PREVALENCE AND INCIDENCE OF ANTIBODIES AGAINST SARS-COV-2 IN CHILDREN AND SCHOOL STAFF MEASURED FOR ONE YEAR IN BELGIUM: A SERO-EPIDEMIOLOGICAL PROSPECTIVE COHORT STUDY – AMENDED PROTOCOL

July 2021

Summary

This protocol amendment describes changes in:

1. Main objective
2. Secondary objectives
3. Study population
4. Questionnaire
5. Testing periods of participants

The **table of changes**, with rationale, the amended protocol, the questions added to the questionnaire and informed consent forms are provided in the following pages. The sections that have been changed are highlighted in yellow.

Table of changes

Changes July 2021:

Change / addition	Rational	Affected protocol section
Testing periods: Extension of the study period from 1/9/2021 tot 31/3/2022 with addition of two testing periods (September 2021 and December 2021)	The pandemic has not been fully controlled and new waves remain possible. The evolution of the pandemic remains unclear, with the circulation of new variants of concern, a still ongoing vaccination campaign and, so far, no vaccine availability for the paediatric population. With the start of the new school year no vaccine will yet be licensed for use in the under 12 year olds, resulting in primary school children remaining a SARS-CoV-2 infection susceptible population while it is unclear how this will be impacted by a (partially) vaccinated adult population. Further surveillance of the seroprevalence in the paediatric population will inform us on the proportion of children with prior infection. Vaccination in the adult population renders further follow-up of school staff less useful. Similarly, the higher likelihood of vaccination among secondary school pupils making further follow-up of this group also less useful. Keeping the same primary school study population, which is selected to be representative for primary school children of 8-9 year old in Belgium, will enable us to continue providing Belgian paediatric estimates and having a one year cohort including the same children over time. This allows us to provide both seroprevalence point-prevalence estimates and follow changes in incidence. Additionally, we will be able to get more insight in waning immunity and how this is captured by diagnostic tools.	3. Methods 3.2. Study design 3.6. Data collection 3.6.1. Data collection procedure 7.6. Study insurance
Target population: Restricted to the primary school pupils	By the start of the school year 2021-2022, vaccination age might have been changed for 12 year olds and older. Therefore we limit the study extension to primary school pupils. We do not foresee to include new schools to the study. In case of decreased participation in the school, we will recruit additional pupils up to a maximum of 25 pupils per school to secure a sufficient sample size for the point seroprevalence estimates.	2. Objectives 3. Methods 3.2. Study design 3.6. Data collection 3.6.1. Data collection procedure 3.6.3. Laboratory specimen collection, transport and analysis – serological test 3.6.5. Special considerations and circumstances 7. Protection of human subjects and Ethical considerations 7.1. Informed consent 7.6. Study insurance
Additional questions:	In the questionnaire that is part of the study and has to be completed by the parents questions on relatives' and (if relevant) child vaccination status will be added.	Appendix b: Additional questions to be added at questionnaire for parents
Additional document: New informed consent parents		Appendix d: Informatie- en toestemmingsformulier (NL+FR) – separately attached

Changes November 2020:

Change / addition	Rational	Affected protocol section
Target population: Inclusion of school staff	To our knowledge there is little information on the prevalence of SARS-CoV-2 antibodies among school staff (including teaching and none-teaching staff). Quantifying the seroprevalence and incidence in both children and school staff can provide insight in and compare infection circulation within both groups. Changes in incidence over time, as well in the light of more general community transmission data, can add to the interpretation of potential differences or similarities in transmission dynamics in these interrelated populations. Additionally, information on seroprevalence among school staff is valuable for the implementation of and advice on protective measures in the school environment and has the potential to be used to define school staff as risk-group, possibly for the assessment of prioritization of vaccination.	1. Background 2. Objectives 3. Methods 3.1. Study population 3.2. Study design 3.4. Sample size: staff 3.5. Sampling procedure 3.6. Data collection 3.6.1. Data collection procedure 3.6.2. Questionnaire 3.6.3. Laboratory specimen collection, transport and analysis – serological test 3.7. Data analysis 6. Potential Biases and limitations 7. Protection of human subjects and Ethical considerations 7.3. Potential direct benefits for participants and indirect benefits for broader society
Testing periods	Due to delay in the validation of the saliva test procedure for the detection of antibodies the first testing period will take place in December 2020 and the second one in February 2021.	3.6. Data collection 3.6.1. Data collection procedure
Additional questionnaires: • Parent registration form: SARS-CoV-2 seroprevalence study among children in Belgium • School staff registration form: SARS-CoV-2 seroprevalence study among school staff in Belgium • SARS-CoV-2 seroprevalence study among school staff in Belgium: base-line questionnaire • SARS-CoV-2 seroprevalence study among school staff in Belgium: follow-up questionnaire	The addition of these questionnaires is linked to the expansion of the target group i.e. adding school staff. The registration forms are added to facilitate the collection of personal data necessary for the communication with the parents/guardians of the children and the staff members. This to avoid hand written (difficult to read) contact information.	Appendix a: registratie formulier ouders (NL + FR) Appendix a: registratie formulier schoolpersoneel (NL+FR) Appendix a: vragenlijst schoolpersoneel: 1ste contact (NL + FR) Appendix a: vragenlijst schoolpersoneel: 2de en 3de contact (NL +FR)
Additional document: Informed consent school staff.		Appendix c: Informatie- en toestemmingsformulier schoolpersoneel (NL+FR)

Sponsor	Sciensano
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Co-investigators and partners	• KUL Mathieu Roelants, PhD, Youth Health Care, Environment and Health • McGill University Joanna Merckx, MD MSc, Epidemiology, Paediatric Infectious Diseases

