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Posted Date: 26 October 2023

doi: 10.20944/preprints202310.1684.v1

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Article

High exposure to SARS-CoV-2 among pregnant women after the first and second waves of COVID-19 in Senegal

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Abstract: This study aims to determine the seroprevalence of SARS-CoV-2 in pregnant women attending antenatal consultations (ANC) in Senegal. A non-redundant consecutive recruitment of participants was carried out between, March and July 2021 during the Delta wave of COVID-19. The plasma samples biobanked at -80°C were subjected to an enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of IgG and IgM antibodies of SARS-CoV-2 using the WANTAI SARS-CoV-2 Ab ELISA (Wantai Biological Pharmacy Enterprise, Beijing). To assess factors associated with seropositivity, bivariate analysis was performed with JMP® Pro Version 15.0.0 software (SAS Institute Inc., Cary, NC, 1989–2021). For frequencies below 5%, chi2 or Fischer tests were performed. In all cases, significant statistical threshold was set at *p-values* considered to be less than 0.05. A total of 1,227 pregnant women were recruited in five health facilities, including two in Dakar (n=166) and one in each of the following regional capitals: Saint-Louis (n=400), Ziguinchor (n=264) and Kédougou (n=397). The median age among was 25 years [18 - 50]. The overall seroprevalence observed was 64.9% (797/1227), with higher exposure rates in Dakar with 71.6% (119/166) and Ziguinchor with 68.1% (180/264). Kédougou and Saint-Louis reported 62.9% (250/397) and 62% (248/400), respectively. According to age group, the seroprevalence was 62.6% (331/528), 66.6% (238/357), 63.5% (148/223) and 67.2% (80/119) respectively for [18-23], [24-29], [30-35] and ≥36 years old. This variability in the seroprevalence of SARS-CoV-

2 according to the localities was not statistically significant (p=0.7024). We found no association between factors such as recruitment period, age, level of education, marital and professional status.

Keywords: SARS-CoV-2; Seroprevalence; Pregnancy and COVID-19; Senegal

1. Introduction

Since the official declaration of Coronavirus disease 19 (COVID-19) as a pandemic by the World Health Organization (WHO) on 11 March 2020 [1], several studies agree that the prevalence of SARS-CoV-2 infection, its contagiousness and COVID-19 death related are largely underestimated, thus facilitating its dissemination within communities [2–8]. In Senegal, the first case was registered on 2 March 2020 [9]. Since then, several strategies for combating and preventing COVID-19 have been implemented. These include the Integrated Disease Surveillance and Response (IDSR) system [10,11], the Community Event-Based Surveillance (CEBS) system [12], the activation of Health Emergency Operation Center [13], the formal identification of COVID-19 cases as defined by WHO including their active case-contact detection [14] in order to identify transmission clusters [15]. During the first wave of COVID-19 (March - November 2020), all confirmed COVID-19 cases were isolated at the Treatment Center of Epidemics (TCE), open for this purpose. The second and third waves of COVID-19 in Senegal covered the periods from December 2020 - March 2021 and July - September 2021 respectively [16]. Except for patients with the most severe form or with high-risk conditions for severe COVID-19 requiring hospitalization, individuals who tested positive with minor or no symptoms, both diagnosed via contact tracing or screening campaign at the Blaise Diagne International Airport and other border crossings in the country, were quarantined with regular follow-up at the out of hospital TCE [7]. In addition to non-pharmaceutical interventions (population sensitization on prevention measures, bans on large public gatherings, mandatory wearing of face mask, closing schools and universities, lockdown restrictions...) [17], the vaccination was introduced on February 23th, 2021 and targeted the population considered at risk i.e. health personnel, the elderly and those living with comorbidities. Gradually, it was extended to the general population, however for pregnant women it was only from August 9th, 2021. Vaccination coverage at the time of the second national COVID-19 seroprevalence survey (November 2-14, 2021) was already low by 18% [18].

