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Seroprevalence of IgG antibodies against SARS coronavirus 2 in Belgium – a prospective cross-sectional study of residual samples

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Research in context

Evidence before this study

This is the first study reporting seroprevalence and seroincidence of IgG against SARS-CoV-2 in the Belgian population. Worldwide, PCR tests are being performed to identify mainly sick people suffering from COVID-19. However, seroprevalence studies are important and feasible to study the proportion of the population that has already been in contact with the virus, which helps to understand the likelihood of asymptomatic infections or infections with mild symptoms.

From 11 March to 11 May, updates on the COVID-19 pandemic by the World Health Organisation as well as bulletins from the Belgian Scientific Institute for Public Health, Sciensano, were consulted daily. Press releases from all over the world were monitored during that period. Google, PubMed as well as the pre-print server medrxiv were consulted by searching the terms “seroprevalence SARS-CoV-2” and “COVID-19”,

Added value of this study

This study reports that seroprevalence increased in Belgium from 2·9% (95% CI 2·3 to 3·6) to 6·0% (95% CI 5·1 to 7·1) over a period of 3 weeks during lockdown (30 March-5 April 2020 & 20-26 April 2020) with seroincidence estimate of 3·1% (95% CI 1·9 to 4·3). Moreover, a significant increase in seroprevalence in the age categories 20-30 and ≥80 and within each sex were reported.

Implications of all the available evidence

Seroprevalences worldwide indicate that an increasing fraction of the population has already been exposed to SARS-CoV-2. The continuous monitoring of seroprevalences is valuable to calibrate the response to the epidemic and to guide policy makers to control the epidemic wave and potential future waves and to avoid a deconfinement strategy leading to a rebound. However, it seems likely that natural exposure during this pandemic might not soon deliver the required level of herd immunity and there will be a substantial need for mass vaccination programmes to save time and lives.

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Abstract

Background In the first weeks of the COVID-19 epidemic in Belgium, a repetitive national serum collection was set up to monitor age-related exposure through emerging SARS-CoV-2 antibodies. First objective was to estimate the baseline seroprevalence and seroincidence using serial survey data that covered the start of a national lock-down period installed soon after the epidemic was recognized.

Methods A prospective serial cross-sectional seroprevalence study, stratified by age, sex and region, started with two collections in April 2020. In residual sera taken outside hospitals and collected by diagnostic laboratories, IgG antibodies against S1 proteins of SARS-CoV-2 were measured with a semi-quantitative commercial ELISA. Seropositivity (cumulative, by age category and sex) and seroincidence over a 3 weeks period were estimated for the Belgian population.

Findings In the first collection, IgG antibodies were detected in 100 out of 3910 samples, whereas in the second collection 193 out of 3391 samples were IgG positive. The weighted overall seroprevalence increased from 2·9% (95% CI 2·3 to 3·6) to 6·0% (95% CI 5·1 to 7·1), reflected in a seroincidence estimate of 3·1% (95% CI 1·9 to 4·3). Age-specific seroprevalence significantly increased in the age categories 20-30, 80-90 and ≥90. No significant sex effect was observed.

Interpretation During the start of epidemic mitigation by lockdown, a small but increasing fraction of the Belgian population showed serologically detectable signs of exposure to SARS-CoV-2.

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Introduction

A cluster of 27 individuals who visited the Huanan seafood market was diagnosed with pneumonia of an unknown aetiology in December 2019 in Wuhan (Hubei Province, China).[1] Subsequent isolation and sequencing of the virus revealed a novel coronavirus: severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), of which bats are the most likely host.[2] It is the third coronavirus crossing species to infect human populations, the previous ones being SARS-CoV and MERS-CoV in China (2002) and in Middle Eastern countries (2012), respectively.[3] Human-to-human transmission of the virus was thought to be limited, however, the emergence of SARS-CoV-2 rapidly turned into a public health emergency of international concern, which indicates efficient human-to-human transmission.[4,5] The World Health Organization (WHO) announced on 11 March 2020, that the outbreak became pandemic.[6] Clinical symptoms caused by the virus include loss of taste and smell, fever, malaise, dry cough, shortness of breath, and respiratory distress. Reported illnesses have ranged from very mild to severe (from progressive respiratory failure to death).[2] In addition, increasing age, male sex, smoking, and comorbidities such as cardiovascular diseases and diabetes have been identified as risk factors for developing severe illness.[7] As of 26 April 2020, a total of 2,796,453 confirmed cases in 215 countries were reported to be infected by SARS-CoV-2 causing coronavirus disease 2019 (COVID-19) and resulting in 193,799 deaths.[5]

