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SARS-CoV-2 Antibody Seroprevalence in Industry Workers in Split-Dalmatia and Šibenik-Knin County, Croatia

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Objectives: To examine seroprevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies in industry workers population sample. **Methods:** From 23 to April 28, 2020, we conducted serological testing for antibodies (Immunoglobulin G (IgG) and Immunoglobulin M (IgM)) on 1494 factory employees living in the Split-Dalmatia and Šibenik-Knin County (Croatia). **Results:** We detected antibodies in 1.27% of participants (95% confidence interval [CI] 0.77–1.98%). In Split facility 13/1316 (0.99%, 95% CI 0.53–1.68%) of participants were tested positive, of which 13/1079 (1.20%, 95% CI 0.64–2.05%) of those living outside the facility and 0/237 (0%, 95% CI 0–1.26%) of those living inside the facility. In Knin facility, 6/178 (3.37%, 95% CI 1.25–7.19%) participants were tested positive for antibodies. **Conclusions:** The study showed relatively small SARS-CoV-2 antibody seroprevalence in the DIV Group population sample.

Keywords: Coronavirus disease, Croatia, IgG, IgM, severe acute respiratory syndrome coronavirus 2, seroprevalence

Learning Objectives

- Discuss issues related to employer responses to the COVID-19 pandemic and the efficiency of protective measures taken.
- Summarize the findings on SARS-CoV-2 antibody seroprevalence among industrial workers in Croatia.
- Discuss the study implications for suppression of viral spread among industrial workers.

Coronavirus disease (COVID-19) is a disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] Due to its rapid spread across the world, the WHO declared it as a global pandemic on March 11, 2020.[2] According to the most recent WHO Situation Report on April 28 (when our research was completed), there were 2,954,222 confirmed cases, which led to death in 202,597 cases.[3]

On February 25, 2020, the first confirmed COVID-19 case in the Republic of Croatia was reported in a male patient who had recently returned from Italy, which was at that time the major hotspot of the disease in Europe.[4–6] As a response, on March 19 the Croatian Government introduced restrictions that limited social gatherings, operation of shops/services, as well as the prohibition of sporting/cultural events and closing of the borders.[7,8] Finally, from March 23, citizens were also prohibited from leaving their place of residence.[9] The response to the COVID-19 crisis by the Croatian Government was seen as one of the most rigorous worldwide, placing it on the top of the stringency scale of the Oxford COVID-19 Government Response Tracker on March 26.[10] Following the decrease of new daily confirmed cases and decrease of basic reproduction number to 0.8,[11] on April 19 the Croatian Government lifted the restriction of prohibiting citizens from leaving their place of residence. On April 27, the Croatian Government also started the gradual loosening of measures in an attempt to reduce the negative economic impact.[12] The last official data by the Croatian Institute of Public Health (April 28, 2020) reported a total number of 2055 confirmed cases (49.9 per 100,000) and 63 deaths (1.5 per 100,000).[13] The total number of tests per thousand was 8.4 (in comparison to Europe's median of 10.8) with a positive rate of 0.02 (Europe's median 0.025).[14]

In Split-Dalmatia County ($N = 454{,}798$) and Šibenik-Knin County ($N = 109{,}375$),[15] the first cases were reported on March 15,[16] and March 19, respectively.[17] According to the data available on the first day of testing (April 23), there were 454 confirmed cases (100 cases per 100,000) in Split-Dalmatia County which made it one of the two most affected counties in Croatia.[16] In contrast, on April 27 in Šibenik-Knin County, 83 cases (76 cases per 100,000) were reported.[17]

As in all of Croatia, many companies in named counties also had to temporarily reduce or completely stop the production during the restrictive measures. However, some of them, employing a great number of people in the county, managed to keep the production in lower quantity by introducing a particular set of protective measures. One of them is the DIV Group, which specializes in the production and trade of screws and other mechanical parts and metal products, as well as shipbuilding.[18] Their two major production sites are located in Split (Split-Dalmatia County), and Knin (Šibenik-Knin County) employing around 2200 people and around 400 people, respectively.[18] The Split facility spreads across around 540,000 m², while the Knin facility comprises the area of about 22,000 m². The employees in both facilities work in different production segments and administration. At the facility in Split, some of the employees live at the facility premises.

