

## Serological survey for IgG antibodies against SARS-CoV-2 from newborns and their mothers

Inquérito sorológico para anticorpos IgG contra SARS-CoV-2 de recém-nascidos e suas mães

Encuesta serológica de anticuerpos IgG contra el SARS-CoV-2 de recién nacidos y sus madres

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### Abstract

*This serological survey, conducted in five Brazilian municipalities, evaluated the use of dried blood spots (DBS), obtained from newborns and their mothers, to detect SARS-CoV-2 IgG antibodies. DBS were obtained from 4,803 neonates aged up to seven days and their mothers, both asymptomatic, at public health care clinics during newborn screening. DBS were processed by ELISA to detect IgG antibodies against SARS-CoV-2 nucleocapsid antigen. Mothers of seropositive neonates were interviewed about sociodemographic characteristics and clinical and laboratory antecedents. Non-satisfactory samples, dyads with incomplete data, and vaccinated mothers were excluded. Of the 1,917 DBS dyads samples analyzed, 14.7% of neonates showed IgG antibodies against SARS-CoV-2. Among seropositive neonates, 73.2% of their mothers were also seropositive. More than half of the mothers with seropositive neonates denied clinical or laboratory suspicion of COVID-19 during pregnancy. Suspicion occurred in the third trimester for 24.6% of the mothers. This study tested an innovative strategy to improve the understanding of COVID-19 antibody dynamics during pregnancy and suggests the feasibility of a universal serological survey in puerperal women and neonates.*

SARS-CoV-2; Newborn; Pregnancy; Dried Blood Spot Testing; Neonatal Screening

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## Background

The knowledge about SARS-CoV-2 and the clinical syndrome resulting from the virus infection (COVID-19) has evolved rapidly. However, knowledge about the impacts of the disease on specific groups, such as pregnant women and neonates, remains incipient<sup>1,2</sup>. Maternal COVID infection during pregnancy has been associated with increased risk of neonatal complications such as prematurity and low birth weight. Such outcomes may have long-term effects, even in the absence of vertical transmission<sup>3</sup>.

Pregnant women can transfer SARS-CoV-2 antibodies to the fetus following immunization or natural infection, regardless of a clinical picture of COVID-19<sup>4,5</sup>. Nevertheless, the extent of transfer of antibodies and persistence of immunity remain unknown<sup>2,4</sup>. Many studies have analyzed the seropositivity of children born to women with previously confirmed infection or who were ill during childbirth<sup>2,4</sup>. However, the exact number of children exposed to SARS-CoV-2 during pregnancy may be underestimated as more than 60% of the COVID-19 cases are asymptomatic<sup>6</sup>.

Dried blood spot (DBS) sampling is a well-established method for detecting antibodies against various infections, including SARS-CoV<sup>7,8,9</sup>. In Brazil, the National Newborn Screening Program (PNTN) uses DBS to screen all newborns for many diseases via the Brazilian Unified National Health System (SUS) in the first-week post-birth<sup>10</sup>. This screening program provides an excellent opportunity for serological surveys, including the evaluation of gestational exposure to SARS-CoV-2.

This study aimed to evaluate the use of DBS obtained during newborn screening to detect IgG antibodies against SARS-CoV-2 in newborns and their mothers.

## Methods

This was a cross-sectional study, with a non-probabilistic sample from five municipalities located in southeast Brazil (Contagem, Nova Lima, Itabirito, Ipatinga and Uberlândia, all at Minas Gerais State). Samples were obtained between April and August 2021, before the large-scale vaccination campaign against COVID-19 in pregnant women was launched. During the blood collection phase, Brazil faced the second wave of the pandemic. At that moment, the incidence rate of COVID-19 in the general population in Minas Gerais State was 107.10 cases per 100,000 habitants, whereas the average incidence for the year of 2021 was 26.72 cases per 100,000 habitants<sup>11</sup>. This study was approved by the Ethics Committee of the Federal University of Minas Gerais (protocol n. 4.525.040; approved on 04/Feb/2021) and corresponds to the pilot phase of a larger research project.