Currently, there is no vaccine or medical treatment available to protect against COVID-19. Therefore, unprecedented measures such as physical distancing, large-scale isolation and closure of borders, schools and workplaces were considered in many countries to mitigate the spread of the disease and to reduce the corresponding pressure on the respective healthcare systems.

In Belgium, the first confirmed case was reported on 4 February 2020, an asymptomatic person repatriated from Wuhan.[8] The first locally transmitted cases were confirmed on 2 March 2020. Thereafter, the number of confirmed COVID-19 cases rapidly increased. The Belgian Scientific Institute for Public Health, Sciensano, reported that as of 30 April 2020, 48,519 cases were confirmed (0·4% of the Belgian population and 14·1% of the tested individuals) of which 7594 died. The majority of the reported Belgian cases are in the age category of 80-89 years (20·4%; 9905/48,519).[9]

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Knowledge on and quantification of the age-specific susceptibility to SARS-CoV-2, and its evolution over time, related to control measures that have been taken, is tremendously important to guide policy makers aiming to control the epidemic wave and potential future waves as a result of an insufficient herd immunity level in the population. These needs were translated into the following research objectives: (1) to constitute a national serum bank on a periodic basis (cross-sectional study design) in order to estimate the seroprevalence in Belgium and its regions and to follow-up trends herein over time and (2) to estimate the age-specific prevalence of antibodies in order to identify age groups that have been infected versus those that are still susceptible as a function of time. The current study presents background seropositivity (overall, by age category, and sex) in the Belgian population using serial serological survey data from the first two collection periods (30 March – 5 April 2020, 20 – 26 April 2020), that covered the first weeks of a lockdown period installed by the Belgian government from 13 March 2020 onwards.

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Methods

Study design

This prospective cross-sectional seroprevalence study is conducted in individuals aged 0-101 years. A serum bank covering all Belgian regions was constituted by collecting residual sera from ten private diagnostic laboratories in Belgium. In each collection period, sera are collected over one week's time. In this study we report on the first two collection periods, 30 March-5 April 2020 (mainly representing exposure before the lockdown) and 20-26 April 2020 (representing exposure prior to and during the start of the lockdown period). In total, up to five collection periods with intervals of three to four weeks are foreseen, with the last one conducted by the end of June. To avoid overrepresentation of subjects with acute and/or severe illness, samples collected in hospitals were excluded. Each laboratory was allocated a fixed number of samples per age group (defined in 10-year age bands: 0-9, 10-19, etc., with the oldest age group consisting of subjects aged ≥90 years), per region (Wallonia, Flanders, Brussels), and per periodical collection period. The number of samples was stratified by sex, to obtain equal numbers of males and females in each age group.

The study protocol was approved by the Ethics Committee of the University Hospital Antwerp-University of Antwerp on March 30, 2020 (ref 20/13/158; Belgian Number B3002020000047) and agreed with inclusion without informed consent, on the condition of the samples being collected unlinked and anonymously.

Sample size

The sample size per periodical collection has been calculated according to: (1) previous experience with various age-specific analyses of seroprevalence data in Belgium,[10] (2) estimates of the number of COVID-19 infected people in Belgium and (3) the estimated evolution of the epidemic curve. Based on case numbers (hospitalized cases confirmed with COVID-19), the overall prevalence of COVID-19 infection at the start of the study was estimated to be about 0·4% (42,797/11,460,000). Based on the hypothesized overall prevalence, a total sample size of 4000 in the first collection round ensures the estimation of the overall prevalence with a margin of error of 0·2%; the precision regarding the age-

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specific prevalence estimates is lower due to the division of samples across the age groups. However, an increase in prevalence was expected during the study period. In total, up to 14,000 sera were planned to be collected, distributed over five periodic collections, and for each data collection target numbers per age group were adapted according to feasibility, sample availability and aiming at maximizing precision and assessing the impact of a change in epidemic control policy. The actual number of samples collected per period are indicated in the result section.

