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Epidemiology

Prospective SARS-CoV-2 cohort study among primary health care providers during the second COVID-19 wave in Flanders, Belgium

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Abstract

Background: Primary health care providers (PHCPs) are assumed to be at high risk of a COVID-19 infection, as they are exposed to patients with usually less personal protective equipment (PPE) than other frontline health care workers (HCWs). Nevertheless, current research efforts focussed on the assessment of COVID-19 seroprevalence rates in the general population or hospital HCWs.
Objective: We aimed to determine the seroprevalence in PHCPs during the second SARS-CoV-2 wave in Flanders (Belgium) and compared it to the seroprevalence in the general population. We also assessed risk factors, availability of PPE and attitudes towards the government guidelines over time.
Methods: A prospective cohort of PHCPs (n = 698), mainly general practitioners, was asked to complete a questionnaire and self-sample capillary blood by finger-pricking at five distinct points in time (June–December 2020). We analysed the dried blood spots for IgG antibodies using a Luminex multiplex immunoassay.
Results: The seroprevalence of PHCPs remained stable between June and September (4.6–5.0%), increased significantly from October to December (8.1–13.4%) and was significantly higher than the seroprevalence of the general population. The majority of PHCPs were concerned about becoming infected, had adequate PPE and showed increasing confidence in government guidelines.
Conclusions: The marked increase in seroprevalence during the second COVID-19 wave shows that PHCPs were more at risk during the second wave compared to the first wave in Flanders. This increase was only slightly higher in PHCPs than in the general population suggesting that the occupational health measures implemented provided sufficient protection when managing patients.

Key Messages

- GPs were more at risk of COVID-19 during the second wave than during the first.
- Their risk of infection was only slightly higher than in the general population.
- Sufficient personal protective equipment was available during the second wave.
- Implemented health measures provided sufficient protection when managing patients.
- Dried blood spot sampling is a valuable alternative for SARS-CoV-2 serosurveillance.

Key words: Coronavirus, general practitioners, personal protective equipment, primary health care, prospective cohort, serology.

Introduction

In 2020, Coronavirus disease 19 (COVID-19) affected many European countries in a first wave that occurred in spring and declined after far-reaching lockdowns, and a second wave that emerged in autumn (1,2). Belgium has been hit particularly hard by this second pandemic wave and even had the highest per capita case numbers in Europe during its epidemic peak (3). While these waves are typically monitored in PCR-confirmed cases, serosurveillance data represents the accumulative number of infections and suggests many more undiagnosed cases (4).

High seroprevalence rates are typically observed among primary health care providers (PHCPs), as they manage the vast majority of symptomatic COVID-19 patients (5). Among the PHCPs, general practitioners (GPs) are essential to organize health care efficiently as they reduce the pressure on hospitals by triaging patients (6). If primary care cannot be delivered safely, the COVID-19 epidemic causes disruption to general public health by impacting the delivery of non-COVID-19 related health care. Furthermore, asymptomatically infected GPs might play a crucial role in transmitting COVID-19 to their patients as a vector or as a source of transmission themselves (7). Indeed, while GPs are typically not involved in aerosol-generating procedures (e.g. in comparison to pulmonologists), they do bear the brunt of an epidemic from frequent contacts with patients in less secure circumstances (8). Therefore, preserving the capacity of GPs and their co-workers throughout the COVID-19 epidemic is essential (9).

This is particularly the case in Belgium where the PHCPs workforce consists of older adults (mean age = 55) who are at higher risk for COVID-19 morbidity and mortality (10). However, while many serological surveys have been performed on health care workers (HCWs) in hospital settings (11–17), surprisingly few surveys followed PHCPs over time (5,18). Here, we evaluated the SARS-CoV-2 seroprevalence rate in a prospective cohort of PHCPs during the second wave in Flanders (the largest of the three regions in Belgium). In the cohort, we also assessed risk factors, attitudes towards the government guidelines and availability of personal protective equipment (PPE) over time.