In response to the COVID-19 pandemic in Senegal, the first laboratory strategy was to test all suspected cases of symptomatic COVID-19. All contacts of confirmed cases were also systematically tested. However, this strategy was changed due to its substantial financial cost and human resources need. A second strategy put in place tested only contacts with COVID-19 symptoms or high-risk of death contacts (i.e., contacts of advanced age and/or with comorbidities such as chronic diseases) [15]. To overcome the constraints related to molecular tests, the lab-based detection methods for SARS-CoV-2 were later expanded to rapid diagnostic antigen tests for decentralized screening while initially exclusively done by the reverse transcription polymerase chain reaction (RT-PCR) or quantitative RT-PCR (qRT-PCR) as a gold standard [19,20]. The serological methods could contribute substantially in monitoring the epidemic dynamics in target population and help control of the rapid global spread of SARS-CoV-2 [20,21].

Despite the necessity of their prenatal consultations, pregnant women have in the majority deserted health facilities for fear of acquiring SARS-CoV-2 infection. The conjunction of these different phenomena, particularly during the critical windows of the second and third waves of COVID-19 in Senegal [16], that could put pregnant women more in a risky situation. The COVID-19 pandemic has so far raised questions about the impact of SARS-CoV-2 infection in pregnant women, particularly on the outcome of pregnancy, the health of the child-mother couple during and after pregnancy [8,22–27]. Among these concerns, the high risk of mortality in pregnant women infected with SARS-CoV-2 compared with age-matched nonpregnant individuals [3,8,23–25]. In addition, the risks of preterm deliveries, complications requiring intensive care and invasive ventilation should also be taken into account [22,28–30]. Unfortunately, despite these concerns related to COVID-19 in

the obstetrical population, there is limited published seroprevalence of SARS-CoV-2 data among pregnant women in Africa [3,6,8,25]. Hence the need to study the extent of exposure to SARS-CoV-2 infection in pregnant women.

Given the health emergency, several initiatives focused on the management of SARS-CoV-2 infection emerged from the first months of the pandemic. On the laboratory component, it was diagnostic strategies that have been pursued, focusing on molecular strategies for viral genetic material, antigen tests, and serological assays, and innovations for improving the diagnostic sensitivity and capabilities [19,20]. In the absence of universal access to RT-PCR or qRT-PCR, and even if it is not recommended to establish the diagnosis of the COVID-19, the serological detection of present and post-exposure constitutes a major alternative in terms of public health strategies. This study aimed to determine the seroprevalence of SARS-CoV-2 infection in pregnant women attending antenatal consultation (ANC) to SARS-CoV-2 in Senegal.

2. Materials and Methods

2.1. Patients and study design

This is an ancillary study of the EPIVHE (Environment and Epidemiology of the hepatitis E virus) project, a seroepidemiological survey of hepatitis E, conducted among pregnant women attending antenatal consultation (ANC) in Senegal. A non-redundant consecutive recruitment of participants was carried out over a period from March to July 2021. The sampling plan, description of the participants' recruitment sites, data collection procedure, inclusion and non-inclusion criteria, socio-demographic and other relevant information to the study were fully described in Diouara et *al.* [31]. During the sampling period, no participant presented clinical signs suggestive of a severe form of COVID-19 and no cases of hospitalization had been reported. None of participant had received a COVID-19 vaccine. Ethical and administrative authorization was obtained from the National Health Research Ethics Committee (N°000130/MSA/CNRES/Sec) and the Ministry of Public Health and Social Action of Senegal (N°00000582/MSAS/DPRS/DR).

2.2. Sample preparation and anti-SARS-CoV-2 antibodies detection

The plasma samples stored at -80°C were subjected to a qualitative assay based on the immunoenzymatic method. The test used was the WANTAI SARS-CoV-2 Ab ELISA (Wantai Biological Pharmacy Enterprise, Beijing) and in accordance with the manufacturer's instructions. It should be noted that this test allows the qualitative detection of total antibodies (including IgM and IgG), markers of the immune response to SARS-CoV-2 in patient suspected of previous infection, or for the detection of seroconversion in patients following know recent infection. The WANTAI SARS-CoV-2 Ab ELISA is a two-step incubation antigen "sandwich" enzyme immunoassay kit, which uses polystyrene microwell strips pre-coated with recombinant SARS-CoV-2 spike protein receptor-binding domain (S1/RBD) antigen.