Sample preparation and analysis

After centrifugation of blood samples, selected residual sera (minimum 0·5 mL) were kept in the fridge (4-8°C) for a maximum of 14 days and finally stored at -20°C. Serology results were obtained through a semi-quantitative test kit (EuroImmun, Luebeck, Germany), measuring IgG antibodies against S1 proteins of SARS-CoV-2 in serum (ELISA). The test was performed as previously described by Lassaunière *et al.*[11] The Dutch Taskforce Serology has compared all available data using the EuroImmun ELISA and determined a specificity of 99,2% and sensitivity ranging from 64·5% to 87,8% in pauci-symptomatic patients and patients with severe disease, respectively, using samples from patients >14 days after onset of disease symptoms [12]. Presence of detectable IgG antibodies indicates prior exposure to SARS-CoV-2, an infection which may be resolved or is still resolving, and possibly protection against reinfection,[11,13]

Data management

Data collected for each sample include: unique sample code, sample date, age (in years), sex, and postal code of the place of residence. From the second collection period onwards, for each sample it is recorded whether or not a COVID-19 diagnostic (PCR) test was requested at the collecting laboratories, and whether or not the test result was positive. Samples were delivered anonymously to the investigators. Triage and check for duplicates was done in the collecting laboratories before anonymization.

Each collection period, data were checked for completeness (based on age, sex, and postal code). Serological results (SARS-CoV-2 antibodies) were linked to the database based on the sample code.

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No further data entry was required. All files were kept on a secured server at the University of Antwerp, with restricted access. Data will be stored for 20 years.

Statistical analysis

The serostatus of an individual was considered to be positive if the measured IgG OD values were ≥1·1, equivocal IgG values were considered negative. Crude seroprevalence estimates are displayed with exact Clopper-Pearson 95% confidence intervals (CIs). For all further analyses, the overall seroprevalence estimate and estimates by 10-year age bands, and sex for each collection period were derived by fitting generalized linear models (binomial outcome distribution) to the serostatus of the weighted samples for each collection period. Weighted seroprevalence estimates are stated with the asymptotic 95% CIs using the design-based standard errors. The overall seroincidence estimate and estimates by 10-year age bands, and sex between collection periods were derived by calculating the difference between the corresponding estimated seroprevalence from generalized linear models (binomial outcome distribution) fitted to the serostatus of the weighted samples including an interaction term for the collection period. Weighted seroincidence estimates are displayed with corresponding 95% CIs constructed using the multivariate delta method to quantify the variability thereabout.[14]

We assigned for each collection period weights to the samples such that they replicate the Belgian population structure according to age, sex and provinces for 2020.[15] Weights are computed by comparing the sample and population frequencies, i.e. we used a complete cross frequency table for sex and 10-year age bands and a marginal distribution for the provinces. Weights were trimmed to a maximum value of 3 to reduce the influence of samples in under-represented strata. All analyses were done with the statistical software R (version 3.6.3) using the package survey (version 4.0).[16,17]

Role of the funding source

The funders had no role in study design, data collection, data analysis, data interpretation, writing or submitting of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Results

A total of 7307 serum samples were collected over two periods (30 March-5 April 2020 and 20-26 April 2020) to measure the anti-SARS-Cov2 IgG sero-status. The regional, age, and sex distribution of these samples are shown in Table 1. More Flemish samples were collected in the first period compared to the second period (56·1% in period 1 vs 45·8% in period 2), but this was taken into account in the estimation of the weighted seroprevalences. The median age of the study population was 55 years and 49 years in the respective collection periods. The planned 400 samples per age category in the first collection round was not reached for the age categories 0-20 and ≥90, however, target numbers per age group were adapted according to feasibility and maximizing precision. Slightly more serum samples originated from females in both collection periods. Figure 1 shows that the samples were collected throughout Belgium (panel A and C) and that positive samples were spread over municipalities across Belgium in both collection periods (panel B and D).

