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IMMUNOGLOBULIN-G SEROPREVALENCE AGAINST SARS-COV-2 AND ASSOCIATED RISK FACTORS IN LAHORE, PAKISTAN; A POPULATION-BASED SURVEY

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ABSTRACT
SARS-CoV-2, a novel beta corona virus, is considered responsible for recent COVID-19 pandemic. Due to high proportions of asymptomatic cases, it is difficult to determine the full scope of virus circulation or its true infection ratio. To get beyond these limitations and understand infection rates at the population level, serology testing is a crucial component. A cross-sectional investigation was carried out. Individuals from various towns were identified for recruitment into the study, which were not diagnosed previously as COVID-19 positive by Polymerase Chain reaction and not vaccinated yet. 384 individuals with >18 years of age were screened for SARS-CoV-2 specific Immunoglobulin G antibodies. A total of 384 people were tested for SARS-COV-2 IgG, and 178 of them tested positive, representing an overall prevalence of 46%. Prevalence detected was 32.4% in 18-30 years age group, 50.8% among 31-50 year and 44.4% in 51-70 years age group. Among genders, Prevalence detected was 47% among male and 45.3% from females. Chi square and binary logistic regression was applied to see the significance and association of the study variables. The 33 participants contacted with positive family member and 22 of them tested positive for IgG, with the odds of having neutralizing antibodies (OR 3.390, 95% CI 1.365-8.418). In addition to SARS-CoV-2 infection, the population's antibody status was associated with protection. The seroprevalence statistics show that Lahore's population lacks herd immunity. If a new strain enters the community, the likelihood of a serious outbreak in the future increases.

Key words: SARS-CoV-2, COVID-19, Seroprevalence, Antibodies, asymptomatic carriers

Introduction
An outbreak of SARS-CoV-2 (the pathogen responsible for COVID-19), started in December 2019 in Wuhan, Hubei Province, China, and spread quickly to other parts of the world. Through its continuous epidemic, the novel virus (SARS-CoV-2) poses major hazards to the entire world (1). The

virus identified in Wuhan City, China, since 12 December, 2019 and was originally named as 2019-nCoV. The virus was officially designated to be associated with Corona virus disease "COVID-19" by the World Health Organization (WHO) on February 11th, 2020 (2). The majority of the time, asymptomatic and symptomatic persons or even pre symptomatic individuals could transmit the infection by droplets. Myalgia, dry cough, dyspnea, fever, and diarrhea are only a few of the clinical symptoms brought on by COVID-19. Most SARS-CoV-2 cases are asymptomatic or have only mild, self-limiting illnesses. On the other hand, serious disease can develop in 10% of cases. In 2020, Wang et al reported the most reliable method for determining SARS-CoV-2 infection is reverse transcription polymerase chain reaction (3). While it does not provide information regarding immunity against the COVID-19, which is required to determine the degree of herd immunity in the community, it may result in errors for the diagnosis of a number of pre-symptomatic and symptomless cases during the latency phase of infection (4, 5).

Initially prevalence is just assumed in the population with a novel coronavirus, to be insignificant due to the virus existence novel in origin. Serological surveillance of antibody detection in a population can therefore provide information on inferences and the degree of infection that roughly corresponds to the cumulative incidence of disease in the population (6). The asymptomatic population may be a source of SARS-CoV-2 infection that spreads quickly throughout the incubation phase and increases infectivity (7, 8). In order to determine the loads of illness in a community and to determine the population's herd immunity during a pandemic, serology testing may be a crucial tool. It also provides information on risk factors for disease, such as age, gender, geography, and overall health of the population. (2, 9)

When a SARS-CoV-2 enters and infects a person, the body produces Immunoglobulin M (IgM) and Immunoglobulin G (IgG), which are qualitatively detected by serologic testing. The body produces IgM as the initial antibody in response to a COVID-19 infection within two weeks. After two weeks, the level of IgM started to fall and IgG took over as the primary antibody to fight infection. In Pakistan, extensive studies of seroprevalence within communities have not yet been carried out. It's crucial to comprehend the dynamics of the current COVID-19 infection serology investigation in order to accurately depict the concept of herd immunity. The primary outcome of this study was to estimate the prevalence of SARS-CoV-2 antibodies and possible factors associated with in a population.