The reported sensitivity and specificity of the test are 94.36% and 100% respectively according to the manufactured [32]. Optical density was read using the MICRO READ 1000 ELISA Plate Analyser (Global Diagnostics B, Belgium). The results are calculated by relating each specimen absorbance (A) value to the plate's cut-off value (C.O.). For the calculation of the cut-off value (C.O.), it was C. O= Nc + 0.16. Nc= the mean of absorbance value for three negative controls. A ratio of signal to Cut-off values was calculated for each sample and a sample was declared to be negative if index < 1, positive if the index was \geq 1, and borderline if the index was range 0.9 to 1.1. All samples declared positive in the first tests were re-tested by WANTAI SARS-CoV-2 Ab in accordance with the manufacturer's instructions.

2.3. Statistical analysis

The SARS-CoV-2 seroprevalence was calculated as the number of individuals with specific SARS-CoV-2 antibodies divided by the total number of individuals in the sample and the confidence level was set at 0.95. The selected general factors examined were, participant's age, residential

locality, recruitment period, level of education, marital and professional status. These were selected due to their potential association to SARS-CoV-2 infection as observed in other studies [3,22–26,28– 30,33]. To assess factors associated with seropositivity, bivariate analyzes were performed with JMP® Pro Version 15.0.0 software (SAS Institute Inc., Cary, NC, 1989-2021). For frequencies below 5, chi2 or Fischer tests were performed. In all cases, the confidence interval was set at 0.95 and the *p-values* < 0.05 were considered significant.

3. Results

3.1. Demographic and socio-economical characteristics

During the EPIVHE seroepidemiological survey [31], we recruited 1227 pregnant women in five health facilities including two in Dakar (gynecological clinic of CHUN Aristide le DANTEC (n=50) and the Gaspard Kamara Health Center (n=116)). The other sites were the Regional Hospital Center (RHC) of Saint-Louis, the Health District of Kédougou and the Health Center of Néma de Ziguinchor, with respectively 400, 397 and 264 participants (Figure 1). The distribution by age group was 43%, 29.1% and 18.2%, respectively for [18-23], [24-29] and [30-35 years old]. Only 9.7% of participant were aged 36 years or over; among them, 3.6% (n=45) were over 40 years old. The median age was 25 years [18 - 50 years].

Regarding the level of education, only 9% of participants had reached a higher level of education. The overall uneducated rate was 31.7% with higher proportion recorded in Kédougou (58.7%). Contrary to other localities, 25.9% of women in Dakar had completed higher education. Only 18.7% of participants had regular income (salaried or self-employed). Among them, 45.8% were residents in Dakar, while 11.8% in Saint-Louis. In addition to the aforementioned socio-demographic and economic characteristics by locality, the marital status and the results of the survey relating to hand hygiene, in particular systematic hand washing, are also presented in Table 1.

3.2. SARS-CoV-2 seroprevalence, variability and potential associated factors

In the current study, the SARS-CoV-2 seroprevalence based on total anti-SARS-COV-2 Ab measurement was 64.9% (797/1227) with higher exposure rates in Dakar 71.6% (119/166) and Ziguinchor 68.1% (180/264). Kédougou and Saint-Louis recorded 62.9% (250/397) and 62% (248/400) respectively (Figure 1, Table 2). The highest seroprevalences were observed in Dakar, the capital, and in Ziguinchor. Although no statistically significant difference was observed according to age and localities, the highest seroprevalences (67.2%) were reported in the ≥36 years group and in Dakar (71,6%) with p-value egal to 0.9275 and 0.7024, respectively (Table 2). In addition, we found no association between factors such as age, level of education, marital and professional status (Table 3).

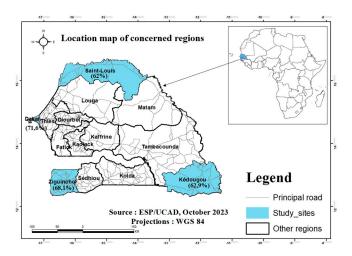


Figure 1. Map of Senegal, with indication of the geographical sites of the study and SARS-CoV-2 antibodies seroprevalence.

