

SARS-CoV-2 transmission in Belgian primary schools of the Federation Wallonie - Bruxelles. An epidemiological pilot study.

Sponsor : UCLouvain

Sponsor's representative

Pr. Annie Robert. Responsable du pôle de recherche EPID Epidémiologie et biostatistique – Institut de Recherche Expérimentale et Clinique, UCLouvain

Principal investigator

Pr. Dimitri Van der Linden. UCLouvain SSS/IREC/PEDI, Pediatric Task Force spokesperson,

Subinvestigators :

Dr. Julie Frère. Pédiatre-infectiologue au CHU Liège, Pediatric Task force member

Dr. Olga Chatzis. Pédiatre-infectiologue aux Cliniques universitaires Saint-Luc, Institut de Recherche Expérimentale et Clinique/PEDI, UCLouvain, Pediatric Task force member

Dr Florence Renard. Médecin scolaire et de santé publique, Conseillère Médicale Promotion de la Santé à l'école (PSE)

Collaborators:

Dr Jean Ruelle. Virologue, chercheur à l'Institut de Recherche Expérimentale et Clinique, UCLouvain,

Pr Benoit Kabamba. Laboratoire de microbiologie et virologie - Cliniques universitaires Saint-Luc

Dr Mathilde de Keukeleire. Chercheur qualifié, EPID, IREC-UCLouvain,

Mme Kelly Cremer. Infirmière, Master en santé publique, Doctorante en EPID (supervision Pr. Annie Robert),

Pr. Laurent Gillet. Laboratoire d'immuno-vaccinologie. Faculté de Médecine Vétérinaire, ULiège,

Pr Marie-Luce Delforge. Microbiologie - Hôpital Erasme - ULB

Dr Els Duysburgh, PhD, épidémiologiste, Sciensano

Dr Nathalie Ribesse (ONE-PSE),

Dr Romain Mahieu. Médecin Direction Santé et Aide aux Personnes, COCOM,

Dr Sophie Lokietek. Médecin AVIQ. Direction prévention promotion et surveillance de la santé,

Dr Joanna Merckx, M.D, M.Sc. épidémiologiste, spécialiste en maladies infectieuses pédiatriques, Département d'épidémiologie, santé au travail et biostatistiques, Université McGill, Montréal, Canada,

Les PSE des écoles impliquées,

La direction et les instituteurs des écoles impliquées

1.0 Synopsis

Title	SARS-CoV-2 transmission in Belgian primary schools of the Federation Wallonie - Bruxelles. An epidemiological pilot study.
Study design	Interventional prospective SARS-CoV-2 infection study.
Study duration	Autumn – winter 2020-2021
General objective	Description and quantification of transmission dynamics of SARS-CoV-2 in primary schools.
Specific objectives	<p>To assess the role of children attending primary school in the spread of SARS-CoV2 during the 2020-2021 pandemic</p> <ul style="list-style-type: none"> • To assess transmission from asymptomatic infected children to other children attending the same school • To assess transmission from asymptomatic infected children to adults (parents and teachers) • To assess transmission from asymptomatic infected adult within the school environment to a child • To assess if a laboratory confirmed case in a classroom has been infected outside the school
Study hypothesis	The investigators hypothesize that children are less likely to become infected in the school environment, compared to the household or outside the school setting. Primary schools do not play a major role in SARS-CoV-2 spreading. They hypothesize that incidence level in schools is mainly a consequence of community transmission ie. the incidence level in households and that the secondary attack rate in schools remains at least 5 times lower than the transmission level in households.
Outcomes	<ul style="list-style-type: none"> • Infection of a child with SARS-CoV-2 secondary to a classmate • Infection of an adult with SARS-CoV-2 secondary to a child • Infection of a child with SARS-CoV-2 secondary to an adult within the school • Infection of a child with SARS-CoV -2 secondary to a household contact or outside the school
Exploratory outcome	Phylogenetic analysis of the SARS-CoV-2 in positive salivary tests by a Whole Genome Sequencing (WGS) test. The aim of this exploratory outcome is to determine potential linkages between cases.