SYNOPSIS

Title	PREVALENCE AND INCIDENCE OF ANTIBODIES AGAINST SARS-COV-2 IN CHILDREN AND SCHOOL STAFF FOR ONE YEAR IN BELGIUM: A SERO-EPIDEMIOLOGICAL PROSPECTIVE COHORT STUDY
Acronym	SeroCoBelChild for “Seroprevalence” “Coronavirus” “Belgium” “Child”
Investigators	Sciensano, KUL, McGill
Objectives	Main objective To determine the prevalence and sero-conversion of antibodies against SARS-CoV-2 in a sample of school-aged children and of school staff (primary and secondary school) and primary school children only in Belgium at different time points. Secondary objectives • Determine the cumulative incidence of SARS-CoV-2 from baseline to 7 months in the complete sample and 1 year among primary school children • Determine the proportion of asymptomatic infections with SARS-CoV-2 in this sample • Gain insight in the role of COVID-19 infection in household members of children and school staff in this sample • Describe children characteristics and investigate potential risk factors for infection among school-age children • Describe characteristics of school staff and investigate potential risk factors for infection among school staff Exploratory objective Will children have to be vaccinated based on incidence of SARS-CoV-2 infection in this sample to create herd immunity?
Duration	12 months, school year 2020-2021 and first semester 2021-2022. Start ~~September~~ December 2020; 3 sampling periods each spread over max 4 weeks (~~Oct~~ Dec 2020, ~~Jan~~ Feb 2020, Apr/May 2021); 2 additional sampling periods for primary school children (September 2021, December 2021)
Design	Prospective observational cohort study
Outcomes	Primary outcome: seroprevalence of antibodies against SARS-CoV-2 in two age groups of school children and school staff (primary school and lower years of secondary schools) in Belgium. Secondary outcomes: • SARS-CoV-2 sero-incidence and seroprevalence over time

	• Cumulative incidence • Proportion of symptomatic sero-positive cases • Participant characteristics associated with test-positivity
Sample size	Total of 1,640 school-aged children and 820 school staff from 82 Belgian schools (41 primary schools and 41 secondary schools) - 44 primary schools with 820 primary school children for 4th and 5th testing period.
Study population	School-aged children and staff of primary and secondary schools in Belgium; For the 4th and 5th testing period only primary school children
Intervention - Data collection	Biological sample: Saliva sample for serological testing. Demographic, socio-economic, risk-behaviour, ~~child~~ infection and prevention control and behaviour related data and clinical symptomatology and illness episodes, family members illness episodes, known class and school positive cases and/or outbreaks.

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1. Background

Since the beginning of 2020 the world is under the spell of the new SARS-CoV-2 virus which causes high morbidity and mortality in adult populations leading to an overload of healthcare resources on a global scale. As of 27th August 2020, around 24,206,817 confirmed COVID-19 cases have already been reported worldwide since the beginning of the epidemics, among which 826,418 deaths(1). For Belgium this number was 83,030 confirmed cases and 9,879 deaths(2).

Based on studies in China, Iceland, the Netherlands and Italy it seems that children are less affected by SARS-CoV-2 infections and play a lesser role in the dissemination of the SARS-CoV-2 virus (3–7). It is unclear to which extend this is due to lesser exposure or an inherent decreased susceptibility to become infected. In Belgium, until the 27th of August 2020, 5,406 (6.5%) confirmed COVID-19 cases were younger than 20 years of age (1,747 (2.1%) between 0 and 9 years of age and 3,659 (4.4%) between 10 and 19 years of age) and Sciensano, the Belgian institute of public health, reported four deaths among the patients younger than 25 years of age (2). The low reported number of cases in children can also be partially explained by the lower testing rates for the diagnosis of acute infection in the paediatric population.

First results of SARS-CoV-2 sero-positivity in Belgium show a seroprevalence among the general population between 4.3% (healthy blood donors between 8 and 10 June) and 6.0% (residual blood samples between 20 and 26 April) and among hospital healthcare workers of 8.8% (representative sample of Belgian hospital healthcare workers between 19 and 24 May)(8). To our knowledge there is no representative data available on seroprevalence among Belgian children and school staff (9).

To get insight in the transmission of SARS-CoV-2 virus in school-aged children, especially in younger or asymptomatic subjects, it is necessary to compile data on infection and past-infection of SARS-CoV-2 virus in the child and (pre-)adolescent population. In early reports, most children were apparently infected within the household (10). In addition, modelling studies of COVID-19 predict that school closures alone would prevent only 2–4% of deaths, much less than other social distancing interventions(11–13). A seroprevalence study following outbreaks in a large school community in Chill showed the highest fraction of infection among teachers, compared to elementary and high school students (14). On the other hand school-based contact-tracing studies found minimal transmission from child or teacher index cases (9,15,16).

Currently studies on rate of infection as well as rate of transmission among children remain scarce. Also the information on SARS-CoV-2 infections among school staff (teachers and others) and the relationship with measured seroprevalence in children is still limited. Nevertheless, such data are important to guide strategies to deal with the current epidemic (e.g. school attendance), and necessary to determine whether children and adolescents will have to be vaccinated in order to achieve herd immunity and to limit the spread of the SARS-CoV-2 virus.

This study will make it possible to better describe the prevalence of circulating antibodies in children and school staff in Belgian primary and secondary schools as well as the incidence of new infections in this subpopulation since they will be followed for 7 months. Additionally, by also questioning household members in the case of the children and by questioning the school staff, we will gain a more in-depth insight in symptomatic COVID-19 disease within households in Belgium, by linking the results of the salivary antibody tests in symptomatic and asymptomatic children with disease information of household members. The study also aims to generate insights in the clinical presentation of the disease among school-aged children and school staff and to evaluate the effect of child behaviour (e.g. participation in outside school activities) on the infection rate among children.

The study will cover all regions[1] in Belgium (Brussels-Capital, Flemish and Walloon Region) and interim data from each wave can guide the Belgian policy makers and CELEVAL (Group of Belgian Experts in charge of COVID-19 related public measures) in their advice on specific COVID-19 related measures for school-age children in order to limit circulation of SARS-CoV-2 in Belgium and to prevent an overload of our healthcare system with a limited societal impact (e.g. school closures). This data might also guide the expert group who will give advice regarding vaccination strategies whether children should be included for vaccination to create herd immunity or not.