 Table 1. Demographic and socio-economical characteristics.

					Study site	s				
	Saint-Louis		Dakar		Kédougou		Ziguinchor		All sites	
Variable	(n = 400)		(n = 166)		(n = 397)		(n = 264)		(n = 1227)	
	Frequency (%)	Media n	Frequency (%)	Median	Frequency (%)	Median	Frequency (%)	Median	Frequency (%)	Medi an
Range of age		•			•	•		•		
18 - 23	112 (28)	20	49 (29.5)	21	256 (64.5)	19	111 (42)	20	528 (43)	20
24 - 29	136 (34)	26	44 (26.5)	27	90 (22.7)	26	87 (33)	26	357 (29.1)	26
30 - 35	101 (25.3)	32	48 (28.9)	32.5	29 (7.3)	30	45	32	223 (18.2)	32
36 and above	51 (12.8)	36	25 (15.1)	37	22 (5.5)	38	21	39	119 (9.7)	38
Educational level										
None	62 (15.5)		25 (15.1)		233 (58.7)		69 (26.1)		389 (31.7)	
Primary	170 (42.5)		46 (27.7)		82 (20.7)		77 (29.2)		375 (30.6)	
Secondary	122 (30.5)		52 (31.3)		75 (18.9)		104 (39.4)		353 (28.8)	
Higher	46 (11.5)		43 (25.9)		7 (1.8)		14 (5.3)		110	
Marital status										
unspecified	11 (2.8)		0 (0)		16 (4.0)		4 (1.5)		31 (2.5)	
Single	8 (2)		6 (3.6)		12 (3)		39 (14.8)		65 (5.2)	
Married	381 (95.3)		160 (96.4)		369 (93)		220 (83.3)		1130 (92)	
Divorced or widowed	0 (0)		0 (0)		0 (0)		1 (0.4)		1 (0.08)	
Regular income (paid work)										
unspecified	6 (1.5)		1 (0.6)		36 (9.1)		4 (1.5)		47 (3.8)	
Yes	47 (11.8)		76 (45.8)		63 (15.9)	•	43 (16.3)		229 (18.7)	
No	347 (86.8)		89 (53.6)		298 (75.1)		217 (82.2)		951 (77.5)	
Systematic hand washing										
unspecified	0 (0)		0 (0)		16 (4)		4 (1.5)	•	20 (1.6)	
Occasionally	0 (0)		0 (0)		0 (0)		6 (2.3)		6 (0.5)	
Yes	392 (98)		166 (100)		350 (88.2)		206 (78)		1114 (90.8)	

No	8 (2)		0 (0)	•	31 (7.8)	48 (18.2)	87 (7.1)	
SARS-CoV-2 se	eroprevalenc	e						
SARS-CoV-2 Ab Positive	248 (62)		119 (71.6)		250 (62.9)	180 (68.1)	797 (64.9)	

Table 2. Variability in SARS-CoV-2 seroprevalence according to age groups and localities.

	Anti-SARS-CoV-2 antibodies		
[Age groups] (%)	n (%)	<i>p</i> -value	
[18–23], <i>n</i> = 528 (43)	331 (62.6)		
[24–29], <i>n</i> = 357 (29.1)	238 (66.6)		
[30–35], <i>n</i> = 223 (18.2)	148 (63.5)	0.9275	
\geq 36 years, $n = 119 (9.7)$	80 (67.2)		
Total (<i>n</i> =1227)	797 (64.9)		
Location (Number of participant)			
Saint-Louis ($n = 400$)	248 (62)		
Dakar ($n = 166$)	119 (71.6)		
Ziguinchor ($n = 264$)	180 (68.1)	0.7024	
Kédougou (<i>n</i> = 397)	250 (62.9)		
Total (n = 1227)	797 (64.9)		

 Table 3. SARS-CoV-2 seroprevalence and potential associated factors.