All neonates aged up to seven days who attended public primary health care clinics for newborn screening accompanied by their mothers were selected to participate in the serological survey. Mothers provided verbal consent to participate with their neonates. We excluded dyads when mothers did not answer the clinical and sociodemographic questionnaire for any reason (n = 31) or were vaccinated for SARS-CoV-2 during pregnancy (n = 13). Samples not satisfactory to be tested were also excluded (n = 17).

Heel puncture was used to collect blood from neonates according to procedures of the PNTN<sup>12</sup>. Blood was obtained from mothers by finger-prick, concomitantly as their neonates. The blood spots were dried on filter papers and sent by mail to be processed at the Center for Newborn Screening and Genetics Diagnosis (NUPAD) of the Federal University of Minas Gerais (UFMG). The DBS specimens were processed by enzyme-linked immunosorbent assay (ELISA), using the Allserum EIA COVID-19 IgG-Dried Blood Spot test (<https://www.mbiolog.com.br>)<sup>11</sup>. The procedure was previously validated by the NUPAD team showing 80% sensitivity (95%CI: 70-87), 99% specificity (95%CI: 95-100), and 90.8% accuracy (95%CI: 86-94) to detect IgG antibodies against SARS-CoV-2 recombinant nucleocapsid antigen (anti-N antibodies).

We used information available in the NUPAD's data system to compare seropositive and seronegative neonate groups. The chi-square test was used to compare categorical variables and the t-test to compare continuous variables (significance level 5%).

Mothers of neonates seropositive for anti-N antibodies answered a telephone interview regarding their sociodemographic characteristics and clinical and laboratory antecedents during preg-

nancy. Mothers were considered to have had COVID-19 during pregnancy if they mentioned at least one of the following conditions: SARS-CoV-2 infection suspected by a physician, had a detectable SARS-CoV-2 RT-PCR test, had a positive antigen test, or had a positive serological test.

Data was stored in an electronic database system used in PNTN. Categorical variables were described using frequencies and percentages and continuous variables using means, standard deviations, and ranges. The agreement between the serological results of mothers and neonates was calculated using the kappa test. Analysis was processed using the Epi Info, version 7.2.3.1 (<https://www.cdc.gov/epiinfo/index.html>), and MedCalc (<https://www.medcalc.org>).

## Results

Blood samples from 4,803 neonates were collected for newborn screening. Of these, 1,917 dyads were eligible for the study (40%). In total, 279 neonates (14.7%) showed SARS-CoV-2 IgG nucleocapsid antibodies in the first-week post-birth. Among seropositive neonates, 73.2% of their mothers were also seropositive (Figure 1). The estimated agreement between mothers and neonates' serological results was 0.74 (kappa).

Table 1 shows the main characteristics of SARS-CoV-2 seropositive and seronegative neonates, as well as their mothers. The seropositive and seronegative groups were similar and, although there was a significant difference between the mean of mothers' age, the percentage of adolescent mothers was also equivalent (Table 1).

More than half of the mothers (54.5%) with seropositive neonates denied clinical or laboratory suspicion of COVID-19 during pregnancy. COVID-19 suspicion was also reported in 4.7%, 16.2% and 24.6%, of the mothers in the first, second and third trimester of the pregnancy, respectively (Figure 2).

## Discussion

Our findings indicate the viability of using DBS to detect SARS-CoV-2 nucleocapsid IgG antibodies in large-scale serological surveys. Previous studies have demonstrated that serological tests using DBS have several advantages over traditional serological techniques. For instance, sample collection and storage are easy and non-invasive, does not require specialized health professionals, and the technique is as reliable as serum samples<sup>7,8,9</sup>. These aspects make the strategy feasible, especially in low-resource settings<sup>7,8,9</sup>.