Study participants/numbers of participating schools	<p>All Belgian primary schools of the Federation Wallonie – Bruxelles that have organized a full resumption of school activities with the control measures recommended by the government, are eligible for the study.</p> <p>Inclusion criteria All children and school attenders from selected schools will be invited to participate.</p> <p>Exclusion criteria Refusal to provide written informed consent before enrolment</p> <p>Sampling procedure Each participating primary school will be considered as a cluster. Eight schools will be chosen by purposive sampling on three criteria: either high (≥ 2 classes per grade) or low size of the school, a school within an area with either a high or a low incidence of SARS-CoV-2 during April 2020, the first wave of covid-19 in Belgium, and a school with either a high or a low socioeconomic level (Belgian 20-point-scale ISE index >14 or <6). As direct partners in the project, physicians in charge of health promotion in schools (PSE teams) will select schools.</p>
Study description	<p>Inclusion visit All participants (children and adults school attenders) will be tested at the inclusion day with</p> <ul style="list-style-type: none"> - A salivary SARS-CoV2 test - A rapid serological test by finger prick (AVIOQ[®]) to determine the seroprevalence of SARS-CoV-2 <p>All participants (children and adults school attenders) will be invited to fill a questionnaire on an electronic format when possible or with a paperback part if not, depending on parents' convenience.</p> <p>Follow-up visits During six consecutive weeks, all participants will be tested once a week with a salivary test. During six consecutive weeks, all participants will be invited to complete a follow-up questionnaire to assess any symptom compatible with a SARS-CoV-2 infection or a contact with a confirmed SARS-CoV-2 case or a travelling return from a risk area.</p>
Endpoints	<ul style="list-style-type: none"> - Time varying number and proportion of children infected with SARS-CoV-2 secondary to an infected classmate (transmission rate inside school but from a child to a child). - Time varying number and proportion of adult school-attenders infected with SARS-CoV-2 secondary to an asymptomatic infected child (transmission rate inside school but from a child to an adult); - Time varying number and proportion of children infected with SARS-CoV-2 secondary to an infected adult school attender (transmission rate inside school but from an adult to a child);

	<ul style="list-style-type: none"> - Time varying number and proportion of children infected with SARS-CoV -2 secondary to a household contact or outside the school (transmission rate from outside school).
Statistical analyses plan	<p>Sample size determination</p> <p>As a pilot study, no formal sample size calculation was performed. The expected number of children is 2400, the expected number of adult school attenders is 200, and the expected number of classes is 72.</p> <p>Initial incidence and then secondary attack rates will be calculated and compared between gender, across age, and between the three kinds of schools using Poisson or Binomial negative regression if overdispersion. Analyses will be weighted to correct for cluster effect of classes (ICC=0.09).</p>

2.0 Background and Rationale

To date, children represent a minority of confirmed cases of COVID-19 worldwide, and it is clear that they are less severely ill when infected (critical illness is very rare: ~1% and death remains extremely rare) than their adult counterparts. In Belgium, the last Sciensano report on children and Covid-19 reveals that children only represent 3% of all infections and 1.7% of all hospitalizations (1). Their role in transmission especially the role of asymptomatic children remains however an unresolved question. Children were, at first, suspected to be a motor of the COVID-19 pandemic, in parallel to influenza infection where school attendance plays an important role in amplifying epidemics. However, more and more data highlight huge differences between the two infections. The role of an individual in the transmission is multifactorial and influenced, among other things, by susceptibility to acquire infection, viral load, symptoms, contact patterns in the community, and social behaviour.

Even with the certainty that children can get infected, available data on probability of viral acquisition state that children have about half the risk of acquiring the virus compared to adults (2). These data are supported by information from contact tracing among households and have been reviewed in four different studies (3-6). Seroprevalence studies show also that children, especially less than 10-year old individuals, have lower rate of past infection (7-10).

However, it is unclear if asymptomatic children with positive PCR on saliva are contagious for their peers in primary school settings and if so, what this secondary attack rate is in this school setting. When quantifying viral load, studies have been conflicting and as well limited in their study design, however viral load does not seem to be majorly influenced by age, and viable viral particles have been retrieved from children's upper respiratory tract specimens (11). These laboratory results must be confronted to clinical observation in order to appreciate their significance. Data on that respect are scarce and what is available emerged from contact tracing in households where children tend to be less identified as index cases. But as they are often asymptomatic or mildly symptomatic, they could be underrepresented as index-cases. In school settings, available data are conflicting but highlight that other factors such as age, or, protective measures such as hand hygiene and social distancing have an impact. In France, transmission is described to have taken place in a secondary school, while in a primary school, transmission likely occurred within households and child transmission mainly reflected the

community presence of infections (12). When studies have been undertaken in settings where more strict protective measures were taken, evidence of transmission within school is limited. Some studies identify no secondary cases (13-15). UK surveillance identifies transmission driven solely by adults (16).

Some argue that, as previously said, these data were collected during lockdown and schools' closure or during careful re-opening and do not reflect the true transmission dynamics. A second often used comment is that only secondary cases linked to symptomatic confirmed cases are taken into account and the role of asymptomatic individuals, mainly children, is underestimated and not measured.

