

COVILLE STUDY

SARS-COV-2 BY RT-PCR AND CONCOMITANT SEROLOGY IN CHILDREN IN PARIS AREA

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

- COVID-19 disease = less frequent and less severe in children than adults
- In France, during the first epidemic wave, the SARS-CoV-2–positive rate was about 2- to 7-fold less for children than adults
- **Questions:**
 - Children could play a role in the spread of the disease?
 - Number of children with no or subclinical symptoms?
 - Children could be the primary drivers of household SARS-CoV-2 transmission?
- ➔ **school closures around the world to try to halt the potential spread of COVID-19**

- *Castagnoli R, JAMA Pediatr 2020.*
- *Wu Z, JAMA 2020.*
- *Ludvigsson JF. Acta Paediatr 2020; 109(6): 1088-95.*
- *Munro APS, Arch Dis Child 2020.*
- *Levy C. <https://medrxiv.org/cgi/content/short/2020051820098863v1> 2020.*

ACTIV Aim of the study → To best approach the spread and dynamics of transmission of SARS-CoV-2 in children



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

Here we combined both RT-PCR testing for SARS-CoV-2 and serology in asymptomatic or pauci-symptomatic children in the Paris area = the most affected region in France, during the COVID-19 epidemic

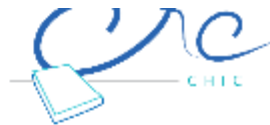


Methods : study design

- Cross-sectional prospective, multicenter study conducted by ACTIV network and Créteil Intercommunal Hospital
- 27 Primary care pediatric private practices in Paris area
- From April 14 to May 12, 2020
- Closing schools and the lockdown decided by the French government for the whole country started on March 17 and finished on May 11, 2020

- **Enrollment of children from birth to 15 years of age**
 - **healthy controls (asymptomatic) = children without any symptoms or signs suggesting infectious disease**
 - *subgroup of children who had symptoms (fever or respiratory or digestive) more than 7 days before enrolment*
 - **pauci-symptomatic children with mild clinical symptoms = children with fever isolated or associated with respiratory signs such as cough, dysphagia, rhinorrhea, diarrhea, vomiting, cutaneous signs, taste loss and/or anosmia**
- **Children were excluded if the clinical condition at enrolment required transfer to pediatric emergency unit or hospitalization**

- Signed consent of parents of the participating children
- Electronic case report form (eCRF) completed by the pediatrician



ACTIV

**Bienvenue sur le site de l'étude "COVILLE :
Prévalence du portage du SARS-CoV2 chez l'enfant
asymptomatique et pauci symptomatique, étude
transversale, prospective, multicentrique,
observationnelle en soins primaires"**

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Methods: Data collected

- **socio-demographic data**
- **history**
- **contact with a person with confirmed COVID-19**
- **contact with a person with suspected COVID-19**
- **clinical symptoms and signs**
- **additional positive biological test**
- **nasopharyngeal (NP) swab for RT-PCR detection of SARS-CoV-2**
- **microsample of blood for micro-method serology**

Methods: 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 $\pm 3\%$, assuming a positivity proportion $< 10\%$, we needed to enroll 300 children per group (asymptomatic and pauci-symptomatic), for 600 patients in total

Pediatricians collected fingerstick whole-blood specimens and used the Biosynex COVID-19 BSS test = rapid chromatographic immunoassay for qualitative detection of IgG and IgM antibodies to SARS-CoV-2 in blood

➤ **Approved by the French national health**

authority <https://covid-19.sante.gouv.fr/tests>

➤ **Diagnostic accuracy of the test (according to the specifications of the manufacturer)**

➤ sensitivity 91.8% [95% CI 83.8-96.6]

➤ specificity 99.2% [95%CI 97.7-99.8]

(<https://www.biosynex.com/laboratoires-hopitaux-tests-covid-19/>)

➤ **Assessment by independent investigators**

confirmed the good diagnostic accuracy of this test among hospital staff with mild disease in

eastern France

Fafi-Kremer S, Bruel T, Madec Y, et al. Serologic responses to SARS-CoV-2 infection among hospital staff with mild disease in eastern France. <https://www.medrxiv.org/content/101101/2020051920101832v2> 2020.



- **Positive serology was defined as :**
 - a case positive for IgM and negative for IgG
or
 - positive for IgM and IgG
or
 - negative for IgM and positive for IgG

- **All other cases were considered: negative serology**

Methods: SARS-CoV-2 RT-PCR (1)

- 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 within 48 hours to the centralized microbiology laboratory, Intercommunal Créteil Hospital
- Before extraction, each NP 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 Allplex™ 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 (viral envelop)



Methods: SARS-CoV-2 RT-PCR (2)

- 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 each reaction mix to verify extraction and determine PCR inhibition. Positive and negative controls were included in each run.