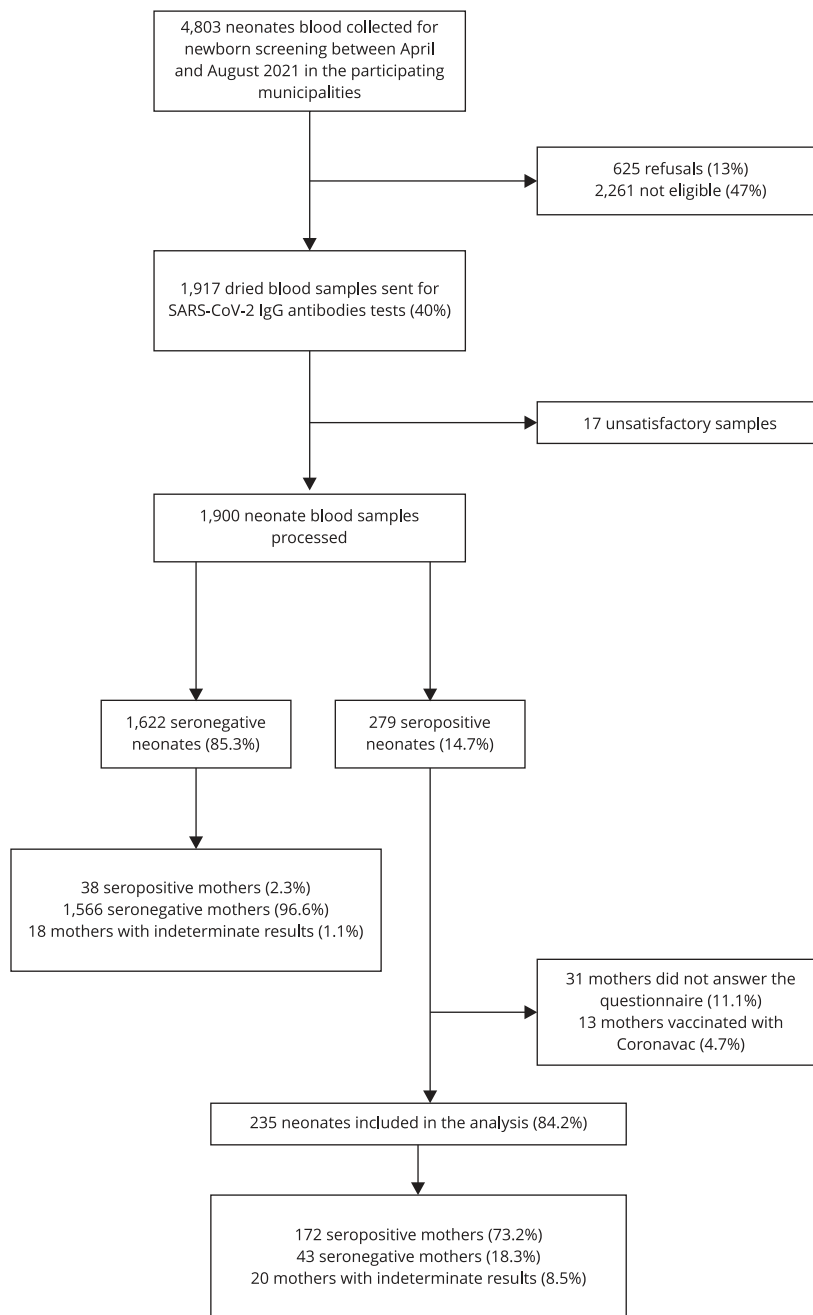
The specimens analyzed in the present study were obtained during the newborn screening in the first-week post-birth following the PNTN routine. Mothers and neonates were asymptomatic at the moment of blood collection. The seropositivity rates for SARS-CoV-2 in our sample was similar to a large Brazilian serologic survey in the same period<sup>13</sup>. The agreement between serological results for mothers and neonates was high, which is consistent with the current literature<sup>2,4</sup>.

Considering vaccinated mothers were excluded and the dynamics of SARS-CoV-2 antibodies<sup>2,4,14</sup>, our findings strongly suggest that, in this sample, the antibodies were produced during pregnancy and transplacentally transferred, reflecting the number of neonates potentially exposed to the virus during the gestational period. Nevertheless, we were unable to demonstrate if seropositive neonates were actually protected against SARS-CoV-2. Studies with a longitudinal follow-up design will reveal the protection conferred by these antibodies<sup>2,4</sup> and the long-term consequences of the in-utero exposure to SARS-CoV-2 on children's health and development<sup>1,3</sup>.