On the other hand, one cannot forget the importance of education and school attendance either, as the WHO pointed out recently. The unanswered question on transmission by children can affect political decisions, economical aspects, wellbeing of children and their families as well as their right for education (17).

It seems, for all these reasons, of importance to assess the transmission dynamics within the school's environment that resume to a full activity according to governmental measures, and, to analyse and sequentially address the impact of asymptomatic individuals.

3.0 Objectives and outcomes

The general objective of our study is to describe the transmission dynamics of SARS-CoV-2 in primary schools in Fédération Wallonie-Bruxelles.

3.1 Specific objectives

Specific objectives of the study are to assess the role of children attending primary schools in the spread of SARS-CoV-2 during the 2020-2021 pandemic

- To assess the child-to-child transmission within primary schools
- To assess the child-to-adult transmission within primary schools
- To assess the adult-to-child transmission within primary schools
- To assess the incidence of asymptomatic children who got infected through contact with an index case outside of the school environment

The investigators hypothesize that children are mostly infected outside school and that primary schools do not play a major role in SARS-CoV-2 spreading. They hypothesize proportional hazards between schools and surrounding areas; an incidence in schools at least 5 times lower than incidence outside the school area and it increases with increasing community transmission rates. Sciensano reports show that the ratio between cases in 10-19 years and cases in 0-9 years remains below 5; as an example, between September 16th and 30th, 2020, it was $4008/731 = 5.5$ despite the same time at school.

3.2 Outcomes

- Infection of a child with SARS-CoV-2 secondary to a classmate
- Infection of an adult school-attender with SARS-CoV-2 secondary to an infected child
- Infection of a child with SARS-CoV-2 secondary to an adult school attender
- Infection of a child with SARS-CoV-2 secondary to a contact outside the school, including the household

3.3 Exploratory outcomes

Exploratory outcomes will be performed pending sufficient or additional resources.

These are anticipated to include assessment of phylogenetic analysis of SARS-CoV-2 in positive salivary tests by a Whole Genome Sequencing (WGS) test. The aim of this exploratory outcome is to determine transmission linkages between cases and reveal transmission patterns inside households and at school, supporting epidemiological investigation.

We will perform WGS tests only on positive rt-PCR salivary tests with a high viral load defined with a Ct lower than 30 (limit of the technique to perform the analysis). Exploratory phylogenetic analysis may involve collaboration with other specialized laboratories and research groups in Belgium and/or abroad. This would involve transfer of coded samples, traceable only by means of a subject identifier.

3.4 Endpoints

- Time varying number and proportion of children infected with SARS-CoV-2 secondary to an asymptomatic infected classmate (transmission rate inside school with the child being the infector).

- Time varying number and proportion of adult school-attenders infected with SARS-CoV-2 secondary to an asymptomatic infected child (transmission rate inside school but from a child to an adult);
- Time varying number and proportion of children infected with SARS-CoV-2 secondary to an infected adult school-attender (transmission rate inside school but from an adult to a child);
- Time varying number and proportion of children infected with SARS-CoV-2 secondary to a household contact or outside the school (transmission rate from in the community).

4.0 Methodology

4.1 Study design

This study is an interventional prospective SARS-CoV-2 infection study.

4.2 Study Period

The study will be conducted during the autumn-winter season 2020-2021 and we expect to start on 15th November 2020.

4.3 Selection of participating schools

All Belgian primary schools of the Federation Wallonie – Bruxelles that have organized a resumption of school activities with the social control measures recommended by the government, are eligible for the study. All children and school attenders attending included schools will be eligible to participate.

Each participating school will be considered as a statistical cluster because children within a school share more correlation together than with children from other schools (children are not an independent and identically distributed (i.i.d.) sample) and an intraclass coefficient (ICC) of 9% will be used in analysis.

We will enrol schools according to the following three criteria:

- School size: small school versus a large one

- Incidence of Sars-CoV-2 infection during the first wave of the pandemic in April 2020
 - Regions/cities with a low incidence (from 1.5 to 4.9 per thousand inhabitants on May 6th, 2020)
 - Regions/cities with a high incidence (from 5.0 to 7.5 per thousand inhabitants on May 6th, 2020)
- Socio-economic level of the school: low socio-economic level versus high level, based on ISE that is an official Belgian 20-point-scale index for all schools of the Federation Wallonie-Bruxelles (FWB).

