preceding weeks, more frequently were positive on serology.

RT-PCR SARS-CoV-2 was more frequently positive for children with positive than negative serology results (12.3% vs 0.6%, p<0.001). Only 3 children had RT-PCR SARS-CoV-2 positivity without any antibody response detected.

Table 3 shows serology and RT-PCR SARS-CoV-2 results for the 543 enrolled children according to contact with a person (adult or child) with suspected or confirmed COVID-19. Only 2 of 275 (0.7%) children without any contact with a person with COVID-19 had positive RT-PCR SARS-CoV-2 results.

On multivariable analysis, serology positivity was associated with contact with a person with proven or suspected COVID-19 (OR 15.1 [95% CI 6.6; 34.6] and 5.8 [95% CI 2.6; 13.2]).

Discussion

This study combines RT-PCR SARS-CoV-2 and serology results to assess the prevalence of SARS-CoV-2 infection in a large cohort of children in the community. In a region strongly affected by the epidemic (Paris area), during the lockdown, as expected, very few children (1.8%) had RT-PCR SARS-CoV-2 positivity, but the proportion with serology positivity (10.7%) was relatively high. Despite the relatively large number of children included (>600), we did not find a significant difference in rate of positive RT-PCR or serology results between asymptomatic and pauci-symptomatic children, which suggests that most children were asymptomatic after a SARS-CoV2 infection.

Among asymptomatic children, those with no history of symptoms during the preceding weeks accounted for two-thirds of children with positive serology results (28/41), which supports the fact that asymptomatic infections are frequent in children. By contrast, history of symptoms during the preceding weeks significantly increased the risk of positive serology. However, on multivariable analysis, the only factor influencing the positivity of RT-PCR or serology was the household contact who previously presented symptoms suggesting COVID-19. Of note, the number of siblings in the family did not significantly increase the probability of a positive RT-PCR or serology result. Several studies have shown that children were usually infected by an adult in the family.[18 22 30 31] In our study, the importance of familial contagion in the modalities of SARS-Cov-2 transmission is suggested by a very low RT-PCR (0.7%) and

serology (3.6%) positivity rate for children without an infected relative and in a period of lockdown.

Among the children with RT-PCR positivity (n=11), only 3 had no antibody response, and 8 were positive for IgG with or without IgM positivity. This finding supports that for these 3 patients, contamination had probably occurred within weeks immediately before enrolment.

We highlight that the frequency of RT-PCR SARS-CoV-2 positivity was significantly higher in children with positive than negative serology results (12.3% vs 0.6%, p<0.001). This finding highlights the difficulties in interpreting the significance of a positive RT-PCR SARS-CoV-2 result without concomitant antibody testing after the epidemic wave. Preliminary reports suggest that children with RT-PCR SARS-CoV-2 positivity and IgG positivity probably had little or no infectivity.[32 33] In a study of 9 patients, attempts to isolate the virus in culture were not successful beyond day 8 of illness onset, which could be related to the decreased infectivity beyond the first week.[34] In the study of Bullard *et al.*, SARS-CoV-2 Vero cell infectivity was observed with only RT-PCR Ct value < 24 and symptom onset to test duration < 8 days.[35] Infectivity was likely low for 8 of the 11 RT-PCR SARS-CoV-2–positive children. Indeed, only 3 children had a Ct value < 31.

Our study has several limitations. First, the role of assumed household transmission has probably been over-estimated because of the well-followed lockdown in France.[36] Indeed, more than 86.5% of children with RT-PCR SARS-CoV-2 or serology positivity had a confirmed or suspected COVID-19 household contact.

However, our rate of positive serology for children in the Paris area was similar to the rate observed for hospitalized children (11.7%) and school children (8.8%).[22 37] Second, the ability to successfully collect NP swabs properly could be more difficult in young children and significantly affect the results and be a factor contributing to the low RT-PCR positivity prevalence observed in our population. However, the pediatricians who performed the study were all involved for many years in a pneumococcal NP carriage study (started in 2001 and currently ongoing) and were particularly well trained to collect appropriately NP samples.[38]

School closure or limitation (reduced number of students or days of attendance) has a major impact on children's development and access to learning.[39] Therefore, the usefulness of school closure or limitation needs evaluation in controlling the COVID-19 epidemic.[40] We plan to renew this study after the full re-opening of schools and daycare centres in the Paris area. To better assess the transmission of SARS-Cov-2 in children and to elucidate their role in the transmission, serial testing of all household members is needed.

Funding. This work was supported by the French Ministry of Health "DGOS PHRC regional IDF 2020 no. AOR20095."

Competing Interests Statement: All other authors declare no competing interests for this study. RC, CL, EV received personal fees and non-financial support from Pfizer. RC reports personal fees from Merck, GSK, Sanofi and AstraZeneca outside the

submitted work. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Contributorship Statement: RC, CL, NO, CJ, OL and EV designed the study. RC, CL, NO, CJ, OL, AS, CB, AE, FC, FCS, AW, OR and EV analyzed and interpreted the data and drafted the article. SB and CL performed the statistical analysis. EV, SA, NS, CR, SLM performed the microbiological analysis. All authors revised and approved the manuscript.

Acknowledgments. We are grateful to the investigators of the COVILLE study Network:

Akou'ou M.H, Auvrignon A, Belaroussi N, Benani M, Cambier Nappo E, Chartier Albrech C, Coicadan L, Condor R, D'Acremont G, D'Ovidio N, De Brito B, Deberdt P, Delatour A, Gorde-Grosjean S, Louvel M, Michot-Cottias A-S, Ravilly S, Seror E, Turberg-Romain C,

We are grateful to Adjemian S, Auffroy O, Begard M, Harant J, Mouaouya M, Receveau F, and Sigere ML, for technical assistance ; Cuquemelle A for secretarial assistance.

We are grateful to the ACTIV team : Ramay I, Prieur C; Prieur A, Borg M, Meyet L, Levy J, and Zemmour E (Association Clinique et thérapeutique Infantile du Val de Marne);

We are grateful to the CRC team: Brussieux M and Hoffart C from the Clinical Reaserch Center of the CHI Créteil.