Methods

Study population and design

In May 2020, we recruited a convenience sample of all PHCPs, i.e. GPs and other PHCPs currently working in the same general practice setting, and physically managing patients/clients in Flanders (Belgium) into a prospective cohort study. Participants that registered for the study and provided written informed consent were asked to self-sample capillary blood by finger-pricking and to complete a baseline questionnaire through a secured online application (basic socio-demographics, health status, stress, the availability and implementation of preventive measures, PPE, attitudes towards the government guidelines and self-discipline of patients) at five different time-points (June–September–October–November and December 2020). The survey included binary (yes/no) and five-point Likert scale answer possibilities ranging from totally agree to totally disagree. The blood samples were stored on the Whatman903 protein saver card and returned via regular mail to the University of Antwerp, where they were preserved in the dark at 4°C. To estimate our primary outcome, i.e. the seroprevalence in PHCPs, based on an expected prevalence of 5% (20%) with a total 95% confidence interval width of 4% (7.2%), a sample size of 500 (PHCPs) was required. An overview of the frequently changing guidelines for PHCPs in place in Flanders during the study period is provided by Domus Medica (19).

Laboratory

All dried blood spots (DBS) were analysed for anti-SARS-CoV-2 IgG antibodies at the Institute of Tropical Medical in Antwerp using an in-house Luminex multiplex immunoassay (MIA), which we described in detail in Mariën et al 2020 (20). In brief, our MIA is a high-throughput platform that allows the simultaneous detection of IgG antibodies against different antigens from SARS-CoV-2: the nucleocapsid protein (NCP), the receptor-binding domain (RBD) and the entire spike protein (S). These antigens are coated with different magnetic beads that can be visualized individually after conjugation with a fluorescent secondary antibody. Using multiple antigens in the same assay increases the specificity of serological testing in contrast to ELISA that usually includes only one antigen. Additional advantages of our MIA are the need for lower serum amounts (<1 µl, ideal for DBS) and the lower cost (as less recombinant antigen is required). In brief, we punched two discs (4 mm) from each DBS in an Eppendorf tube and added dilution buffer (hypertonic Phosphate Buffer Saline buffer) to a final concentration of 1/600. The dilutions were mixed with 1.25×10^6 paramagnetic MAGPLEX COOH-microspheres from Luminex Corporation (TX, USA) that were coupled with recombinant RBD, whole S protein and NCP (BIOCONNECT, the Netherlands). After incubation of beads and diluted sera, biotin-labelled anti-human IgG (1:125) and streptavidin-R-phycoerythrin (1:1000) conjugate were added. The beads were simultaneously read using a Luminex® Bio-Plex 100/200 analyser and expressed as signal-to-noise ratios.

Data analysis

We considered samples to be positive if the fluorescent signal was more than two times the standard deviation plus the mean of the negative controls (n = 96) for all antigens, which corresponds to a specificity of 99% (20). Samples that surpassed the cut-off values for

only two antigens (and if the signal of the third antigen was higher than the mean of the negative controls) were uncertain. Missing serological data (when a participant did not return the DBS at a particular sampling time-point) were imputed if both previous and later serological results could indicate the serostatus (e.g. if a sample was negative on T1 and negative on T3, a missing sample at T2 was considered to be negative on T2). We also considered all seroreverted samples to be positive at later time points, as it is known that most people develop a long-lasting antibody response (at least six months after infection) (20,21). Only positive samples were considered for final seroprevalence and incidence estimates for which confidence intervals were calculated using the binomial distribution (Clopper–Pearson exact method). The incidence was estimated by dividing the number of new seroconverted PHCPs at the end of the month by the number of susceptible PHCPs at the beginning of the previous time-point (represented per 1000 individuals).

To put our data in perspective, we also included seroprevalence estimates from residual and blood donor sera (representing the general population) that were obtained from serosurveillance studies at the University of Antwerp (before the second wave: June and September) (22), and Sciensano and Red Cross Flanders (after the second wave: November and December) (23). These samples were screened on SARS-Cov-2 IgG antibodies using the EuroImmun (June and September) or Wantai (November-December) SARS-CoV-2 antibody ELISAs. Residual sera were acquired from a random selection of ambulatory patients visiting their doctor for any reason including primary care (22). To avoid biased selection of subjects with acute illness (including COVID-19), we excluded samples originating from hospitals and triage centres from the study. Residual samples were allocated to a fixed number of samples per sex and age group (10-year age bands, oldest age group ≥90 years) to assure a generalizable subset of the population.