Crossing those three criteria leads to eight categories of schools and one school of each category will be sampled

- Small school - very low incidence city - low socio-economic level
- Small school - very low incidence city - high socio-economic level
- Small school - very high incidence city - low socio-economic level
- Small school - very high incidence city - high socio-economic level
- Large school - very low incidence city - low socio-economic level
- Large school - very low incidence city - high socio-economic level
- Large school - very high incidence city - low socio-economic level
- Large school - very high incidence city - high socio-economic level

According to the definition of the FWB, investigators consider a small school a school with less than 300 children enrolled in the school and a large one a school with more than 300 children.

To determine regions with the highest and lowest incidence of SARS-CoV-2 infection during the first pandemic wave in Belgium investigators will refer to the epidemiological reports of Sciensano: <https://covid-19.sciensano.be/fr/covid-19-situation-epidemiologique>

Written agreement to proceed has been provided by the Ministry of Education and all concerned “Réseaux d’enseignements” in Fédération Wallonie-Bruxelles. Each school of the FWB is followed by a health promotion team (PSE: promotion de la santé à l’école). Physicians from these teams will propose a purposive sample of schools to enter into the study. A

principal investigator, subinvestigator or a PSE team member will contact the school's director and the organizing committee. In case of refusal, another school of the same category will be invited to participate. The participation of schools will be voluntary.

4.4 Study population

All children registered and attending selected primary schools of the Fédération Wallonie - Bruxelles and all adult's school attenders working in the same selected schools will be the study participants.

4.4.1 Inclusion criteria

- All children registered and attended the selected primary schools of the Fédération Wallonie - Bruxelles and adult's school attenders working in the same selected schools.
- Signed informed assent form by child and informed consent form by theirs parents (or legal guardian) and by adult's school attenders before enrolment

4.4.2 Exclusion criteria

Refusal or absence to provide written informed consent before enrolment

4.4.3 Definitions

4.4.3.1 Asymptomatic versus symptomatic cases

- A positive case: a participant who has a positive PCR salivary test for SARS-CoV-2 detected by systematic weekly screening
- Asymptomatic case: a participant who has a positive salivary test for SARS-CoV-2 during the study period and who did not declare any symptoms compatible with COVID-19
- Symptomatic case: a participant who has a positive salivary test with symptoms compatible with COVID-19 during the study period

4.4.3.2 High versus low risk contact

Definitions of high or low risk contact in the present study are the same as the national definition edited by Sciensano (version 1 october 2020)

https://covid-19.sciensano.be/sites/default/files/Covid19/COVID-19_procedure_contact_FR.pdf

- High risk contact is defined as
 - a person who had face to face contact for at least 15 minutes within a distance of 1.5 meter, without wearing mask or
 - a person with a physical contact
 - a contact with secretions or body fluid
 - who has travelled with a COVID-19 patient for at least 15 minutes, in any vehicle, seated two seats apart

with the index case within the 48 hours before the onset of symptoms, or in the last, 48 hours before the positive test, in case of asymptomatic participants

- Low risk contact is
 - a person who had face to face contact for at least 15 minutes within a distance of 1.5 meter, and wearing the mask correctly
 - a person who had face to face contact for less than 15 minutes within a distance of 1.5 meter (face to face) or
 - a person in the same room for at least 15 minutes with a distance more than 1.5 meter

with the index case within the 48 hours before the onset of symptoms, or in the last, 48 hours before the positive test, in case of asymptomatic participants

School attenders in primary school are wearing a mask when the social distancing cannot be maintained.

5.0 Conduct of the study

Parents and children attending selected schools will be informed of the exact nature of the study with a written version of participant information transmitted by the school director. Parents and children will be invited to participate in a virtual zoom (professional version with secured password) meeting. During this meeting the study objectives, procedures and implications will be explained to the teachers, parents and children. Where/When feasible a

video adapted to each school will be posted on the virtual school platform to be able to reach the maximum of parents and children. Written informed consent will then be obtained from the participants and/or their legal guardian.

Once a week and for six consecutive weeks, all participants of the school will be invited to provide a self-sampled salivary specimen, in the schools and under supervision of medical students on contract.

Currently, procedures only identify symptomatic cases. Interferences with current guidelines will impact results of this study as symptomatic cases and asymptomatic participants with a high-risk contact would be excluded from schools. Indeed, systematic saliva samples will not

Results of the salivary PCR SARS-CoV2 will not be communicated to participants during the study period. Salivary samples of the study will not be analysed routinely but will be treated in batch according to the laboratory capacity of the national platform BIS. The estimated time between sample collection and the availability of the result will be more than a week. Clinical relevance and societal repercussions of the communication to a participant of a positive result so long afterward will be irrelevant. Indeed, it is currently recognized by most experts that contagiousness lasts 7 days after the onset of symptoms. In a study from Taiwan of 100 COVID-19 cases and 2761 close-contacts, secondary attack rates was significantly higher among contacts whose exposure to the index case started within 5 days of symptom onset compared with those exposed later (no secondary cases among the 852 contacts exposed to index case after day 6 of symptom onset) (18).