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Table 1. Characteristics of children enrolled in the study and by pauci-symptomatic and asymptomatic group

	Overall	**Pauci-symptomatic children***	**Asymptomatic children**			
			Previously symptomatic (>7days)**	**No previous symptoms*****	**Total**	**p$**
	n=605	n=283	n=118	n=204	n=322	
Age (years) mean±SD	4.9±3.9	4.8±3.7	5.6±4.3	4.7±4.1	5.0±4.2	0.08
Median	3.8	4.0	4.4	3.4	3.7	

<3 mo.	8 (1.3)	6 (2.1)	0	2 (1.0)	2 (0.6)	
3-30 mo.	218 (36.0)	98 (34.6)	37 (31.4)	83 (40.7)	120 (37.3)	
31 mo. – 5 y.	184 (30.4)	96 (33.9)	34 (28.8)	54 (26.5)	88 (27.3)	0.1
6 y. – 10 y.	134 (22.2)	61 (21.6)	29 (24.6)	44 (21.6)	73 (22.7)	
≥11 y.	61 (10.1)	22 (7.8)	18 (15.2)	21 (10.3)	39 (12.1)	
Sex, male	322 (53.2)	152 (53.7)	65 (55.1)	105 (51.5)	170 (52.8)	0.8
Daycare attendance before lockdown	78 (13.8)	34 (13.0)	6 (5.4)	38 (19.7)	44 (14.5)	
Home	55 (9.7)	24 (9.2)	10 (9.0)	21 (10.9)	31 (10.2)	0.031
Childminder	135 (23.9)	66 (25.2)	29 (26.1)	40 (20.7)	69 (22.7)	
Daycare center	298 (52.7)	138 (52.7)	66 (59.5)	94 (48.7)	160 (52.6)	
School						
Comorbidities	93 (15.4)	45 (15.9)	28 (23.7)	20 (9.8)	48 (14.9)	0.004
Prematurity	35 (6.3)	15 (5.7)	7 (6.1)	13 (7.4)	20 (6.9)	0.8
Siblings						
0	115 (20.6)	57 (21.9)	18 (15.9)	40 (21.5)	58 (19.4)	
1	282 (50.5)	136 (52.3)	61 (54.0)	85 (45.7)	146 (48.8)	0.3
≥2	162 (29.0)	67 (25.8)	34 (30.1)	61 (32.8)	95 (31.8)	

Data are n (%) unless indicated.

$ p compares symptomatic children, asymptomatic children previously symptomatic >7 days and asymptomatic children without previous symptoms

* Pauci-symptomatic children were those with fever isolated or associated with respiratory signs such as cough, dysphagia, rhinorrhea, diarrhea, vomiting, rash, dysgeusia and/or anosmia, during the previous 7 days

** Previously symptomatic (fever or respiratory or digestive) between 7 days and 2 months before enrollment

*** No previous symptoms

Table 2. Results of RT-PCR SARS-Cov-2 testing and serology in children by pauci-symptomatic and asymptomatic group

	Overall	**Pauci-symptomatic children***	**Asymptomatic children**		
			Previously symptomatic (>7 days)**	**No previous symptoms*****	**Total**
	n=605	n=283	n=118	n=204	n=322
RT-PCR					

Overall	11 (1.8) [0.9; 3.2]	7 (2.5) [1.0; 5.0]	1 (0.8) [0.0; 4.6]	3 (1.5) [0.3; 4.2]	4 (1.2) [0.3; 3.1]
Definite positive[$]	5	3	0	2	2
Weakly positive[$ $]	1	1	0	0	0
Presumptive[$ $ $]	5	3	1	1	2
Serology					
IgM+ and/or IgG+	65 (10.7) [8.4; 13.5]	24 (8.5) [5.5; 12.4]	28 (23.7) [$] [16.4; 32.4]	13 (6.4) [$] [3.4; 10.7]	41 (12.7) [9.3; 16.9]
IgM+IgG-	7 (1.2) [0.5; 2.4]	4 (1.4) [0.4; 3.6]	2 (1.7) [0.2; 6.0]	1 (0.5) [0.0; 2.7]	3 (0.9) [0.2; 2.7]
IgM+IgG+	32 (5.3) [3.6; 7.4]	12 (4.2) [2.2; 7.3]	17 (14.4) [$] [8.6; 22.1]	3 (1.5) [$] [0.3; 4.2]	20 (6.2) [3.8; 9.4]
IgM-IgG+	26 (4.2) [2.8; 6.2]	8 (2.8) [1.2; 5.5]	9 (7.6) [3.5; 14.0]	9 (4.4) [2.0; 8.2]	18 (5.6) [3.3; 8.7]

Data are n (%) [95% confidence interval].

[$] p<0.001

* Pauci-symptomatic children were those with fever isolated or associated with respiratory signs such as cough, dysphagia, rhinorrhea, diarrhea, vomiting, rash, dysgeusia and/or anosmia, during the previous 7 days

** Previously symptomatic (fever or respiratory or digestive) between 7 days and 2 months before enrollment

*** No previous symptoms

[$]Definite positive: cycle threshold (Ct) value < 38 obtained for 2 or 3 genes.

[$$]Weakly positive: any result with a Ct value > 38 and < 40.

[$$$]Presumptive: Ct < 38 obtained for only one target.

Table 3. Serology and RT-PCR results for the 543 enrolled children according to contact with a person (adult or child) with confirmed and/or suspected COVID-19