To investigate if PHCPs were significantly more likely to be infected than the general population, we used a generalized linear model (GLM) with serological status as response variable and time and occupation (PHCPs or donor sera) as explanatory variables. Serological status was analysed assuming a logit-link function with a binomial distribution. We did not control for clustering of individuals (PHCPs within practices and blood donors within families) in this analysis, since this information was not available for blood and residual donors and participating PHCPs in Flanders were almost homogeneously distributed over the municipalities (Fig. 1). Answers to the questionnaires were analysed using generalized linear or ordinal logistic regression models depending on the response variable (binary or Likert scale, respectively) (24). To test if the attitudes/responses changed over time, we used time as an explanatory variable in these models. A Bonferroni correction was used to correct p-values for multiple testing. All data analyses and visualizations were performed in R4.1.0 using the packages epiR, gplots, dplyr, rgdal, RColorBrewer, surveillance, Likert, MASS, cowplot, ggpubr (25).

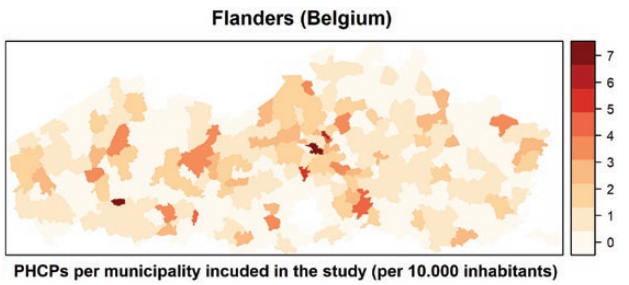

Figure 1. *Spatial distribution of the primary health care providers (PHCPs) in Flanders that were included in the cohort study between June and December 2020. The color scale represents the number of PHCPs per municipality per 10 000 inhabitants*

Results

From all the PHCPs that responded to our call to participate in the study, 698 provided informed consent and returned at least one DBS. The willingness to participate remained high throughout the study, as most participants (n = 604) returned a DBS at more than three-time points. The cohort consisted mainly of GPs (n = 641) and women (n = 520), and the mean age was 41.1 years (SD = 11.6) (Table 1). The seroprevalence of PHCPs after the first COVID-19 wave was 4.6% (95%CI 3.2–6.4%) in June, which is in line with the seroprevalence of blood donors (4.9%; 95%CI 3.1–5.9%) (22) but lower than the seroprevalence of hospital HCWs (9.4%; 95%CI 6.5–13.4%) (15,17) at that time in Flanders. The seroprevalence of both PHCPs and the general population (blood + residual donors) remained stable until September but increased significantly from October to December (df = 1, χ^2 = 75.5, P < 0.00001) (Fig. 2). We detected the highest increase between October and November, which coincided with the peak in confirmed PCR cases in the community. After the second wave, we did observe a small but significant difference in the seroprevalence between PHCPs (13.4%; 95%CI 10.8–16.4%) and blood donors (9.0%; 95%CI 6.2–11.8%; df = 1, χ^2 = 7.9, P = 0.005) (Fig. 2) (23). The odds to being seropositive were significantly higher in PHCPs compared to blood donors [odds ratio = 1.33 (95%CI 1.09–1.62)]. Consequently, the incidence among PHCPs was low in the period June–September (3 cases/1000 individuals; 95%CI 1–10), increased in October (27 cases/1000 individuals; 95%CI 15–42) and reached its highest point in November (40 cases/1000 individuals; 95%CI 26–58) (Supplementary Fig S1).