[1] A stratified selection of all of all municipalities.

2. Objectives

Main objective

To determine the prevalence and incidence of antibodies against SARS-CoV-2 in a sample of school-aged children and school staff (primary and secondary school) and primary school children only in Belgium at different time points.

Secondary objective

- Determine the cumulative incidence of SARS-CoV-2 from baseline to 7 months in the complete sample and 1 year among primary school children
- Determine the proportion of asymptomatic infections with SARS-CoV-2 in this sample
- Gain insight in the role of COVID-19 infection in household members of children and school staff in this sample
- Describe child characteristics and investigate potential risk factors for infection among school-age children
- Describe characteristics of school staff and investigate potential risk factors for infection among school staff

Exploratory objective

- Will children have to be vaccinated against SARS-CoV-2 to create herd immunity?

3. Methods

The methodology is based on and in line with the methodology as described in the WHO document '*Population-based age-stratified seroepidemiological investigation protocol for COVID-19 virus infection*'(11).

3.1. STUDY POPULATION

The study population includes staff and children of primary and secondary schools. The children belong to two predefined age groups. These two age groups are chosen because studies have shown more pronounced symptoms of infection and disease depending on age and because transmission dynamics also vary by age. Adolescents of 15 year and older will not be included as their symptom patterns resembles more disease pathology of adults. Because of pragmatic and practical reasons we will include primary school children from the 3rd grade (age 8-9) and secondary schoolchildren from the 2nd grade (age 13-14). For primary schools, all school staff, teaching as well as supporting/none-teaching staff (e.g. administrative staff) who has contact with pupils (meaning they are at school at the same time as the pupils are at school), will be included in this study. For secondary schools we decided to include only teaching staff, this because due to present implemented corona-measures in secondary schools, contacts between pupils and none-teaching staff in these schools are at present very limited or not existing. An additional criteria for secondary school teachers to be included in this study is that these have to teach at least also in the 1st or 2nd grade of the secondary school. As teachers often teach in different age-groups and have contacts with colleagues teaching other age-groups, limitation to select teachers teaching dedicated age groups will have no value.

The study sample will be recruited in all Belgian schools including Brussels-Capital, and the Flemish and Walloon Regions (including the German speaking community). School attendance in this age group is high in Belgium (Fédération Wallonie, 2019), for which reason recruitment of school-age children through schools is acceptable.

We do not include children and staff in kindergarten. We assume that transmission dynamics in kindergarten are to some extend similar to those in children in primary school up to the 3rd-4th grade (age 10). The latter group however is easier to approach. Participant inclusion and sample collection in younger children in a school environment without the presence of a direct caregiver would require additional resources.

Following are the eligibility criteria:
Inclusion criteria:

- Children primary schools: any pupil from the 3rd grade (or from the 2nd grade if not enough participants from 3rd grade are available in the selected school) who regularly attend school and is present at school at the first testing period
- Children secondary schools: any pupil from 2nd grade who regularly attend school and is present at school at the first testing period
- Staff primary schools: any teaching or supporting/none-teaching staff (e.g. administrative staff) having contact with pupils and is present at school at the first testing period
- Staff secondary schools: any teaching staff teaching at least also in the 1st or 2nd grade of the secondary school and is present at school at the first testing period
- Prior known SARS-CoV-2 infection does not exclude participation

Exclusion criteria:

- Pupils or staff who refuse to have the saliva sample taken
- For children: being a sibling of a study participant

- A close family member included in the study: only one member per nuclear family will be included in the study
- No informed consent form signed (by parent of legal caregiver) available at the recruitment

3.2. STUDY DESIGN

This study will be set up as a prospective cohort study.

Children and school staff will be recruited at the 1st testing period in the participating school. Samples will be taken within a timeframe of maximum 4 weeks for all the participants.

Parents/legal child caregiver and staff will need to complete a questionnaire at each of the testing moments providing basic socio-demographic characteristics (at baseline only) and risk-behaviour and health characteristics including presence of symptoms during the time since the previous testing period (at each of the testing periods). The questionnaire will be completed ~~by a parent of caregiver~~ through a secured online application during the same week as the collection of a saliva sample. ~~from the child.~~

The study will last 12 months. There will be in total 3 testing periods when data and sample collection will be performed for the total study population; in ~~October~~ December 2020 (Baseline), ~~January~~ February 2021 (M3) and April/May 2021 (M6). There will be a 4th and 5th testing period for primary school pupils only: in September 2021 (M9) and December 2021 (M12).

Saliva samples will be sent to the Sciensano laboratory for analysis. Serological test results will be communicated to the child's parent/legal caregiver by a closed letter through the participating school.

Epidemiological data analysis will be performed and reported after each testing period and at the end of the study.

3.3. SAMPLE SIZE: SCHOOL CHILDEREN

Considering a seroprevalence of 6.0% (the highest seroprevalence at present reported among the Belgian general population (17)) an effective sample size of 400 would give us margin of error (half the confidence interval width) as a measure of precision of 2.3%. As seen in Figure 1, we gain some precision by increasing the sample size to 1000 but the difference is only 0.8%. Taking in to account the cost, we considered using a sample size of 400 for this study acceptable. Figure 1 shows both the exact binomial confidence interval and the asymptotic version (normal approximation) because we are potentially dealing with low numbers/prevalence, in which case we should not use a normal approximation.

For operational and practical reasons we opted for cluster sampling, choosing 41 clusters from 4,498 Belgian primary schools and 41 clusters from 1,590 Belgian secondary schools. We assume a design effect of 2 will be largely sufficient to account for variability in SARS-CoV-2 prevalence among children in Belgian primary and secondary schools. Thus we required 19.51 subjects per cluster, which we rounded upwards to 20 subjects. This gives us a necessary total number of 820 children to be recruited/examined per age group.