In the first collection period, IgG antibodies were detected in 100 out of 3910 samples, whereas in the second collection period 193 out of 3397 samples had IgG antibodies. This corresponds to a crude seroprevalence estimate of 2·6% (95% CI 2·1 to 3·1) and 5·7% (95% CI 4·9 to 6·5) in both collection periods, respectively. The weighted overall seroprevalence showed a significant increase from 2·9% (95% CI 2·3 to 3·6) to 6·0% (95% CI 5·1 to 7·1) over a period of 3 weeks (Figure 2, panel A) which is also shown by the overall seroincidence estimate of 3·1% (95% CI 1·9 to 4·3) (Figure 2, panel D). A significant increase in seroprevalence was observed in the age categories 20-30, 80-90, and ≥90 as indicated by the seroincidence estimates (Figure 2, panel B+E). For example, in the 20-30 year olds, seroprevalence increased from 1·4% (95% CI 0·6 to 3·1) to 7·6% (95% CI 4·9 to 11·9) which is reflected in the corresponding seroincidence estimate of 6·2% (95% CI 2·7 to 9.8). The seroprevalence estimates ranged between 1·4% (20-30 years) and 5·9% (0-10 years) in collection period 1 and between 3·8% (60-70 years) and 15·1% (≥90 years) in the second period. Among age categories in collection period 2, the seroprevalence of the oldest category (≥90 years) significantly differed from the seroprevalences of the age categories 10-20 years, 30-40 years, 60-70 years, and 70-80 years. Within each sex a significant increase in seroprevalence was observed (Figure 2, panel C+F) but no

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differences between males and females in seroincidence or in seroprevalence in any of the periods were identified.

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Discussion

This study reports seroprevalence and seroincidence estimates of IgG antibodies against SARS-CoV-2 for Belgium based on 7307 residual sera collected by diagnostic laboratories over 30 March – 5 April and 20 – 26 April 2020, shortly after the start of a national lockdown period to control the emerging COVID19 epidemic. The overall seroprevalence in Belgium was estimated to be 2·9% (95% CI 2·3 to 3·6) and 6·0% (95% CI 5·1 to 7·1) in the first and second collection period. As such, seroprevalence estimates doubled over a period of 3 weeks. More specifically, elderly (≥80 years old) and the 20-30 year old subjects showed higher seroprevalence estimates in the second collection period compared to the first one.

Because little is known about the medical history of subjects of whom residual samples are collected, any potential bias is difficult to identify and control for when estimating seroprevalence. In this study, the potential for selection bias was reduced by enrolling multiple laboratories in Belgium with samples collected from ambulatory patients visiting their doctor for any reason. Samples originating from hospitals were excluded from the study to avoid over selection of subjects with acute and/or severe illness. Residual sera have previously been used in serosurveillance studies in Belgium [10] as in other countries, and can provide valuable and representative information on immunity against infectious diseases for the general population [18].

Stringent containment measures were enforced in Belgium as of 13 March 2020. These included travel bans, closures of schools, shops, factories and social gatherings in an effort to contain the spread of COVID-19 and decrease its burden on public health. These intervention measures slowed down the number of COVID-19 patients that were hospitalized daily. By the first two weeks of the lockdown (25 March 2020), over 500 cases were hospitalized daily, and this growth rate was halved 4 weeks later.[9] As of 30 April 2020, 0·1% of the Belgian population had been hospitalized for COVID-19 ($15{,}239/11{\cdot}46 \times 10^6$) and 0·4% of the Belgian population had tested positive for SARS-CoV-2 ($48{,}519/11{\cdot}46 \times 10^6$) on a total of 345,047 screened patients.[9] Clearly, the reported numbers of COVID-19 confirmed cases represent an underestimation and were influenced by the testing policy as testing was initially focused on the most severe cases, presenting to hospitals. Asymptomatic and mild cases

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were less likely reported as at different stages of the first epidemic wave, varying proportions of symptomatic cases presented to primary care, and varying proportions of these cases were tested. The estimated seroprevalence in the week of 20-26 April 2020 (6·0%, 95% CI 5·1 to 7·1) indicates that far more people had generated antibodies against SARS-CoV-2 and thus had been in contact with the virus than what is expected from the number of COVID-19 confirmed cases reported in Belgium on 30 April 2020 (0·4%). The current seroprevalence study measuring IgG antibodies against SARS-CoV-2 in the general population thus provides information that, in combination with the reported confirmed COVID-19 cases, allows estimating the total number of SARS-CoV-2 infections in Belgium.