The survey showed that PHCPs were potentially at high risk of COVID-19 infections since more than 90% continued working and had physical contact with suspected patients during the entire study period (Fig. 3). The risk seems to decrease towards the end of the year as the number of PHCPs participating in the triaging of patients (Fig. 3) and the overall number of contacts per month decreased (Supplementary Fig S2). During the entire study period, 19–43%

Table 1. *Characteristics of primary health care providers (PHCPs) who participated in at least one testing time point in Flanders (Belgium) between June and December 2020*

	All PHCPs n = 698	GPs n = 641	Other PHCPs n = 57
Age, mean (SD)	41 (12)	41 (12)	40 (11)
Gender[a], n (%)			
- Male	173 (24.8)	166 (25.9)	7 (12.3)
- Female	520 (74.5)	470 (73.3)	50 (87.7)
- Not reported	5 (0.7)	5 (0.8)	
Practice size, n (%)[a]			
- Solo	84 (19.7)	84 (19.9)	3 (7.3)
- Duo	83 (19.4)	83 (19.6)	3 (7.3)
- Group (<8 employees)	139 (32.6)	138 (32.6)	6 (14.6)
- Big group (>7)	112 (26.2)	111 (26.2)	27 (65.85)

[a]If numbers do not add up to the column total, this is due to missing data; numbers of practices for PHCPs = 427, GPs = 423 and other PHCPs = 41.

PHCPs agreed with the statement that they definitely would get infected, 9–20% that they definitely already are infected and 44–54% that they feared to infect household members (Fig. 4). It seems that PHCPs were relatively well-protected at work as more than 95% of PHCPs agreed that they had adequate PPE available from June onwards (Fig. 3). PHCPs agreed to experience more stress than during a busy flu season, but the overall confidence in the government guidelines, scientific task force and imposed measures was generally high and increased significantly over time (Fig. 4). Overall, most PHCPs agreed that patients followed the government guidelines of hygiene and self-quarantine (Fig. 4). From the 112 PHCPs who reported a positive PCR result, the majority claimed that they became infected after contact with a patient (61%), while the minority claimed that they became infected by someone else [relative (16%), colleague (14%) and unknown (8%)].

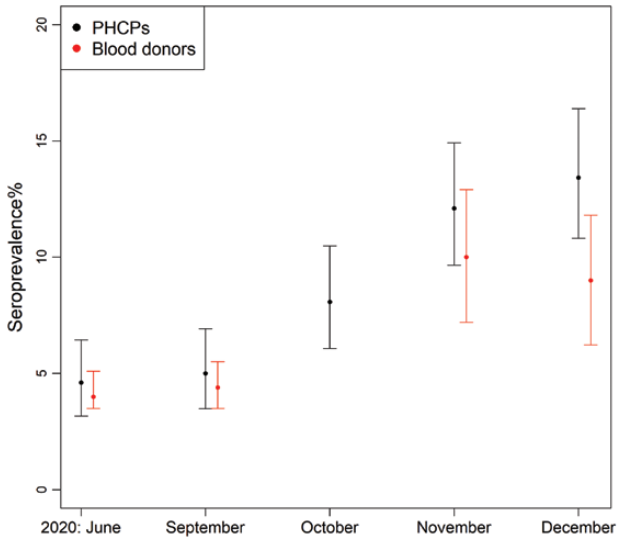

Figure 2. *SARS-CoV-2 seroprevalence in a cohort of primary health care providers (PHCPs; black) and blood donors (red) after the first and during the second COVID-19 wave in Flanders in 2020 at the end of each month. Error bars represent 95% confidence intervals calculated using the binomial distribution*

Discussion

In a cohort of PHCPs, we observed a significant increase of IgG antibodies against SARS-CoV-2 in DBS samples during the second COVID-19 wave in Flanders. We also found a small but significant difference in seroprevalence between PHCPs and the general population, represented by blood and residual serum donors. The PHCPs' seroprevalence pattern was very similar to that of hospital HCWs in Belgium. The latter pattern also remained stable until September (8%) and increased up to 20% in December (17).