It is important to stress that participation to the present study does not exclude participants to follow the national recommendations as detailed in Sciensano procedures, namely a test performing in case of compatible symptoms of SARS-CoV-2 infection or a quarantine in case of a high-risk contact. Investigators will also insist to students and school attenders on the importance of non-pharmaceutical interventions, including physical distancing and mask to prevent SARS-CoV-2 transmission as recommended by Sciensano.

Protocols (ONE as in the Ministry of Education's circular) will be applied as per the standard procedures. The decision to close a classroom will be taken by the PSE and if needed after concertation with the hygiene control inspector of COCOM or AVIQ.

To investigate a possible link between the absence from school of a participant and SARS-CoV-2 infection, the PSE team member will interview this participant later. This interview will continue until 2 weeks after the end of the study to complete data collection.

Any salivary PCR SARS-CoV2 test that comes back positive (with a maximum Ct of 30) will be analysed by a Whole Genome Sequencing (WGS) of the SARS-CoV2 virus (exploratory outcome). Phylogenetic analysis of the virus is expected to determine transmission linkages between cases and to map transmission patterns inside households and at school.

5.1 Inclusion day

All participants, children and adults school attenders, will be tested at the inclusion day with

- a salivary SARS-CoV2 PCR test collected in the school, under investigators supervision
- a rapid serological test by finger prick (AVIOQ[®]) to determine the seroprevalence of SARS-CoV-2
- All participants (children and adult school attenders) will be invited to complete an electronic questionnaire when possible and a paperback questionnaire otherwise.

The questionnaire will address the following information (see attached file) partially inspired of the questionnaire used in the prevalence study *“Prevalence and incidence of antibodies against SARS-COV-2 in children measured for one year in Belgium: a sero-epidemiological prospective cohort study”* (study coordinator: Els Duysburgh, Sciensano).

- Socio-demographic data: gender, birth date, home postal code, a contact email address, a contact mobile number, country of birth of child and parents
- Housing information: number of people in the household, number of siblings, number of sleeping rooms, distance between school and home
- Exposition information: pre- and after-school daycare attending, extracurricular activities, parent's profession, transportation means between home and school

The salivary SARS-CoV2 sample will be performed at school by the participant him or herself under the supervision of the subinvestigators (80 medical students on contract) for minor

participants. An explanatory film on the method of self-performing the saliva sample will be provided to participants. The children and the teachers will use a garling method to produce saliva. The full procedure is explained in the explanatory film. The participant will wear an adapted mask while gargling. A full explanation of the used gargling sampling technic is also well detailed in the attached paper (19)

The procedure will be performed in a well-ventilated space one participant at a time. The medical student who will collect the sample will wear adapted personal protective equipment (PPE) in order to reduce the risk of transmission to as good as 0. The PPE will include FFP2 mask, gloves, glasses and a protective gown.

After collection, salivary samples will be transported to one of the three participating laboratories (UCLouvain - ULiège - ULB) by the medical student.

Teachers of each class will be invited to complete a questionnaire once with the following questions

- Number of children in the classroom
- Size of the classroom
- Ventilation system and heating system of the classroom (CO2 sensor if any)
- Sanitary protocols of the school: eg hand washing recommendations
- Play-time space: indoor or outdoor
- Lunch space: in the classroom, school cafeteria, etc
- Organisation of pre- and after-school daycare attending: separation according to levels or mix of all classes. Number of children attending.

5.2 Follow-up visit

All participants, children attending selected schools and adults school attenders, will be asked to provide a weekly salivary specimen for six consecutive weeks.

All participants, children attending selected schools and adult school attenders, will have to complete a follow-up questionnaire, once a week for six consecutive weeks (See attached file) providing information about

- any compatible symptom with a SARS-CoV-2 infection
- a high-risk contact with a confirmed SARS-CoV-2 case

- social contacts (number of contacts during the last 7 days outside the school environment and outside the household, including travelling abroad)

6.0 Microbiological analyses

6.1 Salivary test

In an epidemiological study with repeated tests, a simple and non-invasive method to test participants is mandatory. The salivary sample collection is simple, painless and can be performed by the participant himself (under supervision for younger participants). A salivary collection device is developed by the University of Liège (Belgium) and currently used by university students of ULiège.