Unlike many other businesses, the DIV group introduced protective measures ahead of the Croatian Government. From February 25, the company implemented hand disinfection stations in all rooms, as well as regular workstation cleaning protocols. All

communal coffee and food vending stations were closed as well. From March 3, all employees had to undergo temperature checks before entering the facility. From March 11, 8 days before national measures took place, they introduced specific internal measures that included self-isolation for those returning from abroad, work from home for part of the business and administrative staff, and vacation for employees whose work could not be continued. The total number of employees working at the aforementioned facilities was reduced to around 1300 and 300, respectively. For those remaining at the workplace, it was mandatory to wear face masks provided by the company and comply with the other protective measures such as disinfection and social distancing. To ensure compliance with methods, the company continually provided information and warnings for employees using multiple internal communication methods. As an additional prevention method during the lockdown, workers living at the facility premises in Split could not leave the facility more than once a week during the national restrictive measures.

Before this study, the company facility in Split reported a total of seven confirmed cases, 20 employees with symptoms of COVID-19, and 52 employees in self-isolation in different periods. However, all the confirmed individuals were in contact with the virus outside of the factory premises while on vacation or working from home.

To preliminarily assess the current state of infection and measures' efficiency, and to ensure safe conditions, the company management decided to screen their employees for SARS-CoV-2 antibodies. The testing was conducted in Split and Knin company facilities, but also in Zagreb offices ($n = 30$) and Samobor facility ($n = 72$), which were not considered in this study due to limited sample size.

Since the prevalence of corona infection in populations is still unknown, it is a priority to gather information from different parts of the world and different target groups. Although real time polymerase chain reaction (RT-PCR) tests are reliable to detect current infection and viral material, they cannot provide information on previous infection or exposure to the virus.[19] For this reason, and due to the lower financial and temporal demands, serological immunoassay tests have been employed in studies.[20–25] The studies published before the time of testing, as well as unofficial study results, report various proportions of antibodies in studied samples, ranging from 2% to 30%.[20–25] These results could indicate that differences may stem from the sampling strategy as well as the population studied, but also from the performances of serological tests.

As occupation-specific populations were still not considered, the aim of this study was to estimate the proportion of company employees that developed antibodies for the SARS-CoV-2 virus and interpret results to assess the efficiency of measures in the working environment.

METHODS

We conducted serological immunoassay testing for SARS-CoV-2 antibodies in 1494 adult employees of DIV Group (in Split 1316 and Knin 178) from a total population of around 1600 active employees working at facility grounds. The study was performed from the 23 to 28 of April 2020, in the last days before the loosening of national restrictive measures in Croatia. DIV Group organized the study in cooperation with the Clinical Department for Pathology, Forensic Medicine and Cytology, University Hospital Center Split; University Department of Forensic Sciences, University of Split; and University of Split School of Medicine (Split, Croatia).

Participants and Recruitment

DIV Group management and its crisis headquarters invited employees to participate in voluntary screening for SARS-CoV-2 antibodies via phone and/or E-mail. The schedule for the screening

was arranged in cooperation with the head of company departments and sub-companies. Each participant was provided with a questionnaire that had to be completed prior to the test and was asked to sign an informed consent form. The questionnaire form contained questions on basic demographic data, symptoms, recent travels abroad, and possible contacts with infected or likely infected individuals.

Test Type and Performance

We used AMP Rapid Test SARS-CoV-2 Immunoglobulin G (IgG)/Immunoglobulin M (IgM) (AMP Diagnostics, AMEDA Laboradiagnostik GmbH, Graz, Austria). The test is intended for the rapid immunochromatographic qualitative detection of IgG and IgM antibodies to SARS-CoV-2 in whole human blood, serum, and plasma samples. According to the manufacturer, for IgM, test sensitivity is 95.7%, test specificity 97.3%, and test accuracy 96.8%. For IgG, test sensitivity is 91.8%, test specificity 96.4%, and test accuracy 95%. Information on the combined test performance (IgM and IgG) was not provided by the manufacturer.[26]

Sample Collection and Testing

In Split, the screening tests were performed inside the company classroom (about $80\,m^2$), with four testing points arranged in semi-circular pattern placed two or more meters away from each other. In Knin, two testing points were established in a company classroom (about $60\,m^2$) at a distance larger than 4 m. Both rooms had a separate entrance and exit points and were manned by a security guard, that was in charge of escorting the participants into the room and checking whether they had protective equipment (face mask). During the screening, the rooms were continuously ventilated.