Although PHCPs were generally concerned about being at high risk of COVID-19 infections, our study reveals that the risk was only slightly higher than the general population (33% more likely based on seroprevalence data). This result is likely explained by the availability and proper usage of PPE as more than 95% of PHCPs had

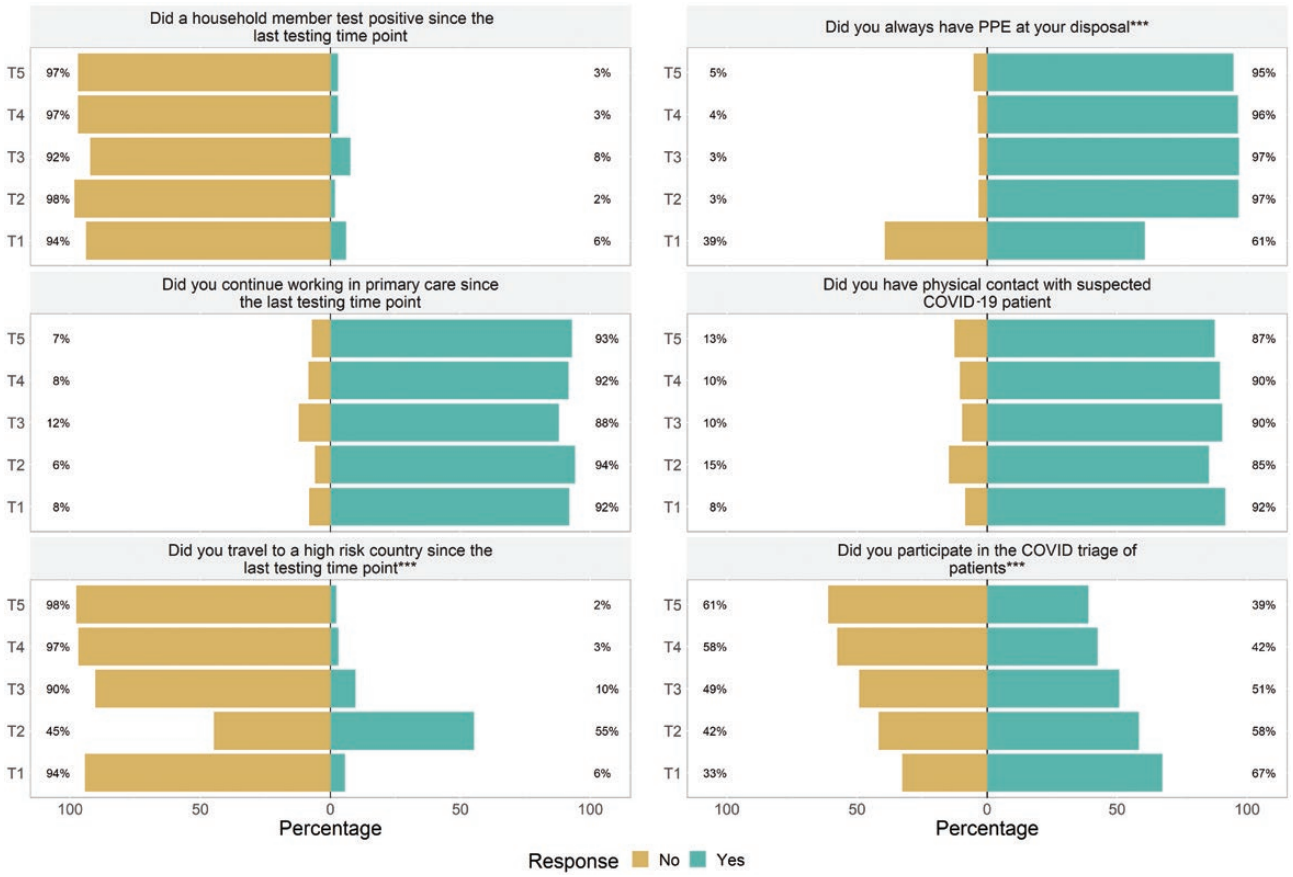

Figure 3. *Binary responses of primary health care providers (PHCPs) on questions related to risk factors of becoming infected with COVID-19 at the end of each month (T1 = June, T2 = September, T3 = October, T4 = November, T5 = December 2020). Asterisks indicate if a significant difference between months was noted (P-values* = 0.05–10^{-4}, **10^{-4}–10^{-7}, ***<10^{-7})*