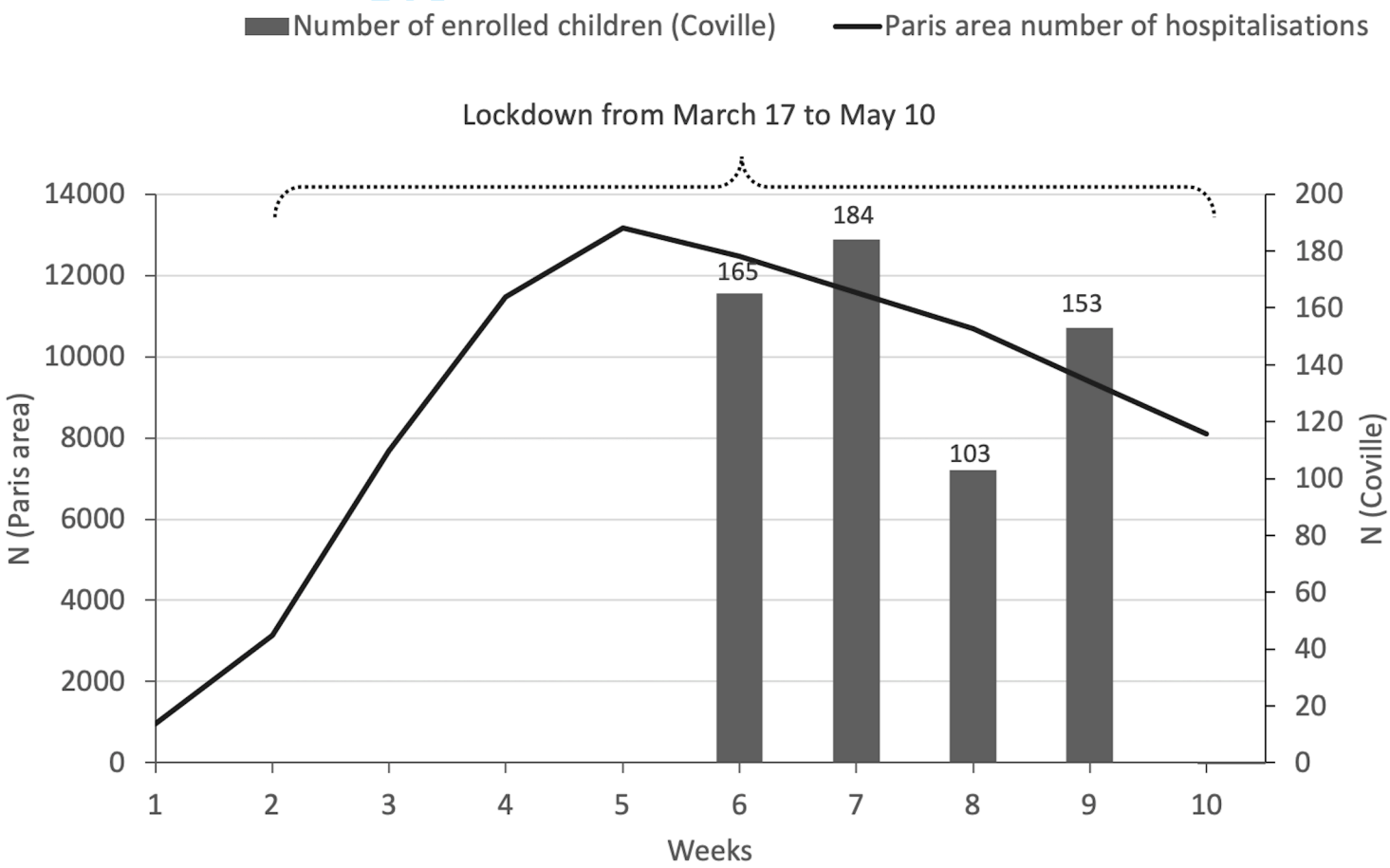
	Enrolled children				
	Overall	**Positive serology**	**Negative serology**	**Positive RT-PCR SARS-CoV-2**	**Negative RT-PCR SARS-CoV-2**
	n=543*	**n=63**	**n=480**	**n=11**	**n=532**
Contact					
Confirmed COVID-19**	93 (17.1) [14.1; 20.6]	29 (31.2) [22.0; 41.6]	64 (68.8) [58.4;78.0]	5 (5.4) [1.8; 12.1]	88 (94.6) [87.9; 98.2]

Suspected COVID-19***	175 (32.2) [28.3; 36.3]	26 (14.9) [9.9; 21.0]	149 (85.1) [79.0; 90.0]	4 (2.3) [0.6; 5.7]	171 (97.7) [94.3; 99.4]
Confirmed/ suspected COVID-19	268 (49.4) [45.1; 53.6]	55 (20.5) [15.9; 25.9]	213 (79.5) [74.1; 84.1]	9 (3.4) [1.5; 6.3]	259 (96.6) [93.7; 98.5]
No contact	275 (50.6) [46.4; 54.9]	8 (2.9) [1.3; 5.7]	267 (97.1) [94.3; 98.7]	2 (0.7) [0.1; 2.6]	273 (99.3) [97.4; 99.9]

Data are n (%) [95% confidence interval].

* 543 available data among 605 enrolled patients
** Confirmed by RT-PCR SARS-CoV-2
*** Suspected symptoms suggestive of COVID-19 because of the limited availability of testing

Figure 1. Number of children enrolled and total hospitalisation during the first COVID-19 epidemic wave in the Paris area



Supplemental table. Description of the 11 patients with positive RT-PCR for SARS-CoV-2

Patients	Target	Result	Ct
Patient 1 (9.4 years)	E gene	-	NA
	RdRP gene	-	NA
	N gene	+	38.45
	IC	+	25.52
Patient 2 (5.1 years)	rs	-	NA
	RdRP gene	-	NA
	N gene	+	34.85
	IC	+	25.04
Patient 3 (4.5 years)	E gene	-	NA
	RdRP gene	-	NA
	N gene	+	36.38
	IC	+	26.04
Patient 4 (5.6 years)	E gene	+	30.91
	RdRP gene	+	32.75
	N gene	+	32.83
	IC	+	26.05
Patient 5 (19 days)	E gene	+	27.20
	RdRP gene	+	28.66
	N gene	+	28.59
	IC	+	25.20
Patient 6 (2.1 years)	E gene	-	NA
	RdRP gene	+	38.91
	N gene	+	38.84
	IC	+	25.91
Patient 7 (4.8 years)	E gene	-	NA
	RdRP gene	+	34.60
	N gene	+	37.52
	IC	+	25.62
Patient 8 (9.5 years)	E gene	-	NA
	RdRP gene	-	NA
	N gene	+	38.63
	IC	+	25.97
Patient 9 (1.8 years)	E gene	-	NA
	RdRP gene	+	35.26
	N gene	+	35.51
	IC	+	25.95
Patient 10 (6.5 years)	E gene	-	NA
	RdRP gene	+	37.59
	N gene	-	NA
	IC	+	25.21
Patient 11 (9.3 years)	E gene	+	28.00
	RdRP gene	+	29.41
	N gene	+	30.14
	IC	+	25.69

Ct, Cycle threshold, IC, internal control, NA, not applicable

BMJ Paediatrics Open

Assessment of SARS-CoV-2 infection by RT-PCR and serology in the Paris area: a cross-sectional study

Journal:	*BMJ Paediatrics Open*
Manuscript ID	bmjpo-2020-000887.R2
Article Type:	Original research
Date Submitted by the Author:	16-Nov-2020
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Keywords:	Epidemiology, Virology

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Assessment of SARS-CoV-2 infection by RT-PCR and serology in the Paris area: a cross-sectional study

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Abstract

Background. Several studies indicated that children seem to be less frequently infected with SARS-CoV-2 and potentially less contagious than adults. To examine the spread of SARS-CoV-2, we combined both RT-PCR testing and serology in children in the most affected region in France, Paris, during the COVID-19 epidemic.

Methods. From April 14, 2020 to May 12, 2020, we conducted a cross-sectional, prospective, multicenter study. Healthy controls and pauci-symptomatic children from birth to age 15 years were enrolled by 27 ambulatory pediatricians. A nasopharyngeal swab was taken for detection of SARS-CoV-2 by RT-PCR and a microsample of blood for micro-method serology.