Methods

Study Setting, Design and Sample size

A population based cross-sectional study was conducted to estimate sero-prevalence in district Lahore, which is the capital city of Punjab province with the highest population. The targeted population was the general population from different towns of Lahore (fig.1). The survey was conducted conveniently by arranging camps in different towns of city, due to some logistic problem and lock down situation during COVID-19 pandemic, probability sampling was not feasible, hence convenient sampling method was adopted and volunteers were enrolled to participate in study, who consented to donate blood sample and data for SARS-COV-2 antibody testing(fig.2). The 384 participants from the different towns were identified for recruitment into the survey, irrespective of prior COVID-19 infection (who are not diagnosed as COVID -19 by PCR and not vaccinated yet).

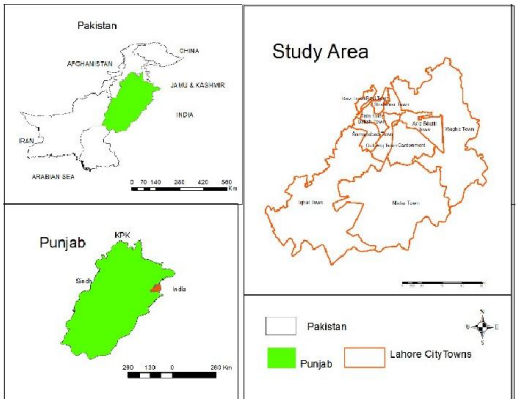

Fig. 1 Map of the Study Area

Sampling, Testing and Data Collection:

Each participant provided a 3 ml sample of venous blood from those who were older than 18. Data was gathered with the use of a predesigned questionnaire that contained close-ended questions related to different characteristics (demographic, socioeconomic, clinical health state of the persons, exposure information and sops regarding COVID-19). Each sample was taken by a certified phlebotomist, who also noted the time and date of sample collection and carried it on dry ice to UVAS's BSL-2 Lab. The blood was centrifuged to separate the serum, which was then stored at -20C.

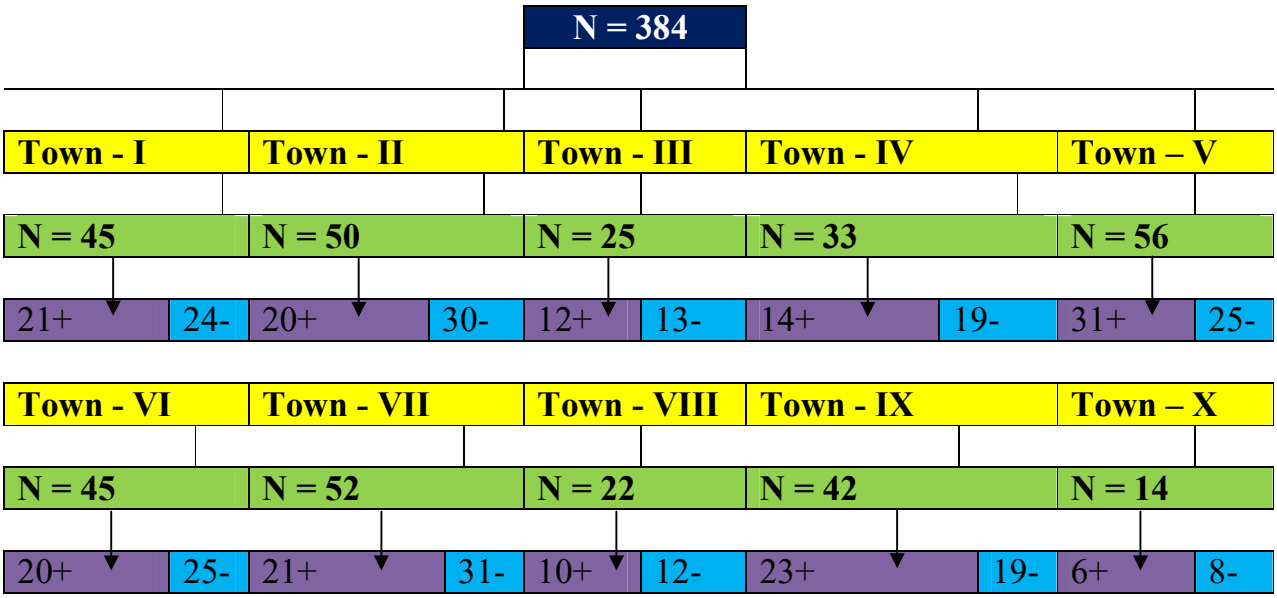

Fig. 2 Flow chart showing positivity and negativity of participants from each town

