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Research Article

Retrospective Search for SARS-CoV-2 during the Winter Season, 2019-2020 in Social Security Population of Mexico

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Abstract

Background: Serological studies worldwide have been conducted to determine the existence of antibodies against the SARS-CoV-2 virus, as well as retrospective virus searches that have shown an unexpected early circulation of SARS-CoV-2 in other countries. **Objective:** To determine the retrospective presence of SARS-CoV-2 in the social security population of Mexico, who tested negative for influenza in molecular tests. **Materials and methods:** Nasopharyngeal/pharyngeal swab samples from influenza-negative cases protocolized in the epidemiological surveillance system from August 2019 to February 2020 were reanalyzed. Samples were randomly selected from all ages and genders; unsuitable samples were excluded. Analysis was performed using the Logix Smart RT-qPCR reagent from Co-Diagnostics, Inc. **Results:** 348 nasopharyngeal/pharyngeal exudate samples were selected during the period from 17 regions of the country. The presence of the RdRP gene of SARS-CoV-2 was not identified in any of the analyzed samples. **Conclusion:** Retrospective search for SARS-CoV-2 virus by RT-qPCR demonstrated absence of positive cases during the 2019-2020 winter season in patients with suspected viral illness.

Keywords: SARS-CoV-2; COVID-19; RT-qPCR; RdRP; Influenza

Introduction

On December 31, 2019, health authorities in Wuhan city, Hubei province, China, reported a cluster of 27 cases of acute respiratory syndrome of unknown etiology, which was linked to a seafood and animal market. On January 7, 2020, Chinese authorities reported the presence of a novel Coronavirus (2019-nCoV) identified as a possible causative etiology of the syndrome. On January 12, 2020, the World Health Organization (WHO) tentatively named this new virus as novel coronavirus 2019 (2019-nCoV). On January 30, 2020, the WHO declared the 2019-nCoV epidemic as a Public Health Emergency of International Concern. On February 11, 2020, the WHO formally named the disease caused by 2019-nCoV as coronavirus disease 2019 (COVID-19). On the same day, the Coronavirus Study Group of the International Committee on Virus Taxonomy named it severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1].

This new virus shares 79% nucleotide sequence similarity with SARS, the virus responsible for the 2002-2003 outbreak [2]. The ACE2 protein was identified as the receptor for SARS-CoV-2 in the cell (this receptor is also used by SARS). This receptor is expressed in about 83% of type II alveolar cells. Men have more ACE2 receptors than women, and individuals of Asian origin express it more than those of African or American origin [3].

Several serological studies have been conducted worldwide to determine the existence of antibodies against the SARS-CoV-2 virus, as there were no solid data on the actual emergence of coronavirus 2 infection (SARS-CoV-2) and it spread in the pre-pandemic period worldwide. Notable studies include those in Italy, where the presence of specific antibodies was investigated in blood samples from 959 asymptomatic individuals, with specific antibodies against SARS-CoV-2 found in 111 of 959 (11.6%) individuals from September 2019 onwards. Another study revealed the circulation of the virus in Italy at the end of 2019 in environmental samples [4]. Similarly, in the United States of America (USA), [5] a study was conducted to determine if reactive antibodies to SARS-CoV-2 were present in the serum of the population before the first identified case in that country (January 19, 2020). Residual samples from 7,389 blood donations from December 13, 2019 to January 17, 2020 were analyzed, showing neutralizing activity and blocking activity of the receptor-binding domain/ACE2 >50%, suggesting the presence of reactive antibodies against SARS-CoV-2, indicating that SARS-CoV-2 may have been introduced into the USA before January 19, 2020 [6]. The lack of specific tests for a new virus limited the possibility of an accurate diagnosis of patient zero and thus determining its exact route.

Similar events have occurred previously in Mexico, with Chikungunya in 2014 and Zika in 2015 in retrospective sample reanalysis [7-9].