Results. Among the 605 children, 322 (53.2%) were asymptomatic and 283 (46.8%) symptomatic. RT-PCR and serology results were positive for 11 (1.8%) and 65 (10.7%) children, respectively, with no significant difference between asymptomatic and pauci-symptomatic children. Only 3 children were RT-PCR–positive without any antibody response detected. The frequency of RT-PCR SARS-CoV-2 positivity was significantly higher for children with positive than negative serology results (12.3% vs 0.6%, $p<0.001$). Contact with a person with confirmed COVID-19 increased the odds of RT-PCR positivity (odds ratio 7.8, 95% confidence interval (1.5, 40.7)) and serology positivity (15.1 (6.6, 34.6)).

Conclusion. In an area heavily affected by COVID-19, after the peak of the first epidemic wave and during the lockdown, the rate of children with RT-PCR SARS-CoV-2 positivity was very low (1.8%), but that of serology positivity was higher (10.7%). Most children with positive RT-PCR results also had positive serology results.

What is already known on this topic?

At this time, several studies suggested that children are less frequently infected with SARS-CoV-2 and potentially less contagious than adults.

- Most of the studies were based on RT-PCR SARS-CoV-2 testing, without antibody assays.

What does this study add?

- This study combining RT-PCR testing and serology assessed the prevalence of SARS-CoV-2 infection in children in an area heavily affected by the COVID-19 pandemic.
- Among a large cohort of children (>600), 11 (1.8%) had positive RT-PCR SARS-CoV-2 results and 65 (10.7%) had antibodies to SARS-CoV-2.
- The only factor associated with RT-PCR SARS-CoV-2 or serology positivity was the presence of a household contact with COVID-19.

Introduction

Since the beginning of the COVID-19 pandemic, reports from several countries indicated that the disease was less frequently reported and less severe in children than adults.[1-3] Worldwide, the number of confirmed pediatric cases seems relatively low, and they account for less than 1% of hospitalized cases and deaths.[1 4] Although most COVID-19 cases in children are not severe, serious COVID-19 illness resulting in hospitalization can occur in this age group, and recently, hyperinflammatory shock, with features similar to atypical Kawasaki disease, was reported in several countries.[5-10]

However, concerns have been raised that children could play an important role in the spread of the disease because community testing has demonstrated a significant number of children with no or subclinical symptoms.[11] Indeed, if as for influenza, children could be the primary drivers of household SARS-CoV-2 transmission, then a silent spread from children who did not alert anyone to their infection could be a serious driver in the dynamics of the epidemic.[12] On the basis of this prevailing hypothesis, school closures were implemented almost ubiquitously around the world to try to halt the potential spread of COVID-19.[13 14]

However, several studies had already shown that when SARS-CoV-2 infection was suspected (compatible clinical signs, contact with a person with COVID-19), the rate of positive RT-PCR SARS-CoV-2 results was lower in children than adults.[14,15] In contrast, in RT-PCR SARS-CoV-2–positive children, the viral load was comparable between children and adults.[16] Furthermore, one study suggested that children shed infectious SARS-CoV-2.[17] However, results from a systematic review of household clusters of COVID-19 revealed that only 3/31 clusters were due to a child index case, and a population-based school contact-tracing study found minimal transmission by child or teacher index cases.[18 19] Finally, other studies suggested that children were potentially less contagious

than adults but the design of these studies does not exclude the possibility of children being more contagious than adults.[16 20-22]

Some countries such as South Korea and Iceland have implemented widespread community testing. Both countries found children significantly underrepresented in cases. In Iceland, this was true in targeted testing of high-risk groups as compared with adults (6.7% < 10 years vs 13.7% ≥ 10 years positive cases), and in (invited) population screening, no child < 10 years old was positive for SARS-CoV-2 as compared with 0.8% in the general population. [23]

Of note, all these studies were based on RT-PCR testing, but serology diagnosis is also an important tool to understand the prevalence and burden of COVID-19.[24] A serology survey tested adolescents in a high school in the north of France, the site of a cluster at the end of February. Of the 242 students tested, 2.7% of children ≤ 14 years old and 40% aged 15-17 years had positive SARS-CoV-2 serology results (IgG), which suggests a difference in susceptibility to SARS-CoV-2 among younger children.[25]

To best approach the prevalence of SARS-CoV-2 in children at a population level, we combined both RT-PCR testing for SARS-CoV-2 and serology in asymptomatic or pauci-symptomatic children (with mild clinical symptoms) in the Paris area, the most affected region in France, during the COVID-19 epidemic.

Patients and Methods

Study population

This was a cross-sectional prospective, multicenter study conducted by the Association Clinique et Thérapeutique Infantile du Val de Marne (ACTIV) network, a research unit expert in epidemiological surveillance and clinical studies in ambulatory pediatric infectious diseases, and the University Intercommunal Créteil Hospital.[26] Primary care

pediatricians (n = 27) took part in the study from April 14, 2020 to May 12, 2020. The strategy of closing schools and the lockdown decided by the French government for the whole country started on March 17 and finished on May 11, 2020.

This study aimed to enroll children from birth to 15 years old who were consulting an ambulatory pediatrician and distributed in two groups: asymptomatic and pauci-symptomatic. Asymptomatic children were defined as children without any symptoms or signs suggesting infectious disease during the previous 7 days. They usually came for vaccination visits. In this group, we defined two subgroups of children: those previously symptomatic (fever, respiratory or digestive symptoms) between 7 days and 2 months before enrollment, and those without any previous symptoms. Pauci-symptomatic children were defined as those with fever isolated or associated with respiratory signs such as cough, dysphagia, rhinorrhea, diarrhea, vomiting, cutaneous signs, taste loss and/or anosmia during the previous 7 days. Children were excluded if the clinical condition at enrollment required transfer to a pediatric emergency unit or hospitalization.

After informing the parents of the participating children and obtaining their signed consent, an electronic case report form (eCRF) was completed by the pediatrician to collect socio-demographic data, history, contact with a person with confirmed COVID-19 by RT-PCR for SARS-CoV-2, clinical symptoms and signs, and additional positive biological tests. We also collected suspected COVID-19 contacts because of the limited availability of testing. Indeed, during the lockdown, the diagnostic RT-PCR SARS-CoV-2 test was mainly available for patients with severe disease and/or healthcare workers, and all symptomatic individuals could not be tested. For all enrolled children, during the same visit, a nasopharyngeal (NP) swab was taken for RT-PCR detection of SARS-CoV-2 and a microsample of blood for micro-method serology.