Methods: SARS-CoV-2 RT-PCR (3)

- NP samples were considered positive when a cycle threshold value (C_t) less than 40 was obtained for any gene
- 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
- The C_t values were used as indicators of the copy number of SARS-CoV-2 RNA in specimens with lower C_t values corresponding to higher viral copy numbers

Statistical analysis

- Data were entered by using an e-CRF (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
- 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 and fundings

- The study protocol was approved by an ethics committee (CPP IDF IX no. 08-022)
- Parents of all infants provided written informed consent
- Funding was received from French Ministry of Health “DGOS PHRC regional IDF 2020 no. AOR20095.”
- The study was registered at [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT04318431) NCT04318431
- Declaration of interests: no competing interests for this study

- From April 14, 2020 to May 12, 2020,
- 27 ambulatory pediatricians in the Paris area enrolled 605 children:
 - 322 (53.2%) asymptomatic
 - 283 (46.8%) symptomatic
- Mean duration from symptom onset to enrollment was 12 ± 15 days (median 5) for symptomatic children.
- Among asymptomatic children, 118 (37%) had history of symptoms during the preceding weeks but more than 7 days before enrolment (mean 40 ± 22 days; median 36)
- Mean age was 4.9 ± 3.9 years (median 3.8) with no significant difference between the two groups

ACTIV Results: In the symptomatic group, main signs and symptoms: (n,%)



- 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%)
- anosmia (5, 3.3%)

Characteristics of children enrolled in the study and by symptomatic and asymptomatic group

	Overall n=605	Symptomatic children n=283	Asymptomatic children n=322	p
Age, mean±SD (years)	4.9±3.9	4.8±3.7	5.0±4.2	0.4
Median	3.8	4.0	3.7	
<3 months	8 (1.3)	6 (2.1)	2 (0.6)	
3-30 months	218 (36.0)	98 (34.6)	120 (37.3)	0.1
31 months – 5 years	184 (30.4)	96 (33.9)	88 (27.3)	
6 years – 10 years	134 (22.2)	61 (21.6)	73 (22.7)	
≥11 years	61 (10.1)	22 (7.8)	39 (12.1)	
Sex, male	322 (53.2)	152 (53.7)	170 (52.8)	0.8
Daycare attendance before lockdown				
Home	78 (13.8)	34 (13.0)	44 (14.5)	
Childminder	55 (9.7)	24 (9.2)	31 (10.2)	0.8
Daycare center	135 (23.9)	66 (25.2)	69 (22.7)	
School	298 (52.7)	138 (52.7)	160 (52.6)	
Comorbidities	93 (15.4)	45 (15.9)	48 (14.9)	0.7
Prematurity	35 (6.3)	15 (5.7)	20 (6.9)	0.6
Brothers/sisters				
0	115 (20.6)	57 (21.9)	58 (19.4)	
1	282 (50.5)	136 (52.3)	146 (48.8)	0.3
≥2	162 (29.0)	67 (25.8)	95 (31.8)	