3.4. SAMPLE SIZE: STAFF

Considering a seroprevalence of a least 10.0% (the latest number reported among the Belgian blood donors was approximately 4% REF; the seropositive rate increased two to 3 fold in both blood donors and leftover samples in April 2020, i.e. starting two weeks after the lockdown was initiated in March) an effective sample size of 400 would give us margin of error (half the confidence interval width) as a measure of precision of 3%. As seen in Figure 1, we gain some precision by increasing the sample

size, but the gain in precision is moderate (e.g. the margin of error decreases only 1% when n = 1000). Taking into account the cost, we considered using a sample size of 400 for this study acceptable. Figure 1 shows both the exact binomial confidence interval and the asymptotic version (normal approximation) because we are potentially dealing with low numbers/prevalence, in which case we should not use a normal approximation. The proposed number of 400 staff members allows to detect a change in sero-provalence over time in the same persons of approximately twice the margin of error with a power of at least 70%, and a twofold difference in prevalence in two equal sized groups (e.g. primary vs secondary school) with a power of approximately 80%.

These numbers apply to the total study population (staff of primary and secondary school), but they will be stratified according to type of school (and province, social background of the school/pupils). In addition, since we will recruit staff members in schools (10/school) and the condition under study is an infectious disease we assume there will be clustering of observations. While the effect of clustering on the standard errors is not yet known, it is common to assume a design effect of two. For a cluster size of 10 (staff/school), this corresponds to an intracluster correlation (icc) of 0.11.

Because of operational reasons we will recruit school staff in the same schools as pupils, meaning the same 41 primary and the same 41 secondary schools. Considering the above sample size calculation this will gives us 410 staff in primary and 410 staff in secondary schools.

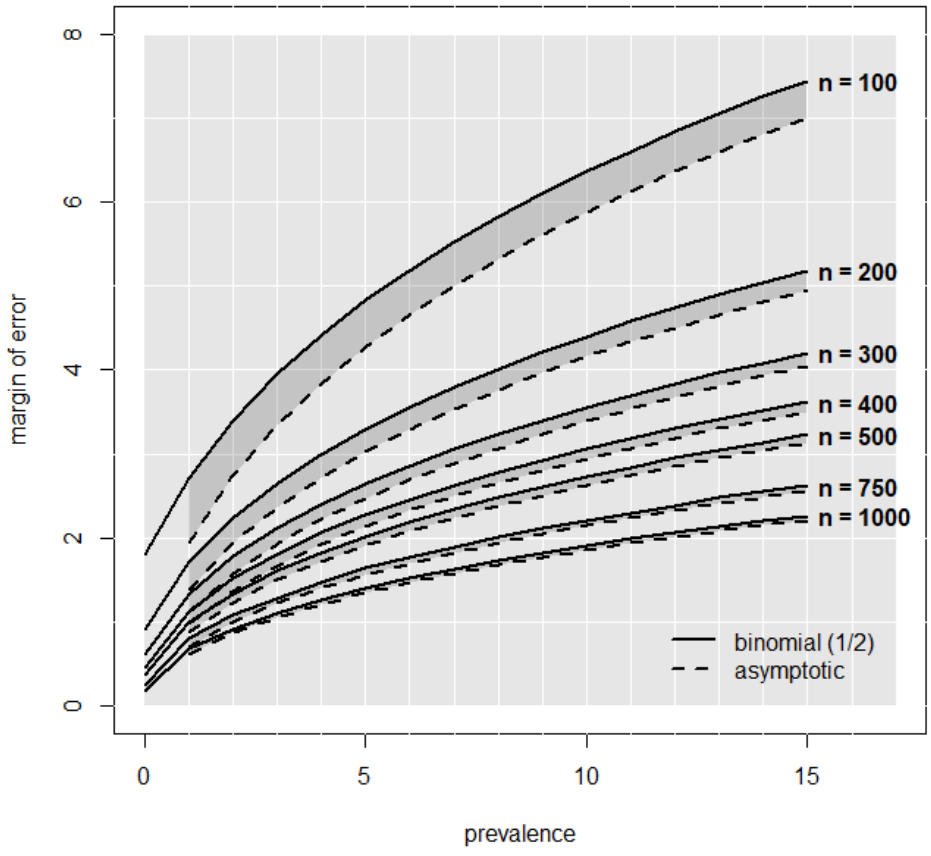

Figure 1: Expected margin of error by prevalence

3.5. SAMPLING PROCEDURE

Children and staff will be recruited in the same schools.
The study uses a two stage cluster sampling design with geographical and socioeconomic stratification:

- **1st stage: Selection of schools (within province selection of clusters; distribution of clusters per province proportional to number of schoolchildren per province; selection probability of schools proportional to the number of pupils)**

  To select the children and staff to be included in the sample, we select 41 clusters for each age group (primary and secondary school children).
  First a number of clusters is assigned to each of the 10 Belgian provinces and Brussels-Capital Region proportional to the number of children attending primary or secondary (first grade) education (data from Fédération Wallonie-Bruxelles and Vlaamse Gemeenschap; retrieved June 2020), but with a cap of 5 clusters for more populous provinces and 4 for less populous provinces. This gives us per age group per province and for Brussels-Capital Region following results:

Province	Number of clusters/schools	Proportion residents 6 – 18 years	Number of arrondissements
Antwerp	5	16.1	3
Limburg	4	7.1	3
East Flanders	5	12.8	6
Flemish Brabant	4	10.5	2
West Flanders	4	9.4	8
Hainaut	5	12.2	7
Liège	4	9.8	4
Luxembourg	1	2.8	5
Namur	2	4.6	3
Walloon Brabant	2	3.9	1 (27 municipalities)
Brussels-Capital Region	5 (4F; 1N)	11.2	19 (municipalities)
Total:	**41**		

  Due to capping 3 clusters were removed from the provinces of Antwerp, 2 from East Flanders, and 1 from Hainaut, West Flanders, and Flemish Brabant. Final weights for the calculation of nationwide or regional prevalence estimates will be based on the distribution children by province of residence according to the most recent data from Statistics Belgium (Statbel, 1/1/2020). The total number of clusters per age group is 23 schools from the Vlaamse Gemeenschap, and 18 schools in total from the Féderation Wallonie-Bruxelles and the German-speaking Community.