Power calculation

To have an appropriate proportion of confirmed RT-PCR SARS-CoV-2–positive patients among asymptomatic and pauci-symptomatic children, with a 95% confidence interval (CI) of ± 3%, assuming a positivity proportion < 10% which was the rate of positive RT-PCR before the beginning of the study, we needed to enroll 300 children per group (asymptomatic and pauci-symptomatic), for 600 patients in total.

Serology

As previously reported[27], pediatricians collected fingerstick whole-blood specimens and used the Biosynex COVID-19 BSS test which was among those approved by the French national health authority.[27] A positive serology result meant that the patient had a previous SARS-CoV-2 infection: positive for IgG or IgM or both. A serology result was considered negative if results for both IgM and IgG were negative.

SARS-CoV-2 RT-PCR

As previously reported[27], pediatricians collected NP specimens transported to the centralized microbiology laboratory (CHIC) for the RT-PCR for SARS-CoV-2 analysis. NP samples were considered positive with a cycle threshold value (Ct) < 40 obtained for any gene. Amplification of two or three targets indicated that SARS-CoV-2 RNA was detected, and amplification of only one target with Ct value < 38 indicated a presumptive positive result. We defined as weakly positive any result with Ct value > 38 and < 40. A sample was considered negative if the internal control was amplified but not the viral target genes. A sample was considered invalid when no amplification was obtained for the internal control.

Statistical analysis

Data were entered by using the eCRF (PHP/MySQL) and analyzed by using Stata/SE v15 (StataCorp, College Station, TX, USA). For the initial analysis (univariate), quantitative data were compared by Student *t* test and categorical data by chi-square or Fisher exact test. Variables (age, clinical signs, contact, siblings and daycare attendance modalities) with $p < 0.20$ on univariate analysis were included in the multivariable model. For this model, we used a logistic regression to estimate odds ratios (ORs) and 95% CIs for factors associated with RT-PCR SARS-CoV-2 and serology positivity. Only variables with a p value <0.05 were kept in the final model. All tests were 2-sided and were considered significant at $p<0.05$.

Ethics

The study protocol was approved by an ethics committee (CPP IDF IX no. 08-022). Parents of all infants and children provided written informed consent. The study was registered at ClinicalTrials.gov NCT04318431.

Patient and Public Involvement

There were no patients or public involved in the research design, process and research findings dissemination.

Results

From April 14, 2020 to May 12, 2020, 27 ambulatory pediatricians in the Paris area enrolled 605 children: 322 (53.2%) children were asymptomatic and 283 (46.8%) pauci-symptomatic. Table 1 presents the characteristics of the enrolled children by group. In the pauci-symptomatic group, the main signs and symptoms were fever (187, 66.3%), cough

(143, 50.7%), pharyngitis (143, 50.7%), rhinitis (137, 48.4%), diarrhea (81, 28.7%), rash (64, 23.0%), vomiting (52, 18.8%), dysgeusia (8, 3.0%) and anosmia (5, 3.3%).

Figure 1 presents the dates of the lockdown and the number of children enrolled, by week, during the first epidemic wave in Paris.[28]

RT-PCR SARS-CoV-2 results were positive for 11 (1.8%) children, with no significant difference between children with and without symptoms (Chi2, p=0.3, Table 2). The supplemental Table 1 shows the details of the 11 positive RT-PCR SARS-CoV-2 cases. Only 3 children had RT-PCR SARS-CoV-2 positivity with Ct value < 31.

On multivariable analysis (supplemental Table 2), contact with a person with proven COVID-19 was the only significant risk factor for RT-PCR SARS-CoV-2 positivity (OR 7.8, 95% CI (1.5, 40.7)).

Table 2 shows the serology results by group. The age distribution of children was similar whatever the serology results, negative or positive: < 3 months, 1.3% vs 1.5%; 3 to 30 months, 37.2% vs 26.2%; 31 months to 5 years, 29.6% vs 36.9%; 6 to 10 years, 21.7% vs 26.2%; ≥11 years, 10.2% vs 9.2%. Serology was positive for 65 of 605 (10.7%) children, and among these, 87.3% had a confirmed or suspected contact. Children previously symptomatic during the preceding weeks, more frequently were positive on serology.

RT-PCR SARS-CoV-2 was more frequently positive for children with positive than negative serology results (12.3% vs 0.6%, p<0.001). Only 3 children had RT-PCR SARS-CoV-2 positivity without any antibody response detected.

Table 3 shows serology and RT-PCR SARS-CoV-2 results for the 543 enrolled children according to contact with a person (adult or child) with suspected or confirmed COVID-19. Only 2 of 275 (0.7%) children without any contact with a person with COVID-19 had positive RT-PCR SARS-CoV-2 results.

On multivariable analysis (supplemental Table 3), serology positivity was associated with contact with a person with proven or suspected COVID-19 (OR 15.1, 95% CI (6.6; 34.6) and 5.8, 95% CI (2.6; 13.2)).

Discussion

This study combines RT-PCR SARS-CoV-2 and serology results to assess the prevalence of SARS-CoV-2 infection in a large cohort of children in the community. In a region strongly affected by the epidemic (Paris area), during the lockdown, as expected, very few children (1.8%) had RT-PCR SARS-CoV-2 positivity, but the proportion with serology positivity (10.7%) was relatively high. Despite the relatively large number of children included (>600), we did not find a significant difference in rate of positive RT-PCR or serology results between asymptomatic and pauci-symptomatic children, which suggests that most children were asymptomatic after a SARS-CoV2 infection.

Among asymptomatic children, those with no history of symptoms during the preceding weeks accounted for two-thirds of children with positive serology results (28/41), which supports the fact that asymptomatic infections are frequent in children. By contrast, history of symptoms during the preceding weeks significantly increased the risk of positive serology. However, on multivariable analysis, the only factor influencing the positivity of RT-PCR or serology was the household contact who previously presented symptoms suggesting COVID-19. Of note, the number of siblings in the family did not significantly increase the probability of a positive RT-PCR or serology result. Several studies have shown that children were usually infected by an adult in the family.[18 22 29 30] In our study, the importance of familial contagion in the modalities of SARS-Cov-2 transmission is suggested by a very low RT-PCR (0.7%) and serology (3.6%) positivity rate for children without an infected relative and in a period of lockdown.

Among the children with RT-PCR positivity (n=11), only 3 had no antibody response, and 8 were positive for IgG with or without IgM positivity. This finding supports that for these 3 patients, infection had probably occurred some time before enrolment.