From the above it is clear that determination of the extent of spread of SARS-CoV-2 is a challenge as typically symptomatic patients are diagnosed. In contrast, mainly asymptomatic and pauci-symptomatic subjects were included in the current study suggesting an **underestimation** of the cumulative incidence of SARS-CoV-2 infection in the population. Moreover, the sensitivity of the serological test used depends on the time since the onset of symptoms,[11] thereby preventing a fraction of the infected subjects to test seropositive if not infected long enough prior to testing. By day 14 after symptom onset, IgG against SARS-CoV-2 are detectable in serum of the majority of patients.[2] Possibly, recent SARS-CoV-2 infected subjects may have been included in the current seroprevalence study of whom antibodies were not yet detectable in blood. In addition, SARS-CoV-2 infected subjects with mild or no symptoms of whom it is reported that they may develop low or no antibodies against SARS-CoV-2, may have been included in this study as well.[19] As such, these pauci-symptomatic subjects may have been falsely seronegative, and thus also cause underestimation of the incidence of infection. This may be partly accounted for in future reports, when available information on whether a diagnostic COVID-19 test was performed together with the outcome of the test will be recorded.

A correlation between **age** and neutralizing antibody level was observed in COVID-19 recovered patients in the study of Wu *et al.*[20] Significantly higher IgG values were seen in elderly patients compared to younger patients with similar disease severity, possibly due to a stronger innate immune response in elderly patients.[20,21] We could not observe any clear age-trend in IgG values in the current

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study, possibly due to the low numbers (n=2-29) of seropositive cases per age category. Gaining insights into the IgG values against SARS-CoV-2 by age will be facilitated as the total number of collected samples increases with every collection period.

Male **sex** has been identified as a risk factor for severe COVID-19 disease. However, SARS-CoV-2 susceptibility is similar for males and females and thus no difference in seroprevalence is to be expected based on sex.[22] Nevertheless, one could argue that symptomatology goes hand-in-hand with the initiation and extent of a humoral response. Since probably no severe symptomatic cases were included in this study, any effect of disease severity and concomitant extent in humoral response in males is not detectable.

The uneven infection rate of SARS-CoV-2 hampers the comparison of seroprevalence between countries. A meta-analysis by Levesque et al.[23] reported a seroprevalence of 14% in Gangelt (Germany, 30 March – 10 April 2020, lockdown by 22 March, 100 households). A Swiss study (Geneva, 6 - 26 April 2020, physical distancing measures by 20 March, 633 households) estimated an increasing seroprevalence, from 3·1% (95% CI 0.2 to 5·99, n=343) to 6·1% (95% CI 2·6 to 9.33, n=416) up to 9.7% (95% CI 6·1 to 13·11, n=576) in three subsequent weeks.[24] A weekly serological study in Sweden (country in 'low-scale' lockdown) showed a seroprevalence of 7·3% (n=1104) in Stockholm in the week of 27 April 2020.[25] Other preliminary serological surveys from EU Member States and USA reported that 1·0 - 3·4% of asymptomatic adult blood donors had antibodies against SARS-CoV-2 virus in the period 20 March – 12 April 2020 (i.e. first month in lockdown in reported countries).[26] These seroprevalence estimates, as well as the seroprevalence estimates obtained in the current study provide a consistent picture and increasing incidence of infections across Europe and North America.