For this study a saline mouth rinse/gargle sample will be used to collect the saliva from participants. This self-collected sampling was tested on 50 participants, aged 4 to 71 years and showed a sensitivity of 98% to detect SARS-CoV-2 by PCR compared with nasopharyngeal swab (19).

6.2. Real-time SARS-CoV-2 PCR

Saliva will be extracted using the MagMAX Viral/pathogen Nucleic Acid Isolation kit on a KingFisher automated platform (ThermoFisher). An unrelated MS-2 phage RNA sequence is added to each specimen to demonstrate the process efficiency. Purified RNA will then be retro-transcribed and amplified on a QuantStudio5 real-time PCR platform with the TaqPath™ COVID-19 RT-PCR kit (Thermo Fisher), which targets ORF1ab, N and S coding sequences using three different sets of primers and probes.

In case of a positive salivary PCR test for SARS-CoV-2 with a maximum Ct of 30, a whole-genome sequencing of the virus will be done at the medical microbiology facility (MBLG) of the UCLouvain (Brussels)

RNA is extracted from the specimen using the QIAamp viral RNA mini kit (QIAGEN). Fifty ng of purified RNA are then retro-transcribed using the Maxima H minus double-stranded cDNA synthesis kit (Thermo Scientific) following the manufacturer recommendations. A DNA NGS-library is then created using the Nextera DNA Flex for enrichment library kit (Illumina). Briefly, 50 to 1000 ng of cDNA are restricted in small fragments in order to create a DNA library of

approximately 300 nucleotides long using bead-linked transposomes (eBLT) (Illumina).

That tagmentation process is followed by a DNA clean-up and an amplification using unique-dual primers (IDT for Illumina UDI set of primers) recognizing the sequence added to each DNA strand during the tagmentation phase and adding a specific sequence to all strands of each sample. Amplified libraries are purified and dosed in spectrophotometry. Libraries are then pooled equimolarly and hybridized with a capture probe panel targeting SARS-CoV-2 as well as 40 other common respiratory viruses (Respiratory Virus Panel v2, Illumina). The enriched pooled library is finally amplified and purified. After a spectrophotometric dosage, the final library is diluted to 100 picomolar and charged on either an iSeq100 or NextSeq550 Mid-output cartridge allowing respectively 8 million or 260 million paired-end reads, in a 2 x 150 bp format. Each sample needs a minimum of 1 million reads to ensure a good coverage of the SARS-CoV-2 genome.

Raw sequences in a fastq format are processed through two different bioinformatic pipelines. First, the Dragen RNA pathogen analysis suite available through the BaseSpace Illumina online platform allows the generation of a consensus sequence and gives a percentage of each viral species detected in the library. Second, the Integrated Database Network System (IDNS) coupled to the advanced sequencing platform NGS pipeline from Smartgene allows the generation of SARS-CoV-2 consensus sequences and sequence comparisons in order to define sequence mutations in nucleotide or amino acid format. Phylogenetic analysis is available with a set of reference or personal sequences. Association of metadata to each sample allows selective analysis and group comparisons.

Whole-genome sequences will be shared on the GISAID open-source international platform. Clade determination and comparison to published SARS-CoV-2 sequences in a phylogenetic tree format is available through the NextClade web site.

6.3. SARS-CoV-2 serology

For Baseline seroprevalence data we will use a lateral flow test antibody IgG/IgM assay (colloidal gold) (Avioq) (Avioq, Bio-Tech, Shandong, China) CE-labelled. Sensitivity for Both IgM and IgG was 68.8% (IC 95% 60.3-76%) and the specificity was 95.8% (IC 95% 88.5-98.6%) (20).

7.0 Statistical analyses plan

7.1 Sample size and statistical analysis

As a pilot study, no formal sample size determination was performed. The expected number of children is 2400 (4 times 200 plus 4 times 400), the expected number of adult school attenders is 200, and the expected number of classes is 72. With an incidence around 0.5-1%, the expected number of cases is 120-240. Initial incidence and then secondary attack rates (as transmission estimator) will be calculated and compared between gender, across age, and between the three kinds of schools using Poisson or Binomial negative regression if overdispersion. Characteristics of classes will be compared between schools using ANOVA with F-tests for continuous variable and chi-square or Fisher exact tests for discrete variables. Any significant difference will be considered for correlation with incidence rates (per classes) and attack rates within the Poisson or Binomial negative regression. A cluster effect (Rho = 1.09 or ICC=0.09) will be assessed for weighting data analysis. Estimates will be presented with their 95% Confidence interval. Differences will be presented as absolute and relative differences.