Shortly after the release of the first SARS-CoV-2 genome, a group from the PAHO in Berlin designed and published a polymerase chain reaction protocol targeting the RdRP, E, and N genes of the new virus [10]. This reaction was evaluated and approved by the WHO for laboratory confirmatory diagnosis. Currently, several kits using this same technology are available on the market, some of which have platforms that allow automation of part of the process, such as the COBAS (Roche) and m2000 (Abbott) platforms. In Mexico, the diagnostic algorithm includes conducting the confirmatory assay on 100% of patients who progress to severe cases or deaths and 10% of ambulatory cases that meet the operational case definition [7].

The Institute of Diagnostic and Epidemiological Reference (InDRE) conducted an intentional search for possible circulation of SARS-CoV-2 in the country, where as of March 1, 2020, 140 samples of Severe Acute Respiratory Infection (SARI) cases negative for influenza and other respiratory viruses were retrospectively analyzed, with negative results for SARS-CoV-2, when at that time five confirmed cases of COVID-19 were reported in Mexico [11].

Likewise, at the Mexican Social Security Institute (IMSS), the circulation of SARS-CoV2 has been identified prior to the official notification of the first case through antibody determination [12]. Hence, the interest in determining the presence of SARS-CoV-2 in the population with social security from IMSS, who tested negative for influenza virus in molecular tests conducted for epidemiological surveillance during the winter season, 2019-2020.

Materials and Methods

Sample Selection

The study universe included samples (nasopharyngeal and/or pharyngeal exudates) collected in IMSS medical units for the influenza diagnostic algorithm with negative results, which were sent to the Central Laboratory of Epidemiology (LCE) from the federal entities according to operational regionalization included in the laboratory epidemiological surveillance system from August 2019 to February 2020, encompassing both sexes. The main inclusion criterion was negativity for influenza virus by RT-qPCR. Samples that did not meet good conditions of volume, concentration, integrity, and purity for analysis were excluded. Additionally, samples that were routinely discarded or showed contamination were also excluded. Samples were randomly chosen using a random number without replacement of the internal sample folio number, disregarding patient identification. With this folio, case data were located in the "Epidemiological Control System

for Laboratory" (SISCEP, by its acronyms in Spanish), to obtain variables such as federal entity, sex, age, clinical condition, onset date of symptoms, and sample collection date.

They were classified into regions according to the National Institute of Statistics and Geography (INEGI, by its acronyms in Spanish) [13] as follows: 1) Central Region (Mexico City, Guerrero, Hidalgo, State of Mexico, Morelos, Puebla, Tlaxcala, and Oaxaca), 2) Central West Region (Aguascalientes, Colima, Guanajuato, Jalisco, Michoacán de Ocampo, Nayarit, Querétaro, San Luis Potosí, and Zacatecas), 3) Northern Region (Baja California, Baja California Sur, Chihuahua, Coahuila, Durango, Nuevo León, Sinaloa, Sonora, and Tamaulipas), and 4) Southeast Region (Campeche, Chiapas, Quintana Roo, Tabasco, Veracruz de Ignacio de la Llave, and Yucatán).

Sample size

The sample size for frequency in a population for proportions with a frequency of 0.50, with confidence limits of 5% and a design effect of 1, resulted in 355 samples.

Laboratory technique

Based on consulted references and considering the type of samples stored in the LCE biobank, selected samples underwent nucleic acid extraction using the Chemagic 360 automated equipment, Perkin Elmer, following the manufacturer's recommendations, and were analyzed using RT-qPCR using the 7500 fast-applied biosystems thermocycler, Thermo Fisher Scientific. The assay used for the identification of the SARS-CoV-2 virus was the commercial reverse transcription polymerase chain reaction (RT-qPCR) technique [10], using the Logix Smart™ Coronavirus 2019 (COVID-19) Test Kit, Co-Diagnostics, Inc. This kit targets the RdRp gene of SARS-CoV-2 and human Ribonuclease P as an endogenous control, with an analytical sensitivity of 99.52% and specificity of 100%, with a detection limit of 4.29 copies per microliter of viral RNA. The presence of an amplification curve for the RdRp gene marker and a Ct threshold value for SARS-CoV-2 less than 45 cycles indicates a positive result. The amplification program used is shown in Table 1.