Next to defining and monitoring the extent of virus spread in a population, evaluating seroprevalence also possibly identifies protective immunity of individuals after infection. The WHO stipulates that as of 24 April 2020, no study has evaluated whether the presence of antibodies to SARS-CoV-2 confers protection against subsequent infection by this virus in humans.[27] Furthermore, even if presence of detectable antibodies against SARS-CoV-2 would be shown to be protective, recent calculations

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reveal that we are still far away from natural herd immunity. Based on the estimated basic reproduction number (R0 ranges from 1·4 to 3·9),[28] 50 – 75 % of a population would need to have protective immunity in order to achieve herd immunity mitigating subsequent waves of COVID-19.[29] Currently, many countries including Belgium are collecting seroprevalence data to guide policy decisions, but it seems likely that natural exposure during this pandemic might not soon deliver the required level of herd immunity and there will be a substantial need for mass vaccination programmes to save time and lives.[30]

Conclusion

Seroprevalence studies are important and feasible to study the proportion of the population that has already been in contact with the virus, which helps to understand the likelihood of asymptomatic infections or infections with mild symptoms. In the current study, the antibody prevalence of 2·9% (95% CI 2·3 to 3·6) by 5 April and 6·0% (95% CI 5·1 to 7·1) by 26 April 2020, indicates that an increasing fraction of the Belgian population has already been exposed to SARS-CoV-2. The seroprevalence estimates reported in this study are valuable to calibrate the Belgian response to the epidemic and to guide policy makers to control the epidemic wave and potential future waves and to avoid a deconfinement strategy leading to a rebound. The latter might be difficult to achieve as the current study results indicated low seroprevalences which are far from required herd immunity levels. Moreover, more research is needed to confirm if seropositivity correlates to protective immunity against the virus.

Data sharing

The authors are willing to share original data on request.

Contributors

SH, JDB and IW interpreted study results and drafted and revised the manuscript. SH also contributed to the study design and planned and performed statistical analysis. SA contributed to the study design, planned statistical analysis, interpreted study results and revised the manuscript. EE contributed to drafting the manuscript. LP and AC contributed to the study design, sample analysis and interpreted

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the study results and revised the manuscript. SDS contributed to sample analysis and revised the manuscript. PB, PVD, NH and HT contributed to the study design, interpreted the study results and revised the manuscript. PVD and HT also conceived the study. NH also planned the conduct of statistical analysis. All authors had access to all of the data and take full responsibility for the integrity of the data, the accuracy of the data analysis, and the finished article. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Declaration of interest

All authors have completed the Unified Competing Interest form available at www.icmje.org/coi_disclosure.pdf and declare: support from research grants from GSK Biologicals, Pfizer, SANOFI, Merck, Themis, Osivax, J&J and Abbott and grants from The Bill & Melinda Gates Foundation, PATH, Flemish Government and European Union, outside the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

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(Evergem), Labo Somedi (Heist-op-den-Berg), Labo LBS (Brussels), Laboratoire Bauduin (Enghien), Medisch labo Bruyland (Kortrijk), Synlab (Luik).

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Figure captions

Figure 1. Map of Belgium at municipality level, collection period 1 and 2; panel A+C: number of samples tested in each municipality, panel B+D: presence of IgG-positive (red) versus exclusively IgG-negative (green) samples in each municipality.

Figure 2. Weighted seroprevalence (A, B, C) and seroincidence (D, E, F) estimates in Belgium overall (panel A+D), by 10-year age bands (panel B+E), by sex (panel C+F).