  To ensure both a geographic dispersion and social stratification within province, we will further assign the clusters using a random starting point and fixed increment factor in an ordered list (population size) of arrondissements[2] (larger arrondissements can be selected more than once), or geographically grouped clusters of municipalities (Walloon Brabant; Brussels Capital Region). A “social-demographic quantile” will be assigned at random to each cluster such that the sum of these quantiles is 1 (e.g. a quartile when 4 clusters are selected in the province). Schools within the geographical region (arrondissement or geographical cluster) will be assigned a similar socio-demographic quantile based on their social index (data available from the Communauté Française and AGODI, Vlaamse Gemeenschap) and number of pupils. One secondary and one primary school will be selected at random within the assigned socio-demographic quantile (if needed, backup schools will be selected at random within the same quantile). The social classification of schools is based on publicly available data, the number of pupils is publicly available for the

[2] For the selection of primary schools it may seem more reasonable to select municipalities, but not all municipalities have a secondary school.

Flemish schools. ~~and will be requested from the responsible agency for the schools from the federation Wallonie-Bruxelles and the German-speaking Community.~~

- **2nd stage: Selection of children and staff in each school (convenient sampling in each cluster)**
  A local study contact will be identified for each participating school. Eligible groups of pupils (classes) will be identified within the school (randomized if applicable), and all children and their parents/legal caregivers from the selected classes will be informed about the study. Children from whom a parent returns the signed informed consent and who agree themselves (assent) are eligible for enrolment in the study. If consent is obtained from more than 20 children, a random selection of 20 children will be drawn using a simple procedure with a mobile app.
  Staff will be informed about the study. Staff who return a signed informed consent are eligible for enrolment in the study. If more than 10 staff members are willing to participate, a random selection of 10 staff members will be drawn using a simple procedure with a mobile app.

3.6. DATA COLLECTION

The selected schools will be contacted by the research assistant (one research assistant for the FR and one for the NL schools) to explain the protocol and obtain their approval. This will be done with the knowledge and involvement of the local CLB (Centrum voor Leerlingen Begeleiding) and PSE (Promotion de la Santé à l'Ecole) units. If approval is given, the schools will choose the eligible classes, taking in to account the guidelines provided by the research team.

3.6.1. Data collection procedure

Biological sample (saliva swab) and survey questionnaire data will be collected simultaneously in ~~October~~ December 2020 (Baseline), ~~January~~ February 2021 (~~M4~~ M3) and April/May 2021 (~~M7~~ M6) and for primary school pupils: September 2021 (M9) and December 2021 (M12). Each of these testing periods should extend for a maximum of four weeks. In each school, one contact person will be designated to support the study locally and communicate with the researchers. This person will also help in informing the participants and provide and collect the informed consent for each of the participants.

3.6.2. Questionnaire

Demographic, socio-economic, risk-behaviour and ~~child~~ infection and control behaviour related data and COVID-19 clinical symptomatology and illness episodes will be collected using a questionnaire at each testing period. Demographic and socio-economic data will only be collected by the baseline questionnaire. Every testing period, information on COVID-19 related symptoms and illness and the contact with (possible) COVID-19 cases will be asked. The questionnaires can be found in the appendix. Questionnaires will be available in French and Dutch.

The questionnaire will be transcribed in LimeSurvey (the data is automatically stored at the secured Sciensano server). Both the French and Dutch version of the questionnaire will be piloted and checked for readability and understanding as well as timing. Adjustments in phrasing can be made to improve the completion of the questionnaire. The questionnaire will be completed online (via computer or tablet/smartphone) by the parents or staff, respectively, during each testing period (background information only at baseline), and will include a pseudonymous ~~child~~ identifier to link the questionnaire data to the result of the biological sample taken from the participant (child or staff) ~~child~~. The web-link to the questionnaire will be provided by the study researchers to the parents of the participating child[3] and participating staff members. Families without internet access, will be invited by the school to complete the questionnaire on a school computer or will receive a paper questionnaire through the

[3] Given that 87% of the Belgian households have an internet connection (federal government economy) we opted to using an online questionnaire, and the questionnaire can also be completed on a smartphone.

school which they can complete and return to the school (under closed envelop). The school will deliver these paper questionnaires to the study researchers who will enter the data in the database.

If questionnaires are missing 1 week after closure of a testing period, a reminder will be sent to the (parents of) participants.

Basic data from the participating schools will be collected. Prior to the different sample periods, the schools will be contacted, periodical school closures and known SARS-CoV-2 outbreaks at the schools will be recorded.

3.6.3. Laboratory specimen collection, transport and analysis – serological test

A biological sample/specimen for serological testing will be collected at each of the 5 testing periods.

Biological sample collection for serological testing will be conducted by 5 teams of 2 nurses (3 teams for the NL schools and 2 teams for the FR schools). If a saliva swab will be suitable for this study (see information below) self-sampling, under the supervision of the nurse, will be performed. The self-sampling will be done in groups of 5 to 10 pupils and can be done outside. Half a day will be needed to sample the students and staff in one school. The sample material for the three sampling periods as well as labelling stickers will be provided by Sciensano.