For each participant, we punctured a finger with a sterile lancet and collected approximately $10\,\mu L$ of whole blood with a disposable pipette. The blood was immediately transferred to the sample well of the test cassette, after which approximately $80\,\mu L$ of buffer was applied in the buffer well. According to the manufacturer's recommendations, the test results were (C—control line; G—test line IgG; M—test line IgM) read between 10 and 15 minutes. Each invalid test (no reaction on control line) and each positive test result was repeated at a different testing point (different test performer, lot, and buffer). All test results were entered into the form and photographically documented in the recommended time interval. During the same day, when participants were tested, the company provided them with the test results over the phone. If a participant was tested positive, he/she was contacted, retested, and forwarded to the local epidemiologic service who arranged additional RT-PCR confirmatory testing. Upon receiving the results, a quarantine/self-isolation measure with health supervision, or other appropriate measures were issued by epidemiologists.

Crisis headquarters maintained further contact with physicians and epidemiologic services to receive information on final results. Additional information about the whereabouts and possible contacts of the positive participants were provided to the researchers by the crisis headquarters.

Statistical Analysis

We provided descriptive statistics on population demographic structure. We calculated the raw proportion of positive test results in the population sample as well as a proportion on a 95% confidence interval (CI). To calculate two-sided 95% CI, Clopper-Pearson exact method in RStudio (version 1.2.5033, RStudio, Inc., Boston, MA) and package GenBinomApps (https://CRAN.R-project.org/package=GenBinomApps) were used. We analyzed the results in the complete sample and separately according to two counties. In the facility in Split, we also analyzed separately the participants who lived on the facility's premises could not leave the facility more than once a week during the national restrictive measures, and the participants living outside the facility to explore

the effect of inside-facility lockdown. Differences between prevalence in different counties were analyzed using a chi-squared test with Yates correction (due to the low number of expected frequencies). The level of statistical significance was set at $P \leq 0.05$.

Ethical Considerations

Ethical approval was attained by the University Department of Forensic Sciences, University of Split, Ethics Committee on April 22, 2020 (2181-227-05-12-19-0003; 024-04/19-03/00007).

RESULTS

Participants' Characteristics

The tested population comprised a wide age population structure, ranging from young adults to elderly population, mostly working in the company as senior consultants and engineers. In total, 1494 participants (88.1% men; median age 46, range 18 to 79) from the DIV group were tested. In Split, there were 1316 participants (89.4% men; median age 46, range 19 to 79), while in the Knin facility, there were 178 participants (78.1% men; median age 45, range 18 to 64). Of the total number of the participants in Split, 237 (18%) were those accommodated inside the facility grounds.

Testing Performance

A total of 1521 immunoassays were used in the study. Six of them were repeated as they did not show a reaction in the control region, while 21 tests were repeated for participants who showed positive first test results. When repeated, all tests showed a reaction in the control region, and tests that were repeated for positive participants demonstrated the same results.

Positive Test Results

Table 1 shows an overview of the screening test results and sample sizes, divided by counties and degree of mobility restrictions.

The seroprevalence of SARS-Cov-2 antibodies in the tested population sample was 1.27%, but ranged from 0% to 3.37%, depending on which population subsample was considered. In the total population sample, the proportion of positive individuals was highest for IgG, followed by IgM and a combination of IgG/IgM antibodies, respectively. Positive test results were detected for 17/1316 men (12 from Split and five from Knin facility) and 2/178 females (one from Split and one from Knin facility). Both females were positive only for IgM antibodies.

All participants living inside facility premises, and with limited mobility during the lockdown measures, tested negative for antibodies. When we excluded them from the overall sample, the proportion of positive participants slightly increased and reached 1.51%.

The difference between proportions of positive participants in Split (with no mobility restrictions) and Knin samples was not statistically significant ($\chi^2 = 3.47$, $P = 0.062$).

Positive Participants

From all positive participants, three of them (one IgM, two IgG/IgM) reported close contact with a person suspected of SARS-CoV-2 infections or with a confirmed case. The positive participants did not report traveling abroad since the beginning of 2020. Table 2 shows symptoms reported to occur since the beginning of 2020 by positive participants.

All IgM positive participants were proceeded to confirmatory testing (RT-PCR). According to feedback received, from a total of six participants with IgM antibodies, two of them were positive for SARS-CoV-2 on RT-PCR, while four participants tested negative.