		Anti-SARS-CoV-2 antibodies						
Educational level	Frequency (%)	n	Prevalence (%)	<i>p</i> -value				
None	389 (31.7)	247	63.4					
Primary	375 (30.6)	254	67.7	0.9175				
Secondary	353 (28.8)	229	64.8					
Higher	110 (8.9)	67	60.9					
Marital status								
unspecified	31 (2.5)	17	54.8					
Single	65 (5.2)	49	75.3	0.7564				
Married	1130 (92)	730	64.6	0.7564				
Divorced or widowed	1 (0.08)	1	100					
Regular income (paid work)								
unspecified	47 (3.8)	26	55.3					
Yes	229 (18.7)	154	67.2	0.7661				
No	951 (77.5)	617	64.8					
Systematic hand washing								
unspecified	20 (1.6)	13	65					
Occasionally	6 (0.5)	4	66.6	0.0040				
Yes	1114 (90.8)	726	65.1	0.9949				
No	87 (7.1)	54	62					

4. Discussion

This is the first large study conducted aiming to document the seroprevalence of antibodies to SARS-CoV-2 in pregnant women in Senegal. The retrospective analysis of sera collected during the

second and third waves of COVID-19 showed a high seroprevalence in pregnant women attending the ANC (64.9%) compared to those obtained during the first (28.4% in November - December 2020) [34] and second national population-based cross-sectional survey (72,2% in November 2021) [18]. In this study, seroprevalence observed among participants in Dakar was higher compared to other localities. Previous studies in Senegal reported this variability in SARS-CoV-2 seroprevalence [34–36]. This difference, even statistically not significant (*p-value* =0.7024), could be explained by the fact that Dakar, the capital city of Senegal, remains the epicenter of the epidemic [15]. It should be noted that Dakar is one of the West African capitals which is strongly connected to other regions of the world. The city has the highest population density and also concentrates most of the economic activities of the country [37]. Furthermore, our results show that the number of participants was low in Dakar (less than 2 times compared to Saint-Louis and Kédougou and 1.5 times for Ziguinchor). At recruitment period, the magnitude of the COVID-19 crisis in Dakar compared to other localities could be the cause of a low attendance antenatal care as it has been reported elsewhere [8,27].

Our findings showed that Ziguinchor was the second locality with the highest SARS-CoV-2 total Ab seroprevalence (68.1%). The seroprevalence founded in the bordering after the first 3 transmission waves were also high, among pregnant women in Gambia (90%), among adults involved in health care and health research (18%) [38], in unvaccinated people living with HIV (27.7%) [39] in Guinea-Bissau, and in general population in Guinea Conakry (42.4%) [40,41]. Ultimately, the high seroprevalence of SARS-CoV-2 total Ab observed in this unvaccinated subpopulation could also be explained by the circulation of the highly transmissible omicron and delta variants during the second and third waves of the COVID-19 epidemic in Senegal [42,43].

The heterogeneity of SARS-CoV-2 seroprevalences observed here is similar to that reported elsewhere. In their review, Lewis et *al.* report a strong heterogeneity both within countries – urban versus rural settings, children versus adults – and between countries and African subregion, that highlights the need for targeted serosurvey and interventions at population level [6]. In Senegal, a national population-based cross-sectional survey [34] and the others studies conducted in healthcare settings [35] and among hemodialysis patients [36] also report this variability in SARS-CoV-2 seroprevalence depending on the locality with high rates observed in Dakar and Ziguinchor in particular.