Despite the available evidence of transplacental antibodies transference<sup>2,4,5</sup>, the persistence of antibodies in children exposed to SARS-CoV-2 during pregnancy is still being discussed<sup>2</sup>. In our sample, for some of the seropositive neonates, the suspicion of infection occurred in the first trimester of gestation; if the mothers were not reinfected, those antibodies may have been produced at least six months before the test.

**Figure 1**

Flow diagram of the study.



**Table 1**

Main characteristics of SARS-CoV-2 seropositive and seronegative neonates and their mothers.

Sample characteristics	Seropositive neonates (N = 235)		Seronegative neonates (N = 1,622)		p-value
	n	%	n	%	
Mothers' age (years) *					0.99 **
< 19	13	5.53	92	5.79	
> 19	222	94.47	1,497	94.21	
Mean ± SD (Min/Max)	29.31 ± 6.65 (14,62/45.43)		28.29 ± 6.45 (14.16/46.45)		0.03 ***
Gestational age (weeks) *					1.00 **
< 37	8	3.47	56	3.54	
> 37	222	96.52	1,528	96.46	
Mean ± SD (Min/Max)	38.87 ± 1.28 (34/42)		38.89 ± 11.27 (32/42)		0.60 ***
Birth weight (g) *					0.56 **
< 2,500	8	3.42	72	4.50	
> 2,500	226	96.58	1,528	95.50	
Mean ± SD (Min/Max)	3,292.02 ± 460.58 (2,250/5,368)		3,210 ± 433.43 (1,985/5,025)		0.19 ***
Gender					0.89 **
Female	111	47.31	778	47.97	
Male	124	52.69	844	52.03	
Neonates' age at blood collection					0.69 ***
Mean ± SD (Min/Max)	4.65 ± 1.4 (1/8)		4.69 ± 1.2 (1/8)		

Max: maximum; Min: minimum; SD: standard deviation.

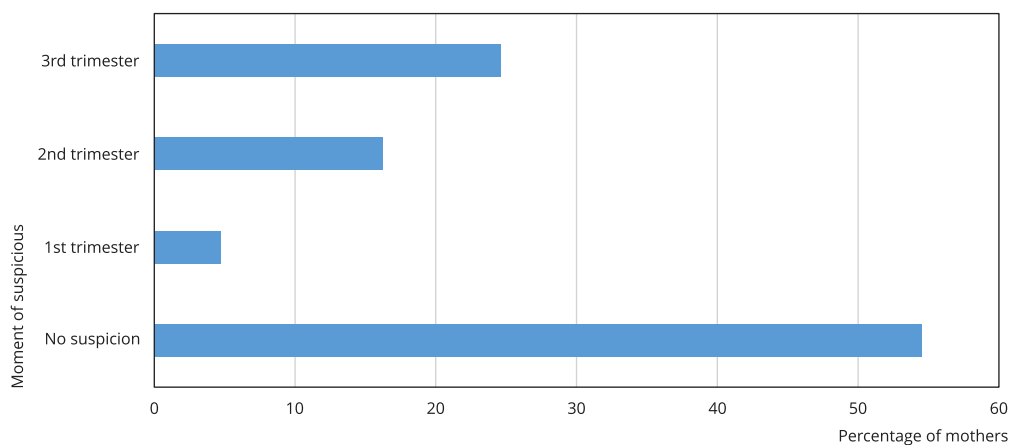
\* Missing data;

\*\* Chi-square test;

\*\*\* t-test.

**Figure 2**

Percentage of mothers with COVID-19 suspicion during pregnancy.



Finally, more than half of the mothers with seropositive neonates had no suspicion of SARS-CoV-2 infection, which indicates the high rate of coronavirus circulation in the community, highlighting the importance of serological surveys and protective measures, especially for pregnant women <sup>1,6</sup>.

We used an innovative design that may optimize an existing public health policy, but the study has limitations. COVID-19 suspicion during pregnancy was reported by mothers. Many of them had not undergone the RT-PCR test; thus, we could not establish when mothers were infected by SARS-CoV-2 during pregnancy with precision. Future studies should follow-up neonates and mothers from the beginning of the pregnancy and sequentially test SARS-CoV-2 antibody, including classes other than IgG, to gather more robust data.