DISCUSSION

The present study showed that seroprevalence of SARS-Cov-2 antibodies in the tested company population sample, ranges, with different inclusion criteria, from 0.77% to 1.98% and from 0.91% to 2.35%. Due to the sample size and considering that study comprised more than 80% of the company's active employees, the results suggest that a relatively small proportion of DIV workers population was exposed to the virus. So, the study demonstrated that the epidemic situation within the company could be controlled by the timely implementation of adequate national and corporate measures despite a large number of industry workers. To the authors' knowledge, this is the first SARS-CoV-2 seroprevalence study conducted in the industry workers population.

The present research was primarily aimed at the population working at DIV Group facilities in Split and Knin and showed that 0.77% to 1.98% of the population had developed antibodies. As it was previously mentioned, some of the participants from Split were living inside the facility with more mobility restrictions. Interestingly, in that subsample, no seropositive individuals were found. Although differences between the Split sample living inside and outside the facilities could not be statistically compared, the results could suggest that the restriction of movement outside the facility (complete lockdown) additionally lowered the transmission factor. These results could speak in favor of quarantine/total lockdown as the most effective measure to stop the spread of the disease. However, the seroprevalence still remained low in the facility population that was not exposed to the total lockdown.

TABLE 1. SARS-CoV-2 Antibody Prevalence According to the Analyzed Population Samples

		IgG+		IgM+		IgG/IgM+		Total Positives for Antibodies	
	n	*n*	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)
Split total	1316	8	0.61 (0.26−1.94)	3	0.23 (0.05−0.66)	2	0.15 (0.02−0.55)	13	0.99 (0.53−1.68)
Split (participants with limited mobility)	237	0	0 (0−1.26)	0	0 (0−1.26)	0	0 (0−1.26)	0	0 (0−1.26)
Split (participants with no mobility restrictions)	1079	8	0.74 (0.32−1.46)	3	0.28 (0.06−0.81)	2	0.19 (0.02−0.67)	13	1.20 (0.64−2.05)
Knin total	178	2	1.12 (0.14−4.00)	3	1.69 (0.35−4.85)	1	0.56 (0.01−3.09)	6	3.37 (1.25−7.19)
Total (no mobility restrictions)	1257	10	0.80 (0.38−1.46)	6	0.48 (0.18−1.04)	3	0.24 (0.05−0.70)	19	1.51 (0.91−2.35)
Total	1494	10	0.67 (0.32−1.23)	6	0.40 (0.15−0.87)	3	0.20 (0.04−0.59)	19	1.27 (0.77−1.98)

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

TABLE 2. Sex, Age, and Symptoms Reported by Positive Participants

Sex	Age	Antibodies	Symptoms						
			General Weakness	Nose Leak	Sore Throat	Cough	Headache	High Body Temperature	Muscle Pain
Split									
M	43	IgM	−	−	−	+	−	−	−
M	59	IgG	−	−	−	−	−	−	−
M	59	IgG	−	−	−	−	−	−	−
M	54	IgG/IgM	−	−	−	−	−	−	−
M	59	IgG	−	−	−	−	−	−	−
M	46	IgG	−	−	−	−	−	−	−
F	26	IgM	+	−	−	−	+	−	−
M	49	IgG	−	−	−	−	−	+	−
M	64	IgG	−	+	−	+	−	−	−
M	55	IgG/IgM	−	+	−	−	−	−	−
M	42	IgG	−	−	−	−	−	+	+
M	51	IgG	−	−	−	−	−	−	−
M	53	IgM	−	−	−	−	−	−	−
Knin									
M	60	IgG	+	+	+	−	+	+	+
M	25	IgM	−	−	−	−	+	−	−
M	62	IgG/IgM	−	−	−	−	−	−	−
M	52	IgG	+	+	−	−	−	−	−
M	46	IgM	−	−	−	−	−	−	−
F	56	IgM	−	+	+	−	−	−	−
Symptoms total:			**3**	**5**	**2**	**2**	**3**	**3**	**2**

Bold values represent the sum of the symptoms.

Overall, results on the company population level could be attributed to the early implementation of company protective measures combined with strict national measures, put in place to mitigate the COVID-19 spread. So, even though the analyzed population could be more exposed than the general Croatian population due to the greater interpersonal contact outside their homes (as they continued to work during the lockdown), low incidence rates imply that they probably were not considerably more affected than the general population. It can be indicative despite the lack of directly comparable serological data in the same time frame. First, positive rates obtained by PCR testing during the same period were around 2%,[14] which is slightly higher but still within 95% CI obtained in this study. As PCR testing was mostly conducted for symptomatic individuals and/or priority groups (highly risk groups due to the occupation or special characteristics, elderly population with chronic diseases, etc),[27] this difference was expected.