Sample type
Since sampling must be done repeatedly in children, a non-invasive sampling method, such as antibody determination in saliva samples, would help to recruit children and keep them in the study for seven months. Therefore, the saliva test, (Oracol or equivalent) (Malvern Medical Developments, UK) has been chosen for this study. The saliva sample method for detection of immunoglobulins in SARS-CoV-2 has been validated in the adult population by Sciensano in a Belgian healthcare workers study (18). Oracol has been validated in the paediatric population for other infectious diseases (Measles, Dengue)(19). A study is currently been performed in The Netherlands, as part of the PIENTER study, where a proportion of the study participants are children making use of saliva testing, and in the Public Health England Laboratories in the UK (personal communication). Proper validation of saliva samples for SARS-CoV-2 in our target population, remains however necessary, given the still limited and non-published evidence on their performance. For this purpose we will validate antibody testing in saliva samples during a separate study. This study takes place in four selected schools in the province of Limburg. Data from the validation study will be used to define feasibility and accuracy, e.g. but not limited to the sensitivity, specificity. Depending on the validity and accuracy of the salivary samples, the study sample type will be selected. In the unlikely case that the performance of the salivary samples is deemed insufficient, all samples in this study will be collected using capillary blood on dried-blood-spots from the participants. The in this study used assay is validated for capillary blood and multiple countries have validated and successfully used capillary blood in the peadiatric population for the collection of antibodies against SARS-CoV-2.

For operational reasons we decided to use the same sample type and procedure for the school staff as the one used for the pupils.

Sample collection
A saliva sample will be collected as per manufacturer and previously in the paediatric population validated instructions. During 1.5 minutes the oral swab will be rubbed with mild pressure against the buccal mucosa of the upper teeth. Samples will be labelled with the study label, data of collection and participant identifier number.

Sample transport
Samples dispatching and collection will be organized by Sciensano using a carrier service. The swaps and biological samples will be delivered and collected at the place of one of the nurses. Collection of

biological samples will be done twice a week. Samples need to be stored at a temperature between 20 and 25°C.

Sample analysis

Analysis of biological samples will be done by Sciensano. A serological test (ELISA) will be conducted on the sample material. The current assay that is used at the laboratory of Sciensano is the Euroimmun (Euroimmun, Medizinische Labordiagnostika, Lübeck, Germany; Cat # EI 2668-9601 G; CE marked March 2020) enzyme-linked immunosorbent assay (ELISA), measuring human antibodies of the class IgG targeting the recombinant S1 domain including the receptor binding domain of the structural protein of SARS-CoV-2. The assay is a medium through-put ELISA requiring in-laboratory testing. The result is read using a microplate reader. As per the manufacturer a ratio is calculated between the optical density (OD) of the patient sample compared to the OD of the calibrator, with a ratio of <0.8 interpreted as negative, between 0.8-1.1 borderline and >1.1: positive, for serum samples. The ELISA test has been validated against the plaque reduction neutralization test (PRNT) (gold standard) by Sciensano. Serum, EDTA plasma and capillary blood (dried blood spot) specimens have been validated by the manufacturer. Accuracy data provided by the manufacturer give a sensitivity of 94.4% (no confidence interval given, n=72, adult samples) and specificity of 100% (in 74 children tested) (https://www.coronavirus-diagnostics.com/antibody-detection-tests-for-covid-19.html) using known negative and positive serum samples. Clinical laboratory validation on adult patients showed a specificity of 100%, with 50 out of 50 samples negative (Charlton et al, 2020) Sensitivity using known positive samples showed an overall all point sensitivity of 63% (95% CI 46-77) and of 88% (95%CI 46-100) in patients more than 21 days post PCR confirmed SARS-CoV-2 infection. Overall, combining literature data (Van Elslande et al, 2020) and *in house* validations, a specificity of 98.6% (494/501; 95%CI 97.1-99.3) and a sensitivity of 95.9% (95%CI 88.8-98.9) at ≥ 15 days post onset of symptoms was observed for the Euroimmun IgG Elisa taken a ratio of 1.1 as cut-off (accuracy 98.3% (565/575)). The laboratory technician will have no insight in the participants identifiers and to previous test results. Test results are qualitative and will be reported as presence versus absence of specific antibodies. After analysis, Sciensano will register the results in a separate data sheet. A child identifier database and a non-identifiable database will be kept. Based on the child identifier (unique registration code), child data on the serological test results will be linked with the questionnaire data.

Sample result

Participants and parents/legal caregivers of participating children will be informed about the result of the~~ir child's~~ serological test after each sampling period. The schools will receive the individual reports under closed envelopes and distribute them to the primary caregivers of the participating children and the participating staff. Informed consent forms and accompanying letters will clearly state that a positive test result only implies that the child has been in contact with the SARS-CoV-2 virus, but that currently no assumptions can be made on protection against COVID-19 disease, unless new convincing scientific data becomes available about protection against re-infection. A contact number will be given in case questions remain regarding the study sample results. Distribution of test results will be done through Sciensano.

3.6.4. Practical considerations

Two junior researchers (one Dutch and one French speaking) will be hired for the duration of the study. They will have implementation, coordinating and administrative responsibilities. Research nurses will be recruited for the sampling school-visit periods. It is the responsibility of each team (Flemish and Wallonia) to coach and supervise the junior researcher, to train the nurses (same training content and instructions will be used for Dutch and French speaking nurses) and to prepare the field visits.

Ten research nurses (5 teams of 2 nurses) will be trained for the sampling periods. The aim is to sample 2 schools (2 x 20 pupils) per day, with 8 schools per week per team. Which would take 2 to 3

weeks to sample 82 schools. Preferably the same nurse teams will be recruited for all three sampling periods.

Standard operating procedures (SOPs) for the study implementation (instructions for schools, the school contact person and the research nurses) will be developed and made available. For the research nurses this includes instructions regarding infection and control procedures, based on the most recent and available knowledge to maximally protect both participants and study personnel. Guidelines (federal – regional, e.g. physical distancing, mask use in public etc.) present at the moment of the field work will be respected.