8.0 Risks - Benefits to participating to the study

The results of the salivary tests will be communicated at the end of the study period to the participants. There is no direct benefit for participants, and we are hoping they will gain a great awareness of better understanding this urgent public health issue for young children. The indirect effects to keep primary schools open even in case of red code are all the benefits for children to attend school and the positive impact on society such as facilitating parents' working especially in case of lockdown when teleworking is extremely challenging for parents looking after young children. Depending on results, this study could provide reassuring data concerning teachers' work conditions.

All Children of participating schools will receive once a week a healthy snack to thank them for participation to the study.

9.0 Ethics

9.1 Declaration of Helsinki and ICH Guidelines for Good Clinical Practice (GCP)

Study investigators will ensure that this study is conducted according to the principles of the latest revision of the Declaration of Helsinki and in full conformity with the ICH Good Clinical Practice (GCP) and local regulatory requirements.

9.2 Informed consent

Written, informed consent must be obtained from all participants (inform assent for children) and their parents (or legal guardians) for children before a participant can be included in the present study.

9.3 Ethics committee and regulatory agency review

The protocol proposed Inform consent form (ICF), and all other study documents, will be submitted to the local ethics committee “Commission d’Ethique hospitalo -facultaire des Cliniques universitaires St-Luc - UCLouvain” in Brussels for approval. The Principal Investigator will submit and, where necessary, obtain approval from the EC for all subsequent substantial amendments to the protocol and ICF.

9.4 Subject confidentiality

This study will be carried out in accordance with the law (July 30, 2018) on the use and processing of sensitive data, the law of patient’s right (August 22, 2002) and the general European regulation on the protection of personal data (May 25, 2018). Identity and participation of patients to the survey will remain strictly confidential.

The manager of confidential data will be Pr. Annie Robert.

All data will be coded: a unique study number containing no personally identifiable information (PII) will identify each subject, and, will be used in the questionnaire, database and sampling. A separate confidential file containing PII will be stored in a secured (locked) location in accordance with data protection requirements. Only the study investigators, the EC and regulatory authorities will have access to the records.

No participant name will be mentioned on samples. A sequential identification number will be automatically allocated to each participant enrolled in the study. This number will identify the participant and will be composed of 12 digits:

- a two-digits number allocated to the school he/she is attending and the replacement if the first school stop for any reason
- a two-digits number allocated to the class, with a first digit for the grade, or 9 for adults outside the class) and a second digit numbering classes within each grade
- a two-digits serial number within the class he/she is in, and above 50 for teachers in charge of the class
- a two-digits sequential number corresponding to adults linked to the child
- a two digits control number (such as modulo 89 of the 8 first digits)
- a two-digits number corresponding to the calendar week of data collection

This unique and automatically checked IDs will allow parents or guardians to enter on a website with a unique password they will receive with ID, in order to fill data for their child. Each child will receive a paperback diary for daily activities and all these books will be collected at the end of the study. Information in the paperback notebooks will be entered after the study in case of no internet access for parents or French problems.

The date of birth of the participant will be mentioned on the sample along with his/her ID number.

The online questionnaires will only be accessible with a login and a password respecting the standards of complexity related to passwords. The data collected will be anonymised. Only the investigators of the study will be aware of the link between the different questions and the participants. The server is hosted by a computing infrastructure at UCLouvain, which is configured to update automatically. The physical location of the server is in the IT manager's office. Access to the said office is limited and secure. Backups of data encoded on the server will be encrypted on an external drive. No remote access will be allowed to the server.

10.0 Samples storage and biobank

Saliva samples, sera and remains of these collected samples will be stored after consent in biobanks.

The biological materials will be stored in the biobank of each participating lab where samples will be analysed. Documentation and location of each collected material and its remain after analysis will be performed at the site of the biobank. The manager of the UCLouvain biobank is Prof. Etienne Marbaix who is responsible for it.

As mentioned, saliva samples will be used to perform a rt-PCR for SARS-CoV-2. Any positive sample will be analysed for viral phylogenetic purposes. All samples will be stored at -80°C, according to the procedure for viral particles preservation. All samples and remains will be held in the biobank for 15 years.

All patients or their parents/tutor will be informed on the sample use and storage. They will also be informed on the strict confidentiality of any patient data and will be assured that all samples will be anonymized.

11.0 Financial and Insurance

This study will be financially supported by research funds of FWB.

The involved parties will be insured, in accordance with the Belgian law 2004022376 “*Loi relative aux expérimentations sur la personne humaine du 7 mai 2004 et mises à jour au 16-11-2018*,” against financial loss resulting from personal injury and/or other damages, which may arise as a consequence of this study.

12.0 Intellectual property

The intellectual property of the present study belongs to UCLouvain.