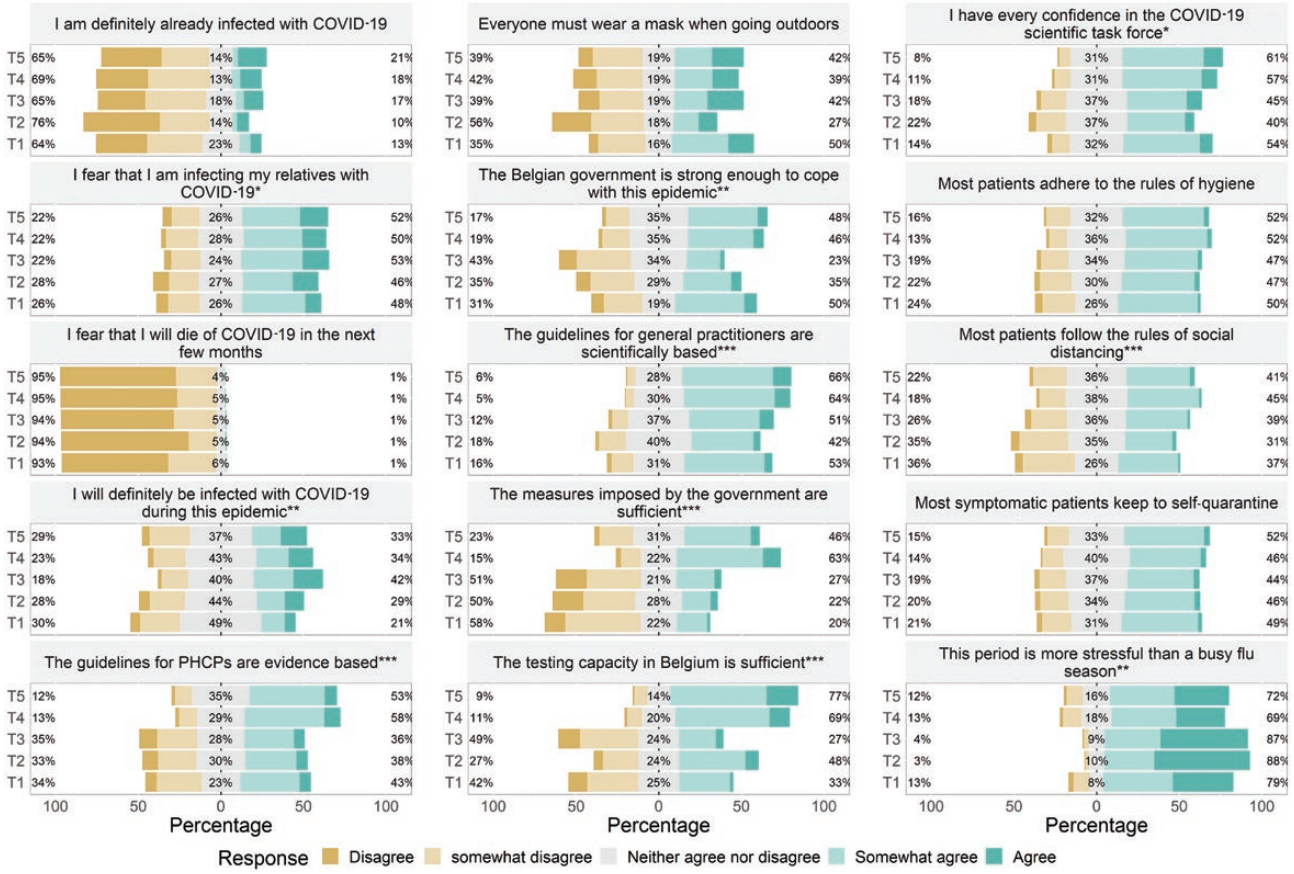

Figure 4. *Likert scaled responses of primary health care providers (PHCPs) on questions related to fear of becoming infected with COVID-19, attitudes towards government guidelines and self-discipline of patients at the end of each month (T1 = June, T2 = September, T3 = October, T4 = November, T5 = December 2020). Asterisks indicate if a significant difference between months was noted (P-values* = 0.05–10^{-4}, **10^{-4}–10^{-7}, ***<10^{-7})*