We highlight that the frequency of RT-PCR SARS-CoV-2 positivity was significantly higher in children with positive than negative serology results (12.3% vs 0.6%, p<0.001). This finding highlights the difficulties in interpreting the significance of a positive RT-PCR SARS-CoV-2 result without concomitant antibody testing after the epidemic wave. Preliminary reports suggest that children with RT-PCR SARS-CoV-2 positivity and IgG positivity probably had little or no infectivity.[31 32] In a study of 9 patients, attempts to isolate the virus in culture were not successful beyond day 8 of illness onset, which could be related to the decreased infectivity beyond the first week.[33] In the study of Bullard *et al.*, SARS-CoV-2 Vero cell infectivity was observed with only RT-PCR Ct value < 24 and symptom onset to test duration < 8 days.[34] Infectivity was likely low for 8 of the 11 RT-PCR SARS-CoV-2–positive children. Indeed, only 3 children had a Ct value < 31.

Our study has several limitations. First, the role of assumed household transmission has probably been over-estimated because of the well-followed lockdown in France.[35] Indeed, more than 86.5% of children with RT-PCR SARS-CoV-2 or serology positivity had a confirmed or suspected COVID-19 household contact. However, our rate of positive serology for children in the Paris area was similar to the rate observed for hospitalized children (11.7%) and school children (8.8%).[22 36] Second, the ability to successfully collect NP swabs properly could be more difficult in young children and significantly affect the results and be a factor contributing to the low RT-PCR positivity prevalence observed in our population. However, the pediatricians who performed the study were all involved for many years in a pneumococcal NP carriage study (started in

2001 and currently ongoing) and were particularly well trained to collect appropriately NP samples.[37]

School closure or limitation (reduced number of students or days of attendance) has a major impact on children's development and access to learning.[38] Therefore, the usefulness of school closure or limitation needs evaluation in controlling the COVID-19 epidemic.[39] We plan to renew this study after the full re-opening of schools and daycare centres in the Paris area. To better assess the transmission of SARS-Cov-2 in children and to elucidate their role in the transmission, serial testing of all household members is needed.

Funding. This work was supported by the French Ministry of Health "DGOS PHRC regional IDF 2020 no. AOR20095."

Competing Interests Statement: All other authors declare no competing interests for this study. RC, CL, EV received personal fees and non-financial support from Pfizer. RC reports personal fees from Merck, GSK, Sanofi and AstraZeneca outside the submitted work. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Contributorship Statement: RC, CL, NO, CJ, OL and EV designed the study. RC, CL, NO, CJ, OL, AS, CB, AE, FC, FCS, AW, OR and EV analyzed and interpreted the data and drafted the article. SB and CL performed the statistical analysis. EV, SA, NS, CR, SLM performed the microbiological analysis. All authors revised and approved the manuscript.

Acknowledgments. We are grateful to the investigators of the COVILLE study Network:

Akou'ou M.H, Auvrignon A, Belaroussi N, Benani M, Cambier Nappo E, Chartier Albrech C, Coicadan L, Condor R, D'Acremont G, D'Ovidio N, De Brito B, Deberdt P, Delatour A, Gorde-Grosjean S, Louvel M, Michot-Cottias A-S, Ravilly S, Seror E, Turberg-Romain C,

We are grateful to Adjemian S, Auffroy O, Begard M, Harant J, Mouaouya M, Receveau F, and Sigere ML, for technical assistance ; Cuquemelle A for secretarial assistance.

We are grateful to the ACTIV team : Ramay I, Prieur C; Prieur A, Borg M, Meyet L, Levy J, and Zemmour E (Association Clinique et thérapeutique Infantile du Val de Marne);

We are grateful to the CRC team: Brussieux M and Hoffart C from the Clinical Reaserch Center of the CHI Créteil.

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Table 1. Characteristics of children enrolled in the study and by pauci-symptomatic and asymptomatic group

	Overall	Pauci-symptomatic children*	Asymptomatic children			p$
			Previously symptomatic (>7days)**	No previous symptoms ***	Total	
	n=605	n=283	n=118	n=204	n=322	
Age (years)						
mean±SD	4.9±3.9	4.8±3.7	5.6±4.3	4.7±4.1	5.0±4.2	0.08
Median	3.8	4.0	4.4	3.4	3.7	
<3 mo.	8 (1.3)	6 (2.1)	0	2 (1.0)	2 (0.6)	
3-30 mo.	218 (36.0)	98 (34.6)	37 (31.4)	83 (40.7)	120 (37.3)	
31 mo. – 5 y.	184 (30.4)	96 (33.9)	34 (28.8)	54 (26.5)	88 (27.3)	0.1
6 y. – 10 y.	134 (22.2)	61 (21.6)	29 (24.6)	44 (21.6)	73 (22.7)	
≥11 y.	61 (10.1)	22 (7.8)	18 (15.2)	21 (10.3)	39 (12.1)	
Sex, male	322 (53.2)	152 (53.7)	65 (55.1)	105 (51.5)	170 (52.8)	0.8

Daycare attendance before lockdown						
Home	78 (13.8)	34 (13.0)	6 (5.4)	38 (19.7)	44 (14.5)	
Childminder	55 (9.7)	24 (9.2)	10 (9.0)	21 (10.9)	31 (10.2)	0.031
Daycare center	135 (23.9)	66 (25.2)	29 (26.1)	40 (20.7)	69 (22.7)	
School	298 (52.7)	138 (52.7)	66 (59.5)	94 (48.7)	160 (52.6)	
Comorbidities	93 (15.4)	45 (15.9)	28 (23.7)	20 (9.8)	48 (14.9)	0.004
Prematurity	35 (6.3)	15 (5.7)	7 (6.1)	13 (7.4)	20 (6.9)	0.8
Siblings						
0	115 (20.6)	57 (21.9)	18 (15.9)	40 (21.5)	58 (19.4)	
1	282 (50.5)	136 (52.3)	61 (54.0)	85 (45.7)	146 (48.8)	0.3
≥2	162 (29.0)	67 (25.8)	34 (30.1)	61 (32.8)	95 (31.8)	

Data are n (%) unless indicated.