Serological Testing

Enzyme linked immune sorbent assay (ELISA) test was used for the anti-SARS-CoV-2 antibodies, which was conducted in a bio safety level 2 facility (BSL-2). Indirect semi-quantitative ELISA technique was used to find IgG antibodies against the SARS-CoV-2 nucleocapsid in human serum, the ID Vet kit used for test.

Statistical Analysis

Statistical analyses were performed with R statistical language. Descriptive analysis for categorical variables was presented as frequency and percentages. The percentage of people with a positive test result for antibodies among all positive cases is known as the seroprevalence. Chi square and binary logistic regression applied to see the significance and association of the study variables.

Results

The final results included 384 eligible subjects who participated over the study period. The participants were divided into four subgroups based on their age: 18.3% were between the age of 18-30; 63.5% were between the age of 31-50; 16.4% were between the age of 51-70, and 0.8% were over the age of 70. Male participants made up (57.6% of the total) while female participants made up (42.4%).

Seroprevalence of SARS-COV-2 IgG antibodies by characteristics of participants

Table 1 showing the seroprevalence of SARS-COV-2 IgG antibodies tested and the results based on participant characteristics. The 24 participants had positive SARSCoV-2 IgG tests, this corresponded to a prevalence of 32.4% in the 18–30 age group, a prevalence of 50.8% in the 31–50 age group; a prevalence of 44.4% in the 51–70 age group; and a prevalence of 66.6% in the >70 age group. Male tested positive 104 and 47% prevalence detected, while females group reported 45.3%. When it comes to education, 40 participants did not receive any education and 22 (5.7 %) of them tested IgG positive with 55% prevalence, 115 participants were matriculate and 54 (30.3 %) of them tested positive with 46.9% prevalence, and 78 participants attended higher secondary school and 37 (20.8%) of them tested positive and 47.4% prevalence detected, 66 participants received a bachelor's degree and 28(15.7%) of them tested positive with a 42.4 % prevalence, 53 participants received a master's degree and 26(14.6%) of them tested positive with a 49% prevalence, 32 were postgraduates and 11(6.2%) tested positive with a 34.3% prevalence. When the occupation of participants was observed with sero-positivity, 12 participants had no occupation and 7(3.9%) of them tested positive with 58.3% percent prevalence, 70 female participants were housewives and 29(16.3%) of them tested positive with 41.4% prevalence, 144 contributors were low skilled workers and 72 (40.4%) of them were positive with 50% prevalence, highly skilled workers were 38 and 14(7.9%) of them tested positive with 36.8% prevalence. 58 participants were academic staff and 28(15.7%) were tested positive with a prevalence of 48.2%, 36 participants were students and 16(9%) of them tested positive with a prevalence of 44.4%. The number of 41(23%) participants with comorbidity tested positive with a prevalence of 45.5%, and 137(77%) participants with no comorbidity tested positive with a prevalence of 46.5%. 76 participants were smokers and 35 (19.7%) tested positive with 46% prevalence; 308 participants were nonsmokers and 143 (80.3%) of them tested positive. Participants who washed their hands during pandemic were 229, and 102(57.3%) of them tested positive with 44.5% prevalence. Participants who did not wash their hands were 76(42.7%), with 49% prevalence. Change of clothes was reported in 194 people, with 87(48.9%) of them testing positive for SARS-COV-2 IgG antibodies; a prevalence of 44.8%. Use of sanitizer reported 101 participants and 41 (23%) of them were tested positive with 90% prevalence. Wearing of mask reported 325 participants and 148 (83.1%) of them were positive with 45.5% prevalence. The participants visited to a hospital during outbreak were 71 and 36 (20.2%) of them tested positive with 50.7% prevalence. 229 participants visited to a crowded area and 111 (62.4%) of them tested positive with 48.4% prevalence. The 50 contributors of study contact with suspected case of COVID-19 and 25 (14%) of them tested positive with 50% prevalence. 8 participants contact with visitor came from abroad and 2 (1.1%) of them tested positive with 25% prevalence. The 33 participant's family members tested positive for COVID-19 and 20 (11.2%) participants catch infection but remained asymptomatic with 60.6% prevalence. The participants reported previously flue like symptoms during interview were 63 and 30 (16.9%) of them tested positive antibody test and 47.6% prevalence detected (Table, 1).