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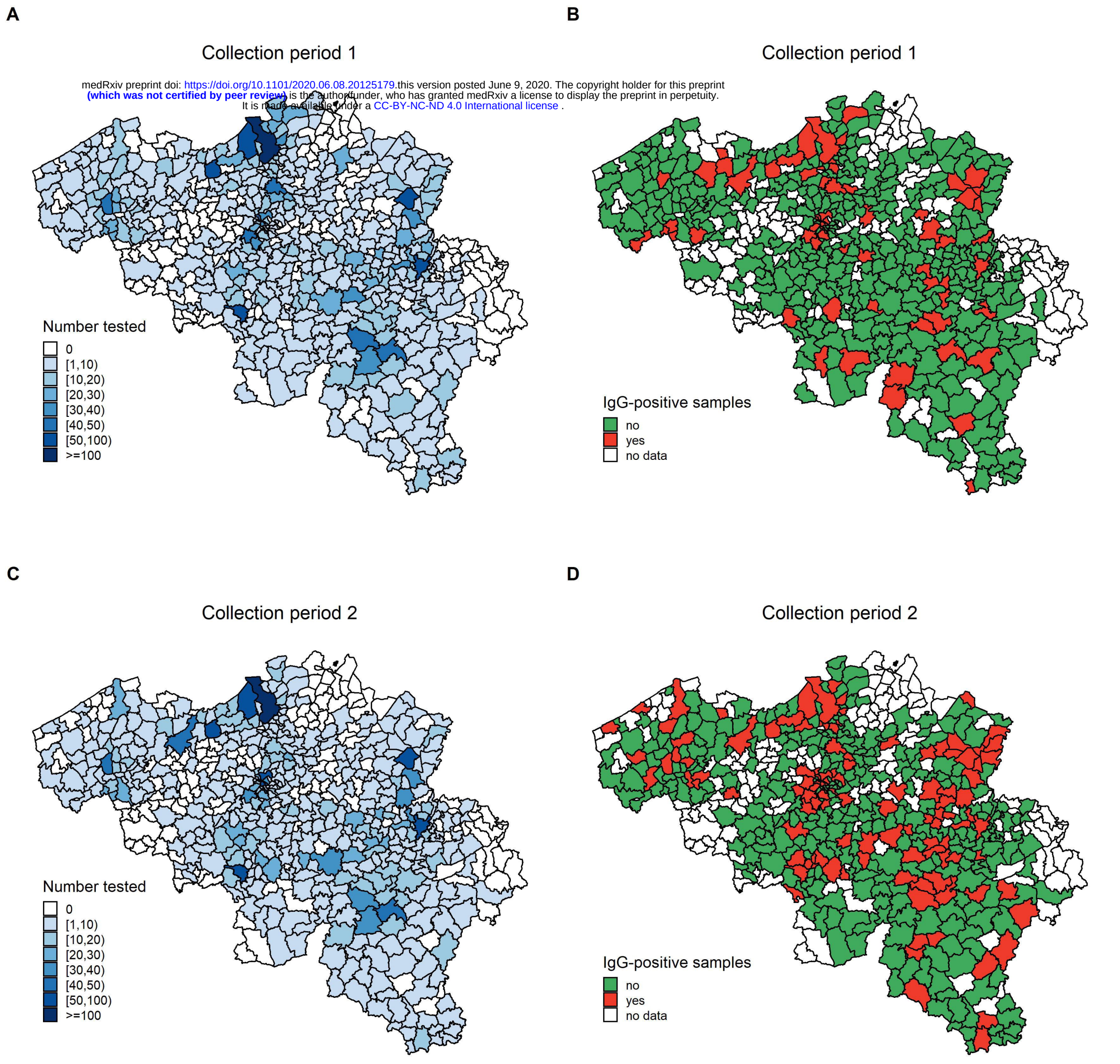