$ p compares symptomatic children, asymptomatic children previously symptomatic >7 days and asymptomatic children without previous symptoms

* Pauci-symptomatic children were those with fever isolated or associated with respiratory signs such as cough, dysphagia, rhinorrhea, diarrhea, vomiting, rash, dysgeusia and/or anosmia, during the previous 7 days

** Previously symptomatic (fever or respiratory or digestive) between 7 days and 2 months before enrollment

*** No previous symptoms

Table 2. Results of RT-PCR SARS-Cov-2 testing and serology in children by pauci-symptomatic and asymptomatic group

	Overall	Pauci-symptomatic children*	Asymptomatic children		
			Previously symptomatic (>7 days)**	No previous symptoms***	Total
	n=605	n=283	n=118	n=204	n=322
RT-PCR					
Overall	11 (1.8) (0.9, 3.2)	7 (2.5) (1.0, 5.0)	1 (0.8) (0.0, 4.6)	3 (1.5) (0.3, 4.2)	4 (1.2) (0.3, 3.1)
Definite positive[$]	5	3	0	2	2
Weakly positive[$$]	1	1	0	0	0
Presumptive[$$$]	5	3	1	1	2
Serology					
IgM+ and/or IgG+	65 (10.7) (8.4, 13.5)	24 (8.5) (5.5, 12.4)	28 (23.7)[$] (16.4, 32.4)	13 (6.4)[$] (3.4, 10.7)	41 (12.7) (9.3, 16.9)
IgM+IgG-	7 (1.2) (0.5, 2.4)	4 (1.4) (0.4, 3.6)	2 (1.7) (0.2, 6.0)	1 (0.5) (0.0, 2.7)	3 (0.9) (0.2, 2.7)
IgM+IgG+	32 (5.3) (3.6, 7.4)	12 (4.2) (2.2, 7.3)	17 (14.4)[$] (8.6, 22.1)	3 (1.5)[$] (0.3, 4.2)	20 (6.2) (3.8, 9.4)
IgM-IgG+	26 (4.2) (2.8, 6.2)	8 (2.8) (1.2, 5.5)	9 (7.6) (3.5, 14.0)	9 (4.4) (2.0, 8.2)	18 (5.6) (3.3, 8.7)

Data are n (%) (95% confidence interval).

* Pauci-symptomatic children were those with fever isolated or associated with respiratory signs such as cough, dysphagia, rhinorrhea, diarrhea, vomiting, rash, dysgeusia and/or anosmia, during the previous 7 days

** Previously symptomatic (fever or respiratory or digestive) between 7 days and 2 months before enrollment

*** No previous symptoms

[$]Definite positive: cycle threshold (Ct) value < 38 obtained for 2 or 3 genes.

[$$]Weakly positive: any result with a Ct value > 38 and < 40.

[$$$]Presumptive: Ct < 38 obtained for only one target.

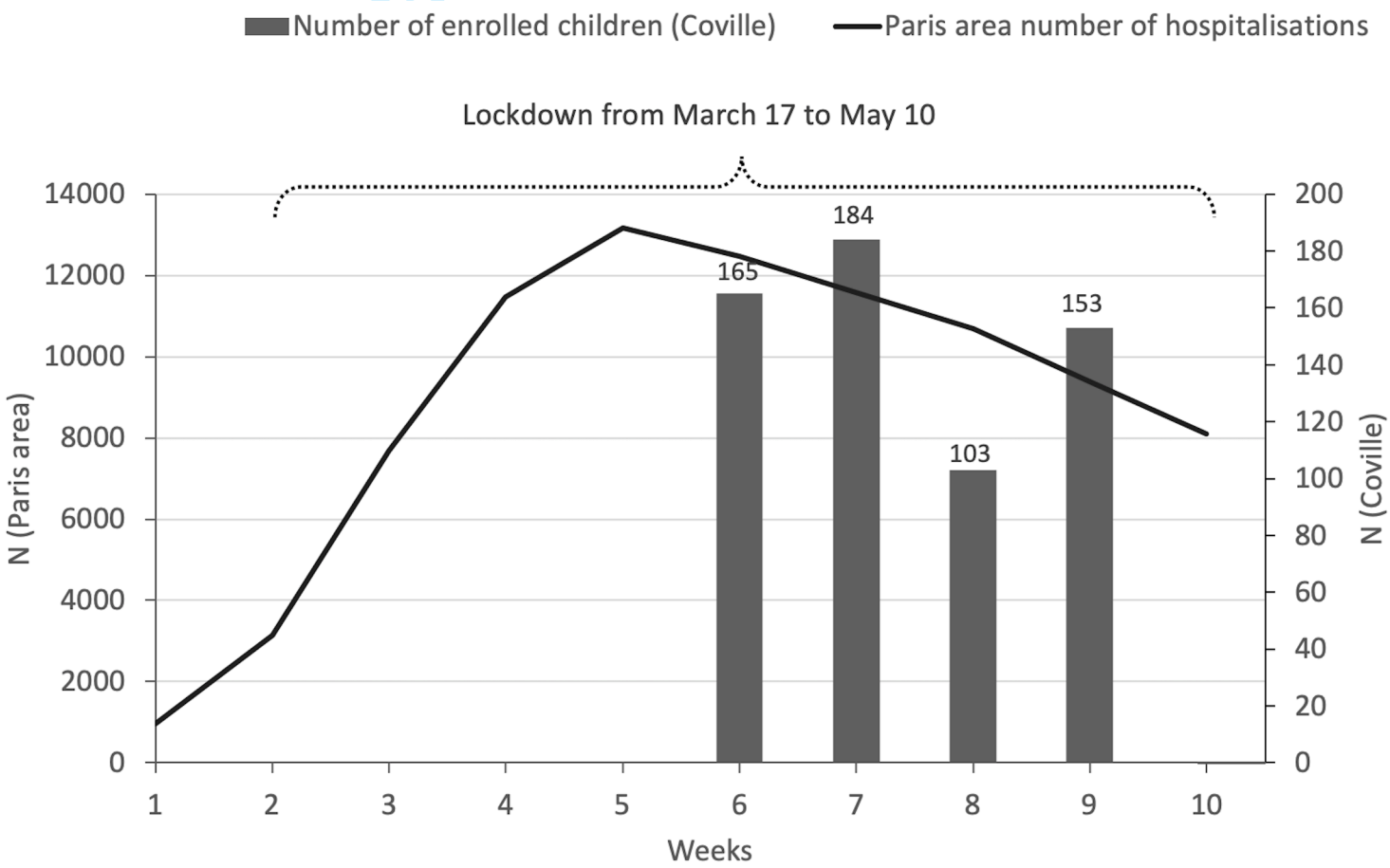
Table 3. Serology and RT-PCR results for the 543 enrolled children according to contact with a person (adult or child) with confirmed and/or suspected COVID-19

	Enrolled children				
	Overall n=543*	**Positive serology** n=63	**Negative serology** n=480	**Positive RT-PCR SARS-CoV-2** n=11	**Negative RT-PCR SARS-CoV-2** n=532
Contact					
Confirmed COVID-19**	93 (17.1) (14.1, 20.6)	29 (31.2) (22.0, 41.6)	64 (68.8) (58.4,78.0)	5 (5.4) (1.8, 12.1)	88 (94.6) (87.9, 98.2)
Suspected COVID-19***	175 (32.2) (28.3, 36.3)	26 (14.9) (9.9, 21.0)	149 (85.1) (79.0, 90.0)	4 (2.3) (0.6, 5.7)	171 (97.7) (94.3, 99.4)
Confirmed / suspected COVID-19	268 (49.4) (45.1, 53.6)	55 (20.5) (15.9, 25.9)	213 (79.5) (74.1,84.1)	9 (3.4) (1.5, 6.3)	259 (96.6) (93.7, 98.5)
No contact	275 (50.6) (46.4, 54.9)	8 (2.9) (1.3, 5.7)	267 (97.1) (94.3, 98.7)	2 (0.7) (0.1,2.6)	273 (99.3) (97.4, 99.9)

Data are n (%) (95% confidence interval).