Secondly, preliminary unofficial serological test results on the general population sample ($n = 1054$) reported by the director of the Croatian Institute of Public Health on July 13 revealed the 2.4% seropositive individuals (précised time frame of sampling not available).[28]

At the time when the study was completed, only a few serological study results have been reported globally, but due to different sampling strategies and population and time frame, they are not directly comparable to our study. For example, a study conducted in Santa Clara County ($N = 1,928,000$), California, USA,[21] which aimed to target the general population, found antibodies in 1.5% (95% CI 1.11 to 1.97%) of the population sample. A study conducted on a general population sample of the island of Jersey, UK ($N = 106,800$), included a total of 855 participants from 438 households. The results showed that the seroprevalence of SARS-CoV-2 antibodies is 3.1% (95% CI, 1.8 to 4.4%).[25] One of the studies, conducted in Gangelt county ($N = 12,529$), which

was a viral hotspot in Germany, comprised a sample of 1000 participants from 400 households. The results stated that 15% of the population developed antibodies to the virus.[22] One study was conducted in Oise in France that targeted participants from virus affected high school (pupils, their family members, and school staff). In the mentioned study, the seroprevalence of antibodies was 25.9% (95% CI 22.6 to 29.4).[23]

Although not directly comparable, due to the application of SARS-CoV-2 sequencing instead of serological testing, a study conducted on the Icelandic population sample provided comprehensive results. It was conducted on approximately 6% of the total Icelandic population and showed different results depending on the sampling patterns.[29] For the targeted population with symptoms or high risk of infection, this study found 13.3% positive cases, while open invitation and random sample provided 0.8% (95% CI 0.6 to 1) and 0.6% (95% CI 0.3 to 0.9) of positive cases, respectively. Therefore, due to the presented differences, it is a priority to include not only different general populations but also different target populations and reveal how efficiently epidemic situation was controlled in different working environments.

The major limitation of the study was the characteristics of serological immunoassay tests, which are currently not sufficiently explored and validated.[19] In our case, the most pronounced drawback of the test reflected in the fact that RT-PCR confirmed two of six IgM positive cases (33%), which was not reported in previous serological studies.[21–23] However, due to the generally low incidence of the disease, this result was not unexpected. Specifically, in such cases, it is possible that a considerable amount of individuals tested positive would not truly be positive. However, it does not imply that people who tested negative truly have antibodies. On the contrary, considering the provided test performance indicators, it is very unlikely for an individual tested negative to be truly positive.[30] This makes such rapid tests an effective tool to prevent the spread of the disease within the working environment.

To further explain possible test inconsistencies, other factors should also be considered. Firstly, most of the tests are still not validated, and, as in our case, only manufacturer data about test performance is available. Along with test performance indicators, test manufacturer also stressed that samples with higher heterophile antibodies or rheumatoid factor could affect the test result.[26] Secondly, WHO stated that serological tests, in general, could be susceptible to cross-reaction with other frequent infections, like human coronaviruses causing common cold.[31] Nonetheless, despite their limitations, these tests can still be a valuable qualitative research tool.[19] Due to their low probability rates of positive cases that tested negative,[30] they could provide credible information about the proportion of the population that was not exposed to the virus.[19]

The present study examined the seropositivity of workers, as well as the related factors, such as contacts, travels, and symptoms. Unfortunately, due to the nature of the study and the great number of testes individuals in the narrow time frame, we could not follow the patients after receiving results and more precisely determine symptoms present at the time of the infection as well as examine the impact of potential comorbidities. These issues were under the control of county epidemiological services. Still, due to the low prevalence of the disease, more comprehensive consideration of these factors would be more appropriate for large-scale general or disease-specific target population studies.

In conclusion, this study showed that restrictions that the DIV group implemented, along with national restrictive measures, had an enormous impact on the suppression of virus spreading. Therefore, our study could contribute to the understanding of the COVID-19 risk in the working and industrial environment and the efficiency of preventive measures. We hope that further studies will additionally explore preventive measures in the industrial context, taking into account that due to the negative economic impact, a total lockdown is no more an option in the "new normality" circumstances.

Interestingly, the workers that did not leave the facility during the pandemic did not exhibit any case of the infection. It could be an additional proof that the quarantine is extremely effective, in the same manner as it was introduced in 1377 in Dubrovnik, as the first documented case of quarantine in the history.[32]

ACKNOWLEDGMENTS

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