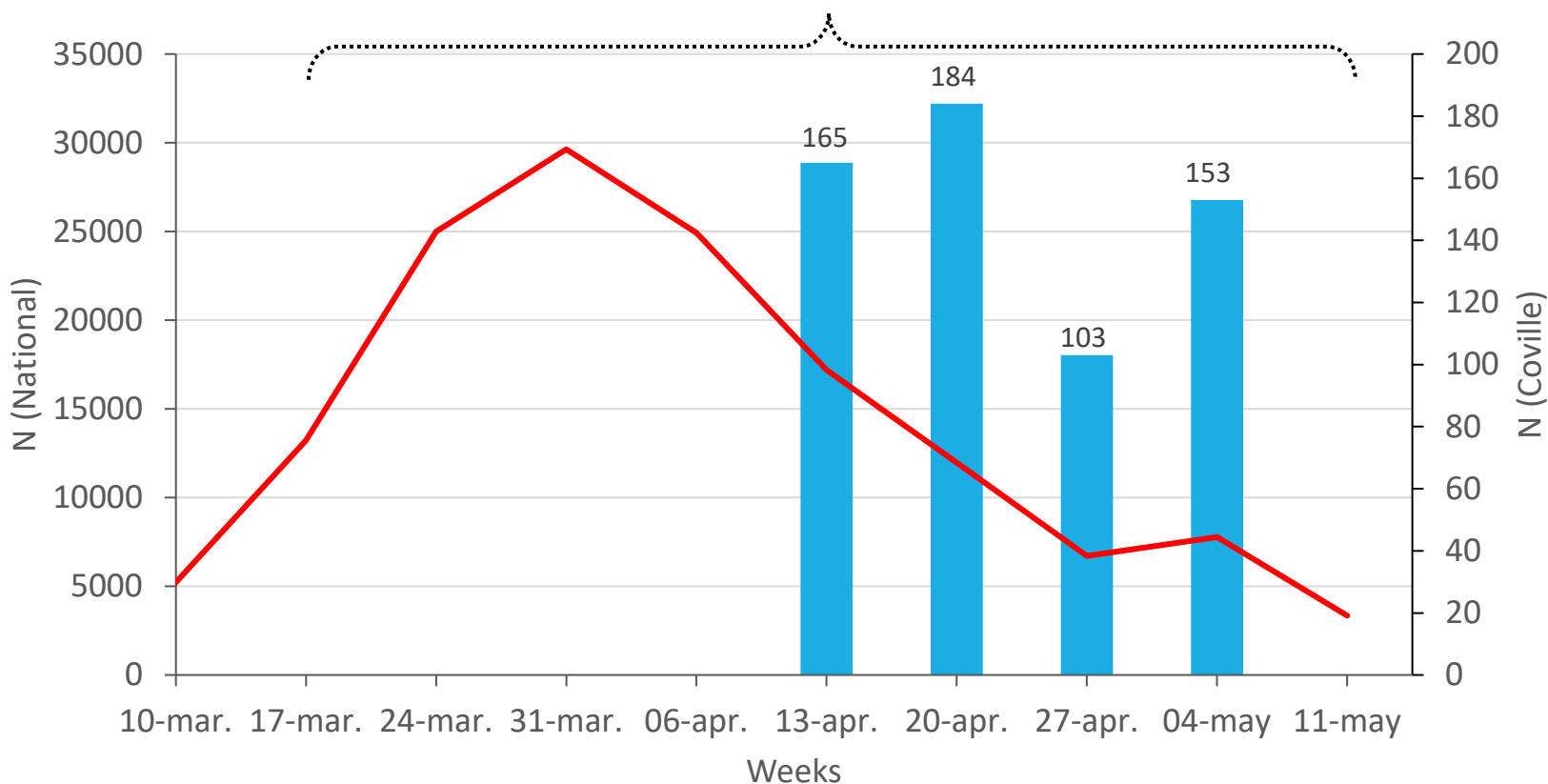
Dynamics of the first COVID-19 epidemic wave in France, the dates of the lockdown and the number of children enrolled by weeks

■ Number of enrolled children (Coville)

— National new Covid-19 cases

Jarlier V. <https://infogram.com/graphiques-covid-1h8n6mymkewj6xo?live>. 2020.

Lockdown from March 17 to May 10



ACTIV Results of RT-PCR SARS-Cov-2 testing and serology in children enrolled in the study and by symptomatic and asymptomatic group



	Overall n=605	Symptomatic children n=283	Asymptomatic children n=322
RT-PCR			
Overall	11 (1.8) [0.9; 3.2]	7 (2.5) [1.0; 5.0]	4 (1.2) [0.3; 3.1]
Definite positive	5	3	2
Weakly positive	1	1	0
Probable	5	3	2
Serology			
IgM+ and/or IgG+	65 (10.7) [8.4; 13.5]	24 (8.5) [5.5; 12.4]	41 (12.7) [9.3; 16.9]
IgM+IgG-	7 (1.2) [0.5; 2.4]	4 (1.4) [0.4; 3.6]	3 (0.9) [0.2; 2.7]
IgM+IgG+	32 (5.3) [3.6; 7.4]	12 (4.2) [2.2; 7.3]	20 (6.2) [3.8; 9.4]
IgM-IgG+	26 (4.2) [2.8; 6.2]	8 (2.8) [1.2; 5.5]	18 (5.6) [3.3; 8.7]

11 patients with positive RT-PCR for SARS-CoV-2

Only 5 children had positive RT-PCR SARS-CoV-2 result for all 3 amplified genes, among which only two children presented C_t between 27 and 30

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

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

ACTIV Results of RT-PCR SARS-Cov-2 testing and serology in asymptomatic children with and without symptoms more than 7 days before enrollment