Contrary to what is usually or even systematically done in northern countries, where RT-PCR tests from nasopharyngeal samples are offered to pregnant women admitted for labour and delivery [20,33]; in Senegal access to molecular diagnostics was still limited. During the sampling period, the use of rapid diagnostic tests including ELISA tests was not authorized for the diagnosis of SARS-CoV-2 infection by local authorities. Only RT-PCR tests are mandatory and intended to establish active infection of SARS-CoV-2 in symptomatic and asymptomatic individuals [15]. Therefore, those with resolved infections or ongoing infections who are no longer testing positive by RT-PCR may be missing. This constitutes a limit for measuring the extent of exposure to SARS-CoV-2 during a pandemic period. In such situation and retrospectively, the use of serological tests based on the detection of antigen, IgM and IgG or IgG antibodies specific to SARS-CoV-2 [20] would make it possible to examine the extent of exposure to SARS-CoV-2. However, diagnostic performance would be influenced by the timing and duration of seroconversion including the IgM and IgG antibodies generation (which varies depending on the patient and disease severity) [20]. Some studies also reported rapid decay of SARS-CoV-2 antibodies over the time [44,45] while others suggest IgG stability for at least 5 months after infection [41]. In this present study, we used the Wantai SARS-CoV-2 Total Ab ELISA, for which satisfactory anti-SARS-CoV-2 antibodies detection performances from samples collected 14 days post-onset of symptoms in both hospitalized patients and those with mild or asymptomatic infections were reported [21,32,46,47]. Moreover, this test has been widely used in other studies conducted in Africa to measure seroprevalence [6,21,34,46,48,49]. Considering the variability of the persistence of anti-SARS-CoV-2 antibodies in serum mentioned above [20,41,44,45] and the possibility of premature sample collection for antibody detection after positive SARS-CoV-2 PCR [20,21,46], we cannot exclude an underestimation of the seroprevalences observed in this study.

Strengths and limitations

The major limitations of this study were absence of information on maternal and perinatal outcomes. Unlike several similar studies, our study does not provide information on gestational age, pre-pregnancy diabetes, pre-pregnancy hypertension, pregnancy outcomes (preterm birth, delivery with or without complications, maternal and neonatal mortality), vertical or horizontal transmission [3,22,24,28–30]. However, besides the heterogeneity of seroprevalences observed between the different localities; this study fills a relative gap in the level of exposure to SARS-CoV-2 of pregnant women, previously unknown at the national level. All of this information demonstrates the relevance and added value of the implementation of SARS-CoV-2 serological-based diagnostics routinely in addition to molecular tests.

5. Conclusions

This study highlighted a high seroprevalence of SARS-CoV-2 (64.9%) in pregnant women attending an antenatal consultation (ANC). These findings showed also that SARS-CoV2 has spread massively in Senegal, despite the few reported cases, as in other African countries. This SARS-CoV-2 exposure rate could be linked to the enrollment period, which overlaps with the second and third waves of COVID-19 in Senegal. These results could help in decision-making regarding of care for pregnant women in a pandemic context.

Author Contributions: Conceived, obtaining funding: A.A.M.D., A.A.; coordinated the work, drafted the manuscript: A.A.M.D., A.A.; performed the work: A.A.M.D., F.T., S.C., A.S., S.D.T., S.S., B.K.; curation: A.A.M.D.; formal analysis: A.A.M.D., A.A.; investigation: A.A.M.D., A.A., S.L., N.M.M., F.D., M.E.F.D., H.D.N., B.B.; supervision: A.A.M.D. validation: A.A.M.D., C.T.K., M.P., AA; reviewed the manuscript: A.A.M.D., F.T., S.C., N.M.M., H.D.N., A.S., S.D.T., S.S., B.K., H.S., Y.D., S.L., F.D., M.E.F.D., B.B., C.M.N., C.T.K., M.P., A.A. All authors have read and agreed to the published version of the manuscript.

Funding: The EPIVHE project was funded by Institut de Recherche pour le Développement (IRD) through its program of young team associated with IRD (JEAI). This ancillary study benefited from additional funds through the SEN'RT-BIOBANKING – 2022-23 project of the École Supérieure Polytechnique (ESP) of UCAD, which enabled the purchase of reagents and consumables.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Senegalese National Ethics Committee for Health Research (N°000130/MSA/CNRES/Sec, protocol code SEN20/31, date of approval: 5 July 2020) and the Ministry of Public Health and Social Action (N°00000582/MSAS/DPRS/DR, protocol code SEN20/31, date of approval: 8 July 2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

Acknowledgments: We thank all the participants in this study as well as the administrative and technical staff of all the health structures that facilitated the implementation of this work. We are grateful to the top management of the Ecole Supérieure Polytechnique (ESP) for their support to the GRBA-BE.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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