Stage 1		**Stage 2**	
Incubation	Activation	Alignment, hybridization	Extension, fluorescence emission
1X 45°C 15 minutes	1X 95°C 2 minutes	50X 95°C 3 seconds	50X 55°C 32 seconds

Table 1: Amplification program for the diagnosis of SARS-CoV-2.

Statistical analysis

Descriptive statistics were used to analyze the samples, using percentages with a 95% confidence interval via the Wilson scale. The t-test was used for the difference in means for age.

Funding

The LCE had its own resources, so the project lacked external funding.

Results

A total of 2,491 samples were processed from July 29 to December 31, 2019, and 2,134 samples from January 1 to March 24, 2020, with negative results for influenza A and B at the LCE. Of these, 191 samples from 2019 and 157 from 2020 were selected (Figure 1), with a median of eight samples per week, predominantly in January 2020 (Figure 2). Out of the 4,625 samples, 348 nasopharyngeal/pharyngeal exudate samples (7.5%) were obtained during the period from August 2019 to February 2020. The distribution included 179 females (51.4%, 95% CI 46.2-56.64) and 169 males (48.6%, 95%CI 43.36-53.8). The age range was from 0 to 99 years, originating from 17 federative entities of the country (Table 2). The overall mean age was 38.5 years, 39.4 years in females, and 37.5 years in males (p=0.5453). Most age groups were balanced, with the 1 to 4 years age group being the most predominant (15.2%), followed by infants under one year (8.0%) and 25 to 29 years (6.3%) (Table 3).