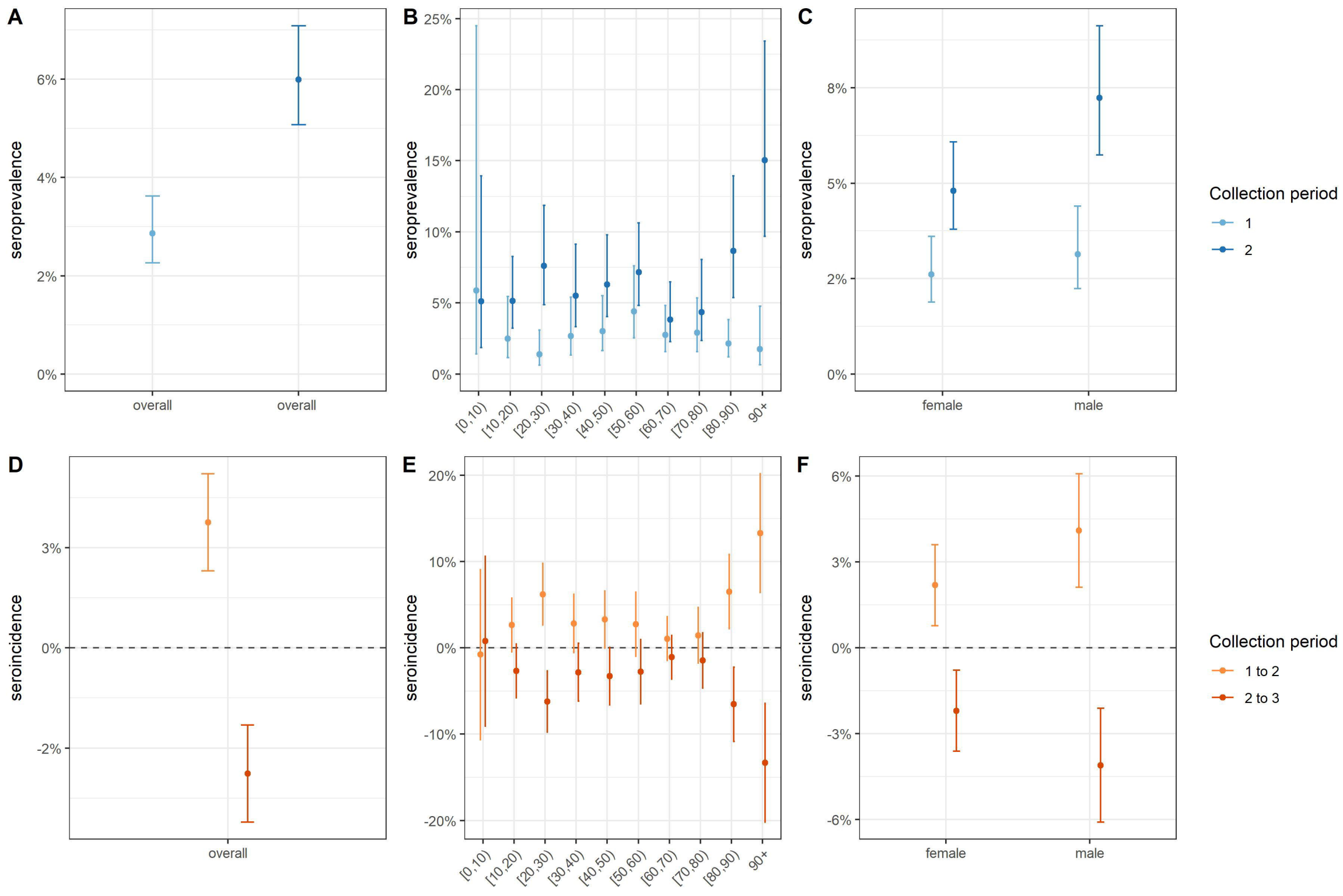
A
B
C
D
E
F
seroprevalence
seroincidence
overall
overall
[0,10)
[10,20)
[20,30)
[30,40)
[40,50)
[50,60)
[60,70)
[70,80)
[80,90)
90+
female
male
Collection period
1
2
1 to 2
2 to 3

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Table 1. Description of the study population, collection period 1 and 2

		Collection period 1		**Collection period 2**	
		(30 March 2020-5 April 2020)		**(20 April 2020-26 April 2020)**	
		n	**%**	**n**	**%**
Number of samples		3910		3397	
Region	**Wallonia**	1511	38·6	1539	45·3
	Flanders	2195	56·1	1556	45·8
	Brussels	204	5·2	302	8·9
Age in years	**0-10**	36	0·9	85	2·5
	10-20	294	7·5	442	13·0
	20-30	436	11·2	375	11·0
	30-40	461	11·8	407	12·0
	40-50	468	12·0	406	12·0
	50-60	498	12·7	430	12·7
	60-70	507	13·0	426	12·5
	70-80	506	12·9	316	9·3
	80-90	493	12·6	315	9·3
	≥90	211	5·4	195	5·7
Sex	**male**	1799	46·0	1599	47·1
	female	2111	54·0	1798	52·9