adequate PPE during the second wave in Flanders. Furthermore, our survey suggests that most PHCPs adhered to the government guidelines, which were judged to be clearly communicated and evidence-based. Their confidence in the government guidelines increased significantly towards the end of the second wave, which might be explained by the increasing PCR-testing capacity and positive news on vaccine effectiveness and availability at the end of the year. Our results contrast with studies from the first COVID-19 wave which reported worldwide poor accessibility to PPE and difficulties of GPs to follow the ever-changing government guidelines (8,26–29). Good infection prevention control and occupational health guidelines might thus explain the limited difference in seroprevalence between PHCPs and the general population in Flanders. Our results align with studies from Germany (Bavaria) and Italy (Sicily) that also found a low rate of past SARS-CoV-2 infections in PHCPs during the first wave (9,18). Together, these few studies suggest that PHCPs can be relatively well protected during COVID-19 waves if unprotected contacts with infected patients are limited (e.g. when sufficient PPE is available and properly used).

Nevertheless, we assume that the limited difference in seroprevalence of PHCPs compared to the general population in December reflects an actual difference, as DBS usually leads to a decrease in sensitivity compared to serum (meaning the actual difference might be higher) (30). Indeed, our direct comparison between PHCPs and blood donors is limited because the samples were different (capillary versus venous blood), stored differently (DBS versus serum tube) and analysed with different techniques (Luminex MIA versus ELISA). We chose DBS sampling because of its convenience of non-invasive sampling and room-temperature storage. Serological point-of-care tests (POCTs) would have substantially improved the timeliness of the test results and the PHCPs could have immediately checked their results (31). We did not use POCTs in this study because their specificity was too low when we conceived this study (32). Nonetheless, our study suggests that DBS sampling is a valuable alternative for SARS-CoV-2 serosurveillance in non-clinical, resource-limited settings. Samples can also be re-used (e.g. to check for co-infections), which is impossible with POCTs. We do not expect that the presence of different SARS-CoV-2 variants would have impacted our seroprevalence and incidence estimates (e.g. due to reduced sensitivity in our assay) (33), given that these strains mainly became prevalent in Belgium after we ended our study (January 2021) (34). Given that older male GPs were somewhat underrepresented, our cohort was less at risk for COVID-19 morbidity and mortality than the average Flemish GP, but not necessarily less susceptible to infection.

Conclusions

The marked increase in seroprevalence and incidence during the second COVID-19 wave shows that PHCPs were more at risk during the second wave than during the first wave in Flanders. This increase was only slightly higher in PHCPs than in the general

population suggesting that the occupational health measures implemented provided sufficient protection when managing patients. They also indicate that the imposed guidelines for PHCPs were effective and should be maintained until herd immunity is achieved.

Supplementary material

Supplementary material is available at *Family Practice* online.

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Declaration

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Ethics approval: ethical approval for the study was given by the institutional review board of the Antwerp University Hospital/University of Antwerp (20/24/315). All participants provided informed consent to participate. We declare that the planning conduct and reporting of the study was in line with the Declaration of Helsinki, as revised in 2013. A research checklist was added following the STROBE guidelines.

Conflict of interest: The funder of the study had no role in the study design; collection, management, analysis, or interpretation of the data, preparation, review, or approval of the manuscript; or decision to submit the manuscript for publication.

Author statement

Conceived the study: S.C. Wrote the paper: J.M. & S.C. Performed the lab experiments: J.M., A.C., D.B. Performed the data analyses: J.M. & S.C. Supervised data collection and laboratory work: J.M., C.L., M.L., S.H., Ad.S., J.V., Avd.B., H.G., Pv.D., S.C., K.A. All authors read and approved the final manuscript.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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