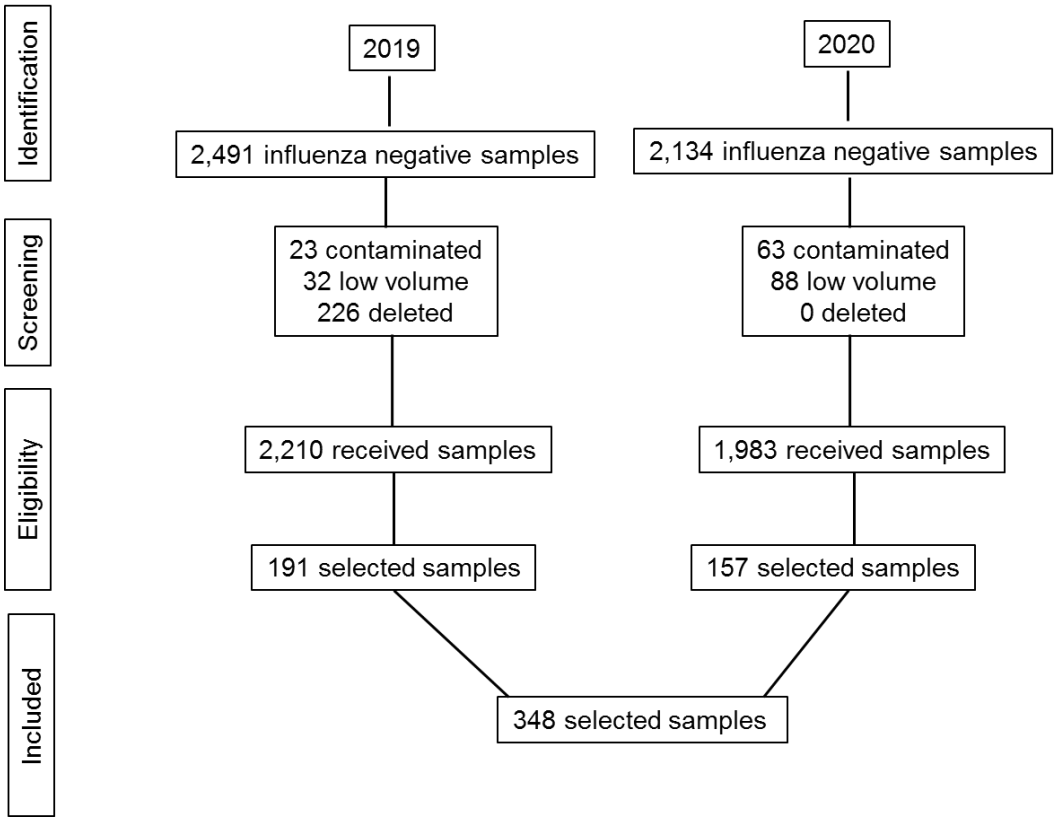

Figure 1: Flowchart of sample selection negative for influenza. Mexico, August 2019 to March 2020.

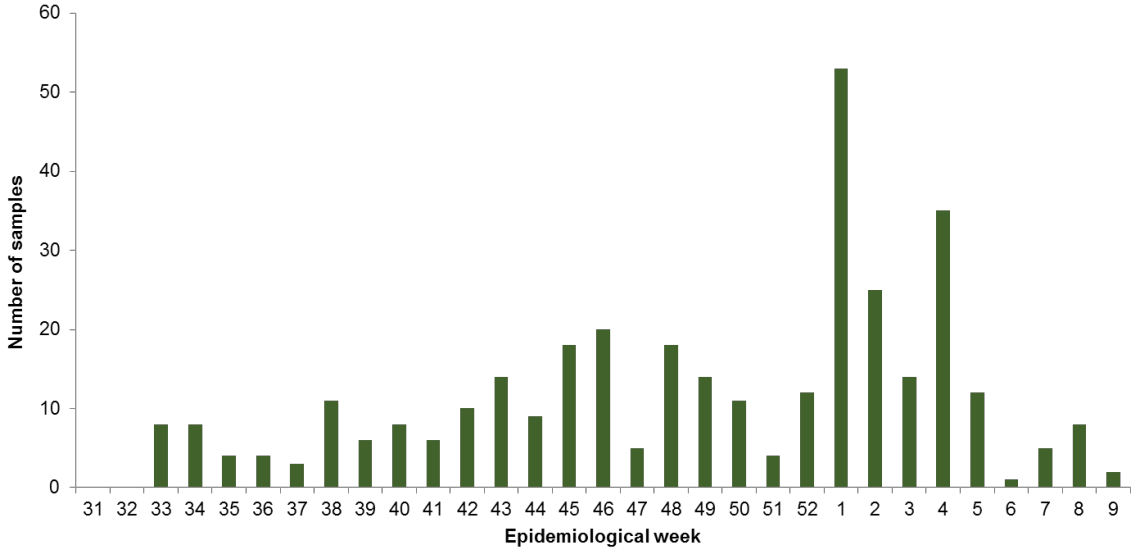

Figure 2: Number of samples negative for influenza with retrospective search for SARS-CoV-2 per epidemiological week. IMSS, week 31 of 2019 to week 9 of 2020.

Region	Estate	Frequency	%
North	Baja California	23	6.6
North	Baja California Sur	27	7.8
Center	Ciudad de México	94	27.0
South	Chiapas	1	0.3
Western Center	Colima	6	1.7
Center	Guerrero	6	1.7
Center	Hidalgo	9	2.6
Center	México	42	12.1
Western Center	Michoacán	1	0.3
Center	Morelos	4	1.1
Center	Oaxaca	22	6.3
Center	Puebla	8	2.3
Western Center	Querétaro	16	4.6
Western Center	San Luis Potosí	15	4.3
North	Sinaloa	1	0.3
Center	Tlaxcala	14	4.0
Southeast	Veracruz	59	17.0
Total		**348**	**100.0**

Table 2: Number of samples analyzed by federative entity for SARS-CoV-2 search. Mexico, August 2019 to March 2020.