Table 1: Seroprevalence of SARS-COV-2 IgG antibodies by characteristics of participants

Variables	Categories	Number of Participants = 384	IgG positive participants = 178	Seroprevelance 95% confidence Interval
Age	18-30	74 (19.3%)	24 (13.5%)	32.4% (22.0-44.3)

	31-50	244 (63.5%)	124 (69.7%)	50.8 % (44.3-57.2)
	51-70	63 (16.4%)	28 (15.7%)	44.4% (31.9-57.5)
	>70	3 (0.8%)	2 (1.1%)	66.6% (9.4-99.1)
Gender	Male	221 (57.6%)	104 (58.4%)	47% (40.3-53.8)
	Female	163 (42.4%)	74 (41.6%)	45.3% (37.5-53.3)
Education	Illiterate	40 (10.4%)	22 (12.4%)	55% (38.4-70.7)
	Matric	115 (29.9%)	54 (30.3%)	46.9% (37.5-56.8)
	Higher secondary school	78 (20.3%)	37 (20.8%)	47.4% (36.0-59.0)
	Bachelor	66 (17.2%)	28 (15.7%)	42.4% (30.3-55.2)
	Masters	53 (13.8%)	26 (14.6%)	49.0% (35.0-7.3)
	Post Graduate	32 (8.3%)	11 (6.2%)	34.3% (18.5-53.2)
Occupation	None	12 (3.1%)	7 (3.9%)	58.3% (27.6-84.8)
	House wife	70 (18.2%)	29 (16.3%)	41.4% (29.7-53.8)
	Low skilled worker	144 (37.5%)	72 (40.4%)	50% (41.5-58.4)
	Highly skilled worker	38 (9.9%)	14 (7.9%)	36.8% (21.8-54.0)
	Health care staff	26 (6.8%)	12 (6.7%)	46.1% (26.5-66.6)
	Academic staff	58 (15.1%)	28 (15.7%)	48.2% (34.9-61.7)
	Student	36 (9.4%)	16 (9%)	44.4% (27.9-61.9)
Comorbidity	Yes	90 (23.4%)	41 (23.0%)	45.5% (35.0-56.3)
	No	294 (76.6%)	137 (77.0%)	46.5% (35.1-36.5)
Smoker	Yes	76 (19.8%)	35 (19.7%)	46.0% (34.5-57.8)
	No	308 (80.2%)	143 (80.3%)	46.4 % (40.7-52.1)
Hands wash	Yes	229 (59.6%)	102 (57.3%)	44.5% (37.9-51.2)
	No	155 (40.4%)	76 (42.7%)	49.0% (40.9-57.1)

Change cloth	Yes	194 (50.5%)	87 (48.9%)	44.8% (37.7-52.1)
	No	190 (49.5%)	91 (51.1%)	47.8% (40.6-55.2)
Use of sanitizer	Yes	101 (26.3%)	41 (23.0%)	90.0% (82.5-95.1)
	No	283 (73.7%)	137 (77.0%)	48.4% (42.4-54.3)
Wearing of mask	Yes	325 (84.6%)	148 (83.1%)	45.5 % (40.0-51.1)
	No	59 (15.4%)	30 (16.9%)	50.8% (37.4-64.1)
Visit to a hospital	Yes	71 (18.5%)	36 (20.2%)	50.7% (38.5-62.7)
	No	313 (81.5%)	142 (79.8%)	45.3% (39.7-51.0)
Visit a crowd area	Yes	229 (59.6%)	111 (62.4%)	48.4% (41.8-55.1)
	No	155 (40.4%)	67 (37.6%)	43.2% (35.3-51.4)
Contact with suspected or confirmed	Yes	50 (13.0%)	25 (14.0%)	50% (35.5-64.4)
	No	334 (87.0%)	153 (86.0%)	45.8% (40.3-51.3)
Contact with visitor came from abroad	Yes	8 (2.1%)	2 (1.1%)	25% (3.1-65.8)
	No	376 (97.9%)	176 (98.9%)	46.8% (41.6-51.9)
Family member tested for covid-19	Yes	33 (8.6%)	20 (11.2%)	60.6% (42.1-77.0)
	No	351 (91.4%)	158 (88.8%)	45.0% (39.7-50.3)
Flu like symptoms	Yes	63 (16.4%)	30 (16.9%)	47.6% (34.8-60.5)
	No	321 (83.6%)	148 (83.1%)	46.1% (40.5-51.7)
Tested for covid-19	Yes	5 (1.3%)	1 (0.6%)	20% (0.5-71.6)
	No	379 (98.7%)	177 (99.4%)	46.7% (41.5-51.8)
Vaccinated	Yes	0 (.0%)	0 (.0%)	0%
	No	384 (100%)	178 (100%)	46.3% (46.1-47.3)