3.6.5. Special considerations and circumstances

- If a school is closed during the planned period of sampling, the sampling will take place as soon as the school reopens. A maximum of 6 weeks after the first school of that sampling period got sampled will be allowed to add the data to the sampling period analysis. On an individual base the case can be made to still collect the data after this 6 week interval, not for inclusion in period data analysis, but to have school based data in relation to the possible outbreak of SARS-CoV-2 virus in this particular school.
- Given the unpredictable future, ad hoc decisions will need to be made when schools in general are closed during the planned sampling period. Postponing the remaining sampling periods will need to be discussed. Alternatively sampling of students on invitations could be considered, if the interest and need for public health is present. The necessary communication and balanced decisions will be made in line with the national guidelines.
- Given the uncertainty about the future development of the COVID-19 epidemic adaptation of sampling periods (more frequent sampling or adding additional sampling period(s) after April/May 2021) and extension of the study is requested.

3.7. DATA ANALYSIS

The analysis will be descriptive for the primary objective. Variables will be described as proportions with a 95% Confidence Interval (95%CI). Several epidemiological parameters/effect measures will be calculated with a 95%CI.
Among others:

- Seroprevalence of SARS-CoV-2: number of children/ number of school staff in whom specific SARS-CoV-2 antibodies was detected by ELISA / total number of children/staff tested.
- SARS-CoV-2 seroconversion rate: Number of children/ number of school staff in whom presence of specific antibodies was detected by ELISA during any of the follow-up tests with a previous sample showing no specific antibody / total number of children/staff followed-up with a baseline negative serum sample, during the study period of 7 months.
- Cumulative incidence
- Incidence rates (using both the total population and the population excluding the non-susceptible from the prior sero-survey testing period)
- Proportion of asymptomatic cases
- Proportion of symptomatic episodes amongst positives versus negatives

A flow diagram of the participants will be created reflecting number of participants approached, eligible and included. The statistical analysis will account for the small differences in sampling probability due to the study design by using specialized procedures for complex (survey) sampling..

For the secondary objectives, uni- and multivariate analysis will be performed assessing risk, protective and predictive factors associated with sero-positivity among children and among staff. Relative risk of sero-positivity in function of specific risk factors (prior to analysis a directed acyclic graphs (DAG) will be constructed and risk factors will be analysed using Poisson regression and effect

measures will be re-calculated and presented as absolute risks and risk differences with their CI). Models will be assessed for presence of confounders and interaction.

When tables will be presented e.g. test positives versus test negatives at the end of the study, no p-values will be presented comparing differences in basic characteristics to avoid chance-findings.

A sensitivity analysis will be performed to estimate bias resulting from the imperfect accuracy of serology testing. If needed other sensitivity analysis will be performed and selection bias will be assessed. Depending on the amount of missing data imputation will be used.

Data analysis will be done by KUL (statistician) and Sciensano using statistical software already available to the analysis team (including STATA 14/ SAS/ R), while having the necessary licenses.

4. Expected outcome

Primary outcome: SARS-CoV-2 seroprevalence at each time point

Secondary outcomes:
- SARS-CoV-2 sero-incidence and seroprevalence over time
- Cumulative incidence
- Proportion of symptomatic sero-positive cases
- Participants characteristics associated with test-positivity

Findings will help to understand the dynamics of COVID-19 among children and support the implementation of preventive and other measures for this population group.

5. Quality assurance

The protocol will be reviewed by the different partners and published on Clinicaltrials.gov.

Questionnaire contents and tool will be piloted for understandability and to check if the tool functions well. Study nurses responsible for sample collection will be trained. Laboratory analysis will be performed at the Sciensano laboratories. A validation study of the oral swabs will be performed prior to the start of the study to assure the validity of the specimen sample among children. A data analysis plan will be available.

6. Potential Biases and limitations

Selection bias
- Due to feasibility choices. For example, participants ~~children~~ chosen will not be completely representative of the school-age children and school staff population of Belgium. This will be documented by comparing characteristics of the participants with census data and other data sources.
- Exclusion bias: Due to non-consent to the study. The non-consent rate will be calculated to estimate the importance of this bias.

Cluster bias (non-independence of events)
If the selected participants belong to the same group (same class, same school, same family, etc.), this can affect the risk of seroconversion. This effect will be minimized by the multiplicity of contact points investigated, correction of the sample size for the number of clusters and the exclusion of multiple children from the same household.

Information bias
- Misclassification
- Imperfect accuracy (and imperfect reference standard for corrections): sensitivity analysis can be performed correcting for imperfect accuracy of serological testing
- Precision - depending on prevalence

Confounding
- Unmeasured confounders.
- Confounders will be investigated using DAG's and adjusted for where justified.

7. Protection of human subjects and Ethical considerations

7.1. INFORMED CONSENT

- Informed consent of participants, or legal tutor representative in the case of minors, is required prior to inclusion in the study and at the moment of the study extension for the primary school children: see the appendix 'Information and consent form'.
- Informed assent: The children will receive age-appropriate information on the study and may refuse to be included in the study. A new informed assent is provided prior to testing period 4 for primary school children.

7.2. POTENTIAL RISKS

- Biological sample (saliva swap) risks are minimal.
- Information of the result can be considered to be psychologically or socially difficult to manage in the current context of a pandemic. The medical staff performing this study will give appropriate advice.

7.3. POTENTIAL DIRECT BENEFITS FOR PARTICIPANTS AND INDIRECT BENEFITS FOR BROADER SOCIETY

The potential benefits are mainly indirect as the data collected will help improve and guide efforts to understand the SARS-CoV-2 pandemic and partially the transmission dynamics in the paediatric population and among school staff. Participants will also receive the test results, unless they do not wish to receive these.

7.4. CONFIDENTIALITY

Participant confidentiality will be maintained throughout the investigation. Two different databases will be created. One will contain identifying data such as names, surnames, telephone numbers, e-mail addresses and a unique registration code assigned to each participant. The other database will contain the study data (laboratory results and questionnaire information) and will have as identifier the unique registration code. These 2 databases will be kept separately and will be protected by a password. If the investigator entrusts the data for statistical processing, only the second database will be entrusted to this third person. As such, data in the database for analysis will be pseudonymised. Only the principal investigator will keep the database containing personal information and the unique registration code (identification number) assigned to each participant/child.