13.0 References

1. https://covid-19.sciensano.be/sites/default/files/Covid19/COVID-19_fact_sheet_ENG.pdf
2. Viner RM, Mytton OT, Bonell C, Melendez-Torree GJ, Ward JL. Susceptibility to and transmission of COVID-19 amongst children and adolescents compared with adults: a systematic review and meta-analysis. medRxiv. 2020.
3. Lei H, Xu X, Xiao S, Wu X, Shu Y. Household transmission of COVID-19-a systematic review and meta-analysis. J Infect. 2020.
4. Madewell ZJ, Yang Y, Longini IM, Halloran ME, Dean NE. Household transmission of SARS-CoV-2: a systematic review and meta-analysis of secondary attack rate. medRxiv. 2020.
5. Goldstein E, Lipsitch M, Cevik M. On the effect of age on the transmission of SARS-CoV-2 in households, schools and the community. medRxiv. 2020.
6. Aleta A, Martin-Corral D, Pastore y Piontti A, Ajelli M, Litvinova M. Modelling the impact of testing, contact tracing and household quarantine on second waves of COVID-19. Nature human behaviour 2020.
7. Pollan M, Perez-Gomez B, Pastor-Barriuso R, Oteo J, Hernan MA, Perez-Olmeda M, et al. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. Lancet. 2020;396(10250):535-44.
8. Stringhini S, Wisniak A, Piumatti G, Azman AS, Lauer SA, Baysson H, et al. Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Geneva, Switzerland (SEROCoV-POP): a population-based study. Lancet. 2020;396(10247):313-9.
9. Paganini G, Conti F, Giacomelli A, Bernacchia D, Rondanin R, Prina A, et al. Seroprevalence of SARS-CoV-2 significantly varies with age: preliminary results from a mass population screening. J Infect. 2020.
10. Weekly Coronavirus 2019 disease Surveillance Report: Public Health England; [Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/920372/Weekly_COVID19_Surveillance_Report_week_37_FINAL_UPDATED.pdf].
11. L'Huillier AG, Torriani G, Pigny F, Kaiser L, Eckerle I. Culture-Competent SARS-CoV-2 in Nasopharynx of Symptomatic Neonates, Children, and Adolescents. Emerg Infect Dis. 2020;26(10):2494-7.
12. Fontanet A, Grant R, Tondeur L, Madec Y, Grzelak L. SARS-CoV-2 infection in primary schools in northern France: A retrospective cohort study in an area of high transmission. medRxiv. 2020 (Preprint)
13. Heavey L, Casey G, Kelly C, Kelly D, McDarby G. No evidence of secondary transmission of COVID-19 from children attending school in Ireland, 2020. Euro Surveill. 2020;25(21).
14. Macartney K, Quinn HE, Pillsbury AJ, Koirala A, Deng L, Winkler N, et al. Transmission of SARS-CoV-2 in Australian educational settings: a prospective cohort study. Lancet Child Adolesc Health. 2020.
15. Yung CF, Kam KQ, Nadua KD, Chong CY, Tan NWH, Li J, et al. Novel coronavirus 2019 transmission risk in educational settings. Clin Infect Dis. 2020.
16. Ismail SA, Saliba V, Lopez Bernal JA, Ramsay ME, Ladhani SN. SARS-CoV-2 infection and transmission in educational settings: cross-sectional analysis of clusters and outbreaks in England. medRxiv. 2020 (Preprint).
17. Faust SN, Munro APS. It's Time to Put Children and Young People First During the Global COVID-19 Pandemic. JAMA Pediatr. 2020.
18. Cheng HY, Jian SW, Liu DP, Ng TC, Huang WT. Contact Tracing Assessment of COVID-19 Transmission Dynamics in Taiwan and Risk at Different Exposure Periods Before and After Symptom Onset. JAMA Intern Med. 2020.

19. Goldfarb D, Tilley P, Al-Rawahi G. Self-collected Saline Gargle Samples as an Alternative to Healthcare Worker Collected Nasopharyngeal Swabs for COVID-19 Diagnosis in Outpatients. medRxiv. 2020 (Preprint)
20. Montesinos I, Gruson D, Kabamba B. Evaluation of two automated and three rapid lateral flow immunoassays for the detection of anti-SARS-CoV-2 antibodies. Journal of clinical Virology. 2020

14.0 Annexes

14.1 Inclusion questionnaires

14.1.1 For children

14.1.2 For school attenders

14.2 Follow-up questionnaires

14.2.1 For children

14.2.2 For school attenders

14.3 Inclusion questionnaire for parents

14.3 Inclusion questionnaire for teachers