Factors associated with the presence of neutralizing antibodies.

Analysis of factors associated with neutralizing antibodies of participants who were IgG positive by ELISA showed in (fig.3). Higher odds of seropositivity were observed for age of participants (OR 1.521, 95% CI 1.008-2.296). As 1 year of age increases the risk of COVID-19 increases 1.5 times. The 33 participants contacted with positive family member and 22 of them tested positive for IgG,

with the odds of having neutralizing antibodies (OR 3.390, 95% CI 1.365-8.418). Apart from age of participants and contact with positive family member, other factors including gender, occupation, comorbidity, alcohol, smoking, visit to a hospital, visit to crowd place, contact with suspected or confirmed and any flue like symptoms showed during pandemic were not associated with the presence of neutralizing antibodies. The odds ratio was less than 1 for adopting SOPs e.g hand washing, use of mask, use of sanitizer and clothes changing daily during pandemic was found as a protected factor.

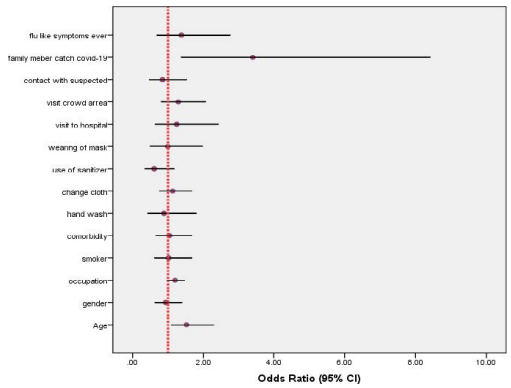

Fig. 3 Factors associated with the presence of neutralizing antibodies

Discussion

This study aims to provide seroprevalence among general population of Lahore at peak of COVID-19 pandemic and lock down situation in August 2021. Serum antibodies against severe acute respiratory syndrome coronavirus 2 (SARS- COV-2) indicate past infection. A total of 384 people tested for SARS-COV-2 IgG and 178 of them resulted positive with 46% prevalence overall. The magnitudes of contributors who were positive on antibody test for IgG had stable concentrations of neutralizing antibodies even those individuals' remains asymptomatic. The seroprevalence evaluations are 45- 47 times greater, respectively established on confirmation by a positive ELISA result. Results of the current study reported that a large number of persons who had been exposed to infection and remained undetected due to subclinical infection. The findings of study showed that vast exposure of the SARS- COV-2 to people accounts for the higher rates of COVID-19. The seroprevalence results matched with other studies conducted in Pakistan, a study reported from Karachi blood bank an estimate of 36% prevalence that included health care staff positivity 13% and industrial labor 50%(10). Initially reports of seroprevalence from world-wide in large populated regions showed high prevalence. A seroprevalence survey conducted in Iran at peak of COVID-19 pandemic with 33% prevalence(11). In our study high seroprevalence (50.8%) of SARS- COV-2 IgG was detected in 31-50 years of age and (44.4%) in 51-70 years of age group. Though, the young population indicated a high prevalence than older age groups reported, signifying more contact to the infection but disease susceptibility vary from asymptomatic to symptomatic in young population. The findings showing that young people exposed to SARS- COV-2 and remain asymptomatic, it may be this group of population had more exposure in community, educational institute, offices and public places. The reason for asymptomatic may be due to higher level of immunity in this age group. A seroprevalence study conducted in Indonesia the maximum prevalence reported (14.8%) between age groups 40–49 years (12). Antibody response among gender was different; male showed 47% seroprevalence that was comparatively higher than females that was 45.3%. Our findings matched with other studies reported from different countries. China reported the infection among females was less due to COVID-19 as compared to male gender. Previous studies also reported that male were more susceptible to infection than females from SARS- COV & MERS- COV that were conducted in mice(13, 14). Comorbidity status of study participants showed 45.5% prevalence. It has been well known that certain comorbidities commonly exist in any individual in every population. For example,