7.5. BIOLOGICAL SPECIMEN

The biological specimen will be stored after entering the biobank (located at Sciensano) for a duration of maximum 10 years after which they will be destroyed. Specimens might be used for additional COVID-19 related testing. If the participant wishes its biological specimen to be destroyed upon completion of the study, this will need to be mentioned in the informed consent form.

7.6. STUDY INSURANCE

We have an insurance for this study with Ethias. Insurance reference is: 45.437.023
An ethical committee review has been sought for this protocol from the Ethics Committee Research of UZ Gent and ethics approval was received ……2021. Ethical committee clearance: B.U.N.: B6702020000744.

7.7. DATA PROCESSING AND PROTECTION (GDPR)

Data processing and protection for this study complies with the provisions of the regulations for the protection of Personal Data, including but not limited to the EU General Data Protection Regulation 2016/679 of 27 April 2016 and its implementing decrees. Questions and other issues regarding data protection will be addressed to Sciensano Data Protection Officer, e-mail address: dpo@sciensano.be.

The General Data Protection Regulation (GDPR, the European legislation governing the protection of personal data, applies to this study. The legal basis on which the data are processed is consent.

- See Article 6 § 1 (a) of the GDPR
- See Article 9 § 2 (a) of the GDPR

7.8. DISTRIBUTION OF RESULTS

The study protocol will be registered at Clinicaltrials.gov.
Anonymous study results should be made accessible and available as soon as possible after every testing period and at the end of the study to public health authorities involved in management of the COVID-19 epidemic in Belgium. This can be done through a policy brief or presentation. Sciensano will coordinate the distribution of results. These results will be also published on the relevant websites of these institutions.

The general public will also be informed regarding these anonymous results through press communications. This will be done by the communication departments of Sciensano and of the study partners.

Scientific peer-reviewed publications (possible short publication, regular paper) will be prepared to add to the body of evidence and availability for the global scientific community and public health decision makers.

Appendices

a. Questionnaires:
   - Parents: 1ste contact,
   - Parents: 2nd and 3rd contact,
   - Schools: baseline
   - Schools: 2nd and 3rd testing period
   - Parent registration form
   - School staff registration form
   - School staff: baseline
   - School staff: 2nd and 3rd testing period

b. Additional questions to be added at questionnaire for parents at 4th and 5th testing period

1. Werd uw kind gevaccineerd tegen het coronavirus (COVID-19)? ○ Ja, mijn kind kreeg één dosis van een vaccin ○ Ja, mijn kind kreeg twee dosissen van een vaccin ○ Neen, mijn kind kreeg nog geen vaccin 1.1. Indien optie 1: Datum van de eerste dosis: _____ 1.2. Indien optie 2: Datum van de tweede dosis: _____ Indien u de exacte datum van de vaccinatie niet kent, kan u aangeven wanneer uw kind ongeveer werd gevaccineerd. 1.3. Met welk vaccin werd uw kind gevaccineerd ? ○ Ik weet het niet ○ Het Pfizer-BioNTech (Comirnaty) vaccin ○ Het Moderna vaccin ○ Het AstraZeneca (Vaxzevria) vaccin ○ Het Johnson & Johnson (Janssen) vaccin ○ Een ander vaccin, namelijk: _____	1. Votre enfant a-t-il été vacciné contre le coronavirus (COVID-19)? ○ Oui, mon enfant a reçu une première dose d'un vaccin. ○ Oui, mon enfant a reçu deux doses d'un vaccin. ○ Non, mon enfant n'a pas encore été vacciné 1.1. En cas d'option 1: Date de la première dose: _____ 1.2. En cas d'option 2: Date de la deuxième dose: _____ Si vous ne connaissez pas la date exacte de la vaccination de votre enfant, vous pouvez indiquer une date approximative. 1.3. Avec quel vaccin votre enfant a-t-il été vacciné? ○ Je ne sais pas ○ Vaccin de Pfizer-BioNTech (Comirnaty) ○ Vaccin de Moderna ○ Vaccin d'AstraZeneca (Vaxzevria) ○ Vaccin de Johnson & Johnson (Janssen) ○ Un autre vaccin, notamment: _____
2. Zijn de volwassenen (personen van 18 jaar en ouder) die onder hetzelfde dak wonen als uw kind gevaccineerd tegen COVID-19? ○ Ja, iedereen is gevaccineerd ○ Sommige zijn gevaccineerd, sommige niet ○ Nee, niemand is gevaccineerd. ○ Ik wens deze vraag niet te beantwoorden. 2.1. Indien antwoordmogelijkheid 1: Is iedereen volledig gevaccineerd* tegen COVID-19? ○ Ja ○ Neen (Volledige vaccinatie* = één dosis van het Johnson & Johnson vaccin ; twee dosissen van een ander vaccins)	2. Est-ce que les adultes (18 ans et plus) vivant sous le même toit que votre enfant sont vaccinés contre la COVID-19 ? ○ Oui, tout le monde est vaccinés ○ Certains le sont vaccinés, d'autres ne le sont pas. ○ Non, personne n'est vacciné. ○ Je ne souhaite pas répondre à cette question. 2.1. Si option 1 : Est-ce que tout le monde est complètement vaccinés* contre la COVID-19 ? ○ Oui ○ Non (Vaccination complète* = une dose pour le vaccin Johnson & Johnson ; deux doses pour un autre vaccin)

c. Informed consent form child (to be signed by parent/legal caregiver) and age appropriate informed assent forms for first part of the study (December 2020 – June 2021)
d. Informed consent and assent forms for study extension to be completed prior to testing period 4.
e. Informed consent form school staff

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