* 543 available data among 605 enrolled patients
** Confirmed by RT-PCR SARS-CoV-2
*** Suspected symptoms suggestive of COVID-19 because of the limited availability of testing

Figure 1. Number of children enrolled and total hospitalisation during the first COVID-19 epidemic wave in the Paris area



Supplemental Table 1. Description of the 11 patients with positive RT-PCR for SARS-CoV-2

Patients	Target	Result	Ct
Patient 1 (9.4 years)	E gene	-	NA
	RdRP gene	-	NA
	N gene	+	38.45
	IC	+	25.52
Patient 2 (5.1 years)	rs	-	NA
	RdRP gene	-	NA
	N gene	+	34.85
	IC	+	25.04
Patient 3 (4.5 years)	E gene	-	NA
	RdRP gene	-	NA
	N gene	+	36.38
	IC	+	26.04
Patient 4 (5.6 years)	E gene	+	30.91
	RdRP gene	+	32.75
	N gene	+	32.83
	IC	+	26.05
Patient 5 (19 days)	E gene	+	27.20
	RdRP gene	+	28.66
	N gene	+	28.59
	IC	+	25.20
Patient 6 (2.1 years)	E gene	-	NA
	RdRP gene	+	38.91
	N gene	+	38.84
	IC	+	25.91
Patient 7 (4.8 years)	E gene	-	NA
	RdRP gene	+	34.60
	N gene	+	37.52
	IC	+	25.62
Patient 8 (9.5 years)	E gene	-	NA
	RdRP gene	-	NA
	N gene	+	38.63
	IC	+	25.97
Patient 9 (1.8 years)	E gene	-	NA
	RdRP gene	+	35.26
	N gene	+	35.51
	IC	+	25.95
Patient 10 (6.5 years)	E gene	-	NA
	RdRP gene	+	37.59
	N gene	-	NA
	IC	+	25.21
Patient 11 (9.3 years)	E gene	+	28.00
	RdRP gene	+	29.41
	N gene	+	30.14
	IC	+	25.69

Ct, Cycle threshold, IC, internal control, NA, not applicable

Supplemental Table 2. Risk factors of positive RT-PCR for SARS-CoV-2 by univariate and multivariate analysis

	Positive RT-PCR SARS-CoV-2 n=11	Negative RT-PCR SARS-CoV-2 n=532	OR (95%CI)	p	aOR (95%CI)	p
Age						
<3 mo.	1 (12.5%)	7 (87.5%)	1			
3-30 mo.	2 (0.9%)	216 (99.1%)	0.1 (0, 0.8)	0.033		
31 mo. – 5 y.	4 (2.2%)	180 (97.8%)	0.2 (0, 1.6)	0.1		
6 y. – 10 y.	4 (3.0%)	130 (97.0%)	0.2 (0, 2.2)	0.2		
≥11 y.	0	61 (100%)	-	-		
Contact						
None	2 (0.7%)	273 (99.3%)	1		1	
Suspected COVID-19	4 (2.3%)	171 (97.7%)	3.2 (0.6, 17.6)	0.2	3.2 (0.6, 17.6)	0.2
Confirmed COVID-19	5 (5.4%)	88 (94.6%)	7.8 (1.5, 40.7)	0.015	7.8 (1.5, 40.7)	0.015
Fever						
No	3 (1.1%)	268 (98.9%)	1			
Yes	8 (3.1%)	254 (96.9%)	2.8 (0.7, 10.7)	0.1		
Rhinitis						
No	3 (0.9%)	325 (99.1%)	1			
Yes	7 (3.5%)	195 (96.5%)	3.9 (1.0, 15.2)	0.051		

Supplemental Table 3. Risk factors of positive serology by univariate and multivariate analysis

	Positive serology n=63	Negative serology n=480	OR (95%CI)	p	aOR (95%CI)	p
Daycare attendance before lockdown						
Home	3 (3.9%)	75 (96.1%)	1			
Childminder	4 (7.3%)	51 (92.7%)	2.0 (0.4, 9.1)	0.4		
Daycare center/School	50 (11.6%)	383 (88.4%)	3.2 (1.0, 10.7)	0.052		
Siblings						
No	7 (6.1%)	108 (93.9%)	1			
Yes	55 (12.4%)	389 (87.6%)	2.2 (1.0, 4.9)	0.06		
Contact						
None	8 (2.9%)	267 (97.1%)	1		1	
Suspected COVID-19	26 (14.9%)	149 (85.1%)	5.8 (2.6, 13.2)	<0.001	5.8 (2.6, 13.2)	<0.001
Confirmed COVID-19	29 (31.2%)	64 (68.8%)	15.1 (6.6, 34.6)	<0.001	15.1 (6.6, 34.6)	<0.001
Respiratory signs						
No	19 (8.3%)	210 (91.7%)	1			
Yes	41 (13.5%)	262 (86.5%)	1.7 (1.0, 3.1)	0.06		
Dysgeusia / Anosmia						
No	53 (10.6%)	445 (89.4%)	1			
Yes	3 (23.1%)	10 (76.9%)	2.5 (0.7, 9.4)	0.2		
Rash						
No	42 (10.1%)	375 (89.9%)	1			
Yes	15 (15.8%)	80 (84.2%)	1.7 (0.9, 3.2)	0.1		
Cough						
No	28 (8.8%)	289 (91.2%)	1			
Yes	31 (14.6%)	181 (85.4%)	1.8 (1.0, 3.0)	0.04		
Rhinitis						
No	28 (8.5%)	300 (91.5%)	1			
Yes	31 (15.4%)	171 (84.6%)	1.9 (1.1, 3.3)	0.017		
Chest pain						
No	58 (11.2%)	461 (88.8%)	1			
Yes	2 (33.3%)	4 (66.7%)	4.0 (0.7, 22.2)	0.1		