In summary, the strategy tested in the present study can improve the current understanding of the dynamics of SARS-CoV-2 antibodies during pregnancy and suggests the feasibility of a universal serological survey in puerperal women and neonates using DBS.

## Contributors

G. S. M. A. Pinheiro and C. R. L. Alves contributed to the study conceptualization, methodology, investigation, data curation, writing, and review. V. M. G. O. Azevedo, A. A. Bentes, G. C. Januário, J. N. Januário, M. L. Cintra, and S. M. A. Lemos contributed to the study conceptualization, methodology, investigation, writing, and review. A. B. A. Souza and L. G. Pires contributed to the study investigation, writing, and review. All the authors approved the final version of the manuscript.

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## Additional informations

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## Resumo

*Este inquérito sorológico, realizado em cinco municípios brasileiros, avaliou o uso de sangue seco em papel filtro (DBS), obtidas de recém-nascidos e suas mães, para detectar anticorpos IgG SARS-CoV-2. DBS foram obtidas de 4.803 neonatos com até sete dias de vida e suas mães, ambos assintomáticos, em unidades de saúde pública durante a triagem neonatal. DBS foram processadas por ELISA para detectar anticorpos IgG contra o antígeno do nucleocapsídeo SARS-CoV-2. As mães de neonatos soropositivos foram entrevistadas quanto às características sociodemográficas e antecedentes clínicos e laboratoriais. Foram excluídas amostras insatisfatórias, díades com dados incompletos e mães vacinadas. Das 1.917 amostras analisadas, 14,7% dos neonatos apresentaram anticorpos IgG contra SARS-CoV-2. Entre os recém-nascidos soropositivos, 73,2% era filho de mulheres também soropositivas. Mais da metade das mães com recém-nascidos soropositivos negaram suspeita clínica ou laboratorial de COVID-19 durante a gravidez. A suspeita de COVID-19 ocorreu no terceiro trimestre para 24,6% das mães. Este estudo testou uma estratégia inovadora para melhorar a compreensão da dinâmica de anticorpos contra SARS-CoV-2 durante a gravidez e sugere a viabilidade de realização de um inquérito sorológico universal em puérperas e neonatos.*

*SARS-CoV-2; Recém-Nascido; Gravidez; Teste em Amostras de Sangue Seco; Triagem Neonatal*

## Resumen

*Esta encuesta serológica, realizada en cinco municipios brasileños, evaluó el uso de manchas de sangre seca (DBS), obtenidas de recién nacidos y sus madres, para detectar anticuerpos IgG contra el SARS-CoV-2. Se obtuvieron DBS de 4.803 recién nacidos de hasta siete días de edad y sus madres, ambos asintomáticos, en clínicas de salud pública durante el cribado neonatal. Las DBS se procesaron mediante ELISA para detectar anticuerpos IgG contra el antígeno de la nucleocápside del SARS-CoV-2. Se entrevistó a madres de recién nacidos seropositivos sobre características sociodemográficas y antecedentes clínicos y de laboratorio. Se excluyeron muestras no satisfactorias, díadas con datos incompletos y madres vacunadas. De las 1.917 muestras de diadas DBS analizadas, el 14,7 % de los recién nacidos mostró anticuerpos IgG contra el SARS-CoV-2. Entre los recién nacidos seropositivos, el 73,2% de sus madres también eran seropositivas. Más de la mitad de las madres con recién nacidos seropositivos negaron sospecha clínica o de laboratorio de COVID-19 durante el embarazo. La sospecha ocurrió en el tercer trimestre para el 24,6% de las madres. Este estudio probó una estrategia innovadora para mejorar la comprensión de la dinámica de anticuerpos de COVID-19 durante el embarazo y sugiere la viabilidad de una encuesta serológica universal en mujeres puérperas y recién nacidos.*

*SARS-CoV-2; Recién Nacido; Embarazo; Pruebas con Sangre Seca; Tamizaje Neonatal*

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