	Overall n=322	With symptoms n=118	Without symptoms n=204
RT-PCR			
Overall	4 (1.2) [0.3; 3.1]	1 (0.8) [0.0; 4.6]	3 (1.5) [0.3; 4.2]
Definite positive	2	0	2
Weakly positive	0	0	0
Probable	2	1	1
Serology			
IgM+ and/or IgG+	41 (12.7) [9.3; 16.9]	28 (23.7) [16.4; 32.4]*	13 (6.4) [3.4; 10.7]*
IgM+IgG-	3 (0.9) [0.2; 2.7]	2 (1.7) [0.2; 6.0]	1 (0.5) [0.0; 2.7]
IgM+IgG+	20 (6.2) [3.8; 9.4]	17 (14.4) [8.6; 22.1]*	3 (1.5) [0.3; 4.2]*
IgM-IgG+	18 (5.6) [3.3; 8.7]	9 (7.6) [3.5;14.0]	9 (4.4) [2.0; 8.2]

* p<0.001

Children with history of symptoms during the preceding weeks, more frequently were positive on serology

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	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
Probable	1 (0.2)	4 (6.2)	0	3 (9.4)	1 (3.9)

- $p < 0.001$: 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+

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%, $p < 0.001$). Only 3 children were RT-PCR SARS-CoV-2-positive without any antibody response detected.

ACTIV 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]

* 543 available data on 605 enrolled patients

- 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
- Among the positive serology, 87.3% had a confirmed or suspected contact

- On multivariate analysis, **contact with a person with COVID-19 household was the only significant risk factor for RT-PCR–positive SARS-CoV-2 infection** **Odds Ratio 7.8, 95% CI [1.5; 40.7]**
- On multivariate analysis, **positivity on serology** was associated with contact with a person with **proven, Odds Ratio 15.1 [95% CI 6.6; 34.6]** or **suspected COVID-19, Odds Ratio 5.8 [95% CI 2.6; 13.2]**

- First to combine 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), no significant difference in the rate of positive RT-PCR or serology results between asymptomatic and pauci-symptomatic children

→ This fact supports that it is unlikely the cluster screening of symptomatic children in the community will be relevant

- Among asymptomatic children, those with history of symptoms during the preceding weeks accounted for 1/3 of children with positive serology results (13/41)

→ asymptomatic infections are frequent in children

- History of symptoms during the preceding weeks increased significantly the risk of positive serology
- 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
 - *This was noted whether this infection was proven by RT-PCR or unproven due to the absence of availability of the test*
 - *The number of siblings in the family did not significantly increase the probability of a positive RT-PCR or serology result*

→ children were usually infected by an adult in the family

Zhu Y, Children are unlikely to have been the primary source of household SARS-CoV-2 infections. doi: 10.1101/2020.03.26.20044826 [published Online First: 30 March 2020]

- Very low rate of positive RT-PCR for SARS-CoV-2: 0.7%
- Low rate of positive serology : 2.9%

Without contact with a suspected or confirmed COVID-19 case

- Of the children positive on RT-PCR (n=11)
 - only 3 had no antibody response → contamination had occurred during the 2 weeks before enrollment
 - and 8 were positive for IgG with or without IgM positivity
- The frequency of positivity on RT-PCR for SARS-CoV-2 significantly higher in children with positive serology (12.3%) than those with a negative one (0.6%, $p < 0.001$)
- difficulties in interpreting the significance of a positive RT-PCR SARS-CoV-2 result without concomitant antibody testing after the epidemic wave
- children positive on RT-PCR for SARS-CoV-2 and positive for IgG probably had little or no infectivity
- Low infectivity : only 5 children had positive RT-PCR SARS-CoV-2 result for all 3 amplified genes, among which only 2 children presented Ct between 27 and 30 (suggesting not very high viral load)

- **The role of assumed household transmission: over-estimated ?**
 - probably because of the well-followed lockdown in France
 - 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

- **Our rate of positive serology does not reflect the epidemiologic situation in the Paris area for children?**
 - probably because COVID-19 families already affected were more likely to consult and agree to participate in the study, the population of parents of enrolled children were possibly over-represented of COVID-19–affected families as compared with the general population

- Our data : consistent with the Fontanet et al. study, finding seropositivity in 10.2% of siblings of students in the cluster high school
- The importance of familial contagion in the modalities of SARS-Cov-2 transmission is underlined 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
- School closure or limitation (reduced number of students or days of attendance) has a major impact on children's development and access to learning
- The usefulness of school closure or limitation needs evaluation in controlling the COVID-19 epidemic
- Available data suggest that the role of children in the dynamics of the adult epidemic is probably modest, but further studies are necessary
- Plan to renew this study after the re-opening of schools and day care centre in the Paris area to better assess the transmission of SARS-Cov-2 in children