diabetes as well as COPD often observed with hypertension and coronary heart diseases. So, people with existing diseases are expected more to catch new infection. Significantly, we have found that people with comorbidities are more at risk of COVID-19(15). The participant of study belongs from different occupations as low skilled worker reported 18.7% prevalence, 3.6% high skilled worker, 3.1% among health care staff, 7.2% prevalence in academic staff and 4.1% prevalence among students. The finding of this study suggests that working people of study population went outside from home are more prone to infection due to maximum exposure with asymptomatic carrier or may be not taking precautionary measures seriously at public places. Especially, health care staffs are more at risk because they have to work with suspected or confirmed patients of COVID-19. As many studies conducted around the world from health care personnel's are more at risk of risk of COVID-19. Our results matched with study conducted from health care personnel at Shaukat Khanum Memorial Cancer Hospital and Research Center, Lahore, Pakistan (16). The occupation related seroprevalence estimated from general population study in Iran that explained a low acceptance of the general population with hand washing, wearing of mask, social distancing in high risk environments such as hospitals, public offices and teaching institutions (17). WHO also indicated occupational risk for COVID-19 as well as other studies shown results for occupational risk at work place due to frequent social interactions that is possibility of asymptomatic spread (6, 18). The odds ratio was less than 1 for adopting SOPs e.g hand washing, use of mask, use of sanitizer and clothes changing daily during pandemic was found as a protected factor. The study findings matched with a KAP survey conducted in the Germany, as concerns of preventive measures, the finding of the survey establish that 95% of the participants assumed that preventive measures are important to control the pandemic. The respondents considered the government directed safety measures as primarily appropriate and applicable (19). The reported results from other studies confirm that close contact of people with COVID-19; particularly those members lived in the same house might increases the viral transmission. Proper isolation and quarantine from other family members can be mostly challenging and not possible in metropolitan areas and not as much of comfortable scenarios. Whereas quarantine for the duration of the lockdown would decrease the number of possibly infected contacts (20).

Conclusion

The overall seroprevalence of SARS CoV-2 antibodies in Lahore population was reasonably high in this study as of May 2021) but still lowest on the base of herd immunity. The seroprevalence varied in the different variables of participant by age and gender. Younger people were more at risk to be seropositive than other age groups. The family members of positive individuals were more expected to catch COVID-19. Mostly, respondents wear the masks outside, maintain the required physical distance whenever possible and wash hands properly. The results of study supported to evaluate the relation of asymptomatic infections in different age groups, gender, occupation and other characteristics of the pandemic. The study made significant findings that can provide better understanding of COVID-19 asymptomatic cases, which may possibly be beneficial for the response to a upcoming epidemic waves.

Limitations

A random sampling for participant's selection from population was not possible due to lockdown. Serum samples were analyzed from participants who willingly consent about to be tested as a volunteers. So, the clinical characteristics of the asymptomatic cases might differ from the infected population. We tried to reduce the bias by providing age, gender and occupation based standardized estimates, which may helpful to validate the bias. This study only identified IgG antibodies; the magnitude of the immunity is unknown at this time.

Declarations

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Conflict of Interest: The authors have no competing interests to declare.

Ethical Approval: The ethical committee approved letter to the researchers for research purpose. This study was performed in line with the principles of the declaration of Helsinki. Approval was granted by the Ethics Committee of University of Veterinary and Animal Sciences (Date 5-5-2020 / No088-IRC/BMR).

Consent to participate: Informed consent was obtained from all individual participants included in the study.

Consent for publication: All authors read and approved the final manuscript for submission and publication.

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