Age	Frequency	Percentage
<1	28	8.0
1 to 4	53	15.2
10 to 14	8	2.3
15 to 19	4	1.1
20 to 24	18	5.2
25 to 29	22	6.3
30 to 34	17	4.9
35 to 39	18	5.2
40 to 44	21	6.0
45 to 49	12	3.4
5 to 9	13	3.7
50 to 54	16	4.6
55 to 59	17	4.9
60 to 64	18	5.2
65 to 69	16	4.6
70 to 74	15	4.3
75 to 79	19	5.5
80 to 84	15	4.3
85 to 89	10	2.9
90 and more	8	2.3
Total	**348**	**100.0**

Table 3: Distribution of analyzed samples by age group.

According to clinical condition, 254 were hospitalized (72.9%, 95%CI 68.09-77.38), 92 were managed ambulatory (26.5%, 95%CI 22.08-31.31), and two resulted in deaths (0.6%, 95%CI 0.1578-2.071). Among the included samples, five were from pregnant women (average gestational age of 25.8 weeks).

Regarding the samples, the mean time between sample collection and symptom onset was 2.2 days (range 0-7 days, standard deviation=0.04). The average volume of aliquots was 1.5 mL, and the average temperature during transport to the laboratory was 4.3°C.

SARS-CoV-2 detection was performed using RT-qPCR, and in 100% of the samples, the RdRP gene of SARS-CoV-2 was not detected.

Discussion

A retrospective intentional search for the nucleic acids of the SARS-CoV-2 virus was conducted on samples negative for influenza from a reference epidemiological laboratory, prior to the official notification of the first case of COVID-19 in Mexico; the virus was not detected in any of them via RT-qPCR.

The Mexican Ministry of Health published, on February 27, 2020, an analysis of 125 samples of Severe Acute Respiratory Infection (SARI) retrospectively, aiming to find the SARS-CoV-2 virus without success [14], days after the official notification of the first five cases. By March 1, 2020, there were 140 samples from 21 of the 32 federative entities, which is consistent with the results obtained from the re-analysis of 348 samples from 17 states. However, a study conducted at the IMSS revealed that a) the observed seroprevalence at the beginning of the pandemic was 3.5% in February 2020, b) 40 cases with IgG were detected 17 days before the official notification of the first COVID-19 case in Mexico, and c) the activity of neutralizing antibodies in all patients was 86.1% [12]. This phenomenon of retrospective identification was observed in other countries, such as Italy [4,5] and the USA [6].

Regarding studies on antibody determination, it has been reported that there is potential for cross-reactivity with other coronaviruses, which would determine a true positive if the molecular test was reactive to SARS-CoV-2. This prompted the search for nucleic acids in samples negative for the prevailing

virus prior to the identification of SARS-CoV-2; hence, the major selection criterion was negativity for influenza [6,12].

At the time of the sample selection cutoff, Mexico was reporting its first COVID-19 cases notified in Mexico City, Chiapas, Chihuahua, and Sinaloa. Additionally, the identification of suspected cases has been based on an operational definition of suspected COVID-19 case with clinical criteria and a history of travel or stay in Hubei Province, China, or contact with a confirmed case or a case under investigation up to 14 days before the onset of symptoms [15]. Before the pandemic stage, the operational definitions of cases were based on mild clinical data for influenza-like illness and those requiring hospitalization for SARI [16].

In a similar retrospective search study [17], out of 58 samples from severe patients, only one sample tested positive for SARS-CoV-2, and the patient was hospitalized on December 27, 2019, without a history of travel to China. These results can be explained by the presence of viral infection in individuals with asymptomatic or subclinical courses [18,19], as well as its potential for sustained person-to-person transmission. A study analyzing 7,666 SARS-CoV-2 genomes revealed a common ancestor between October 6 and December 11, 2019, indicating the initial transmission to humans [20]. The RT-qPCR assay protocol used in the Corman, et al. study has been replicated in other studies [10].

Strengths of the study include: 1) reprocessing of the samples was done in a laboratory accredited for diagnostic competence by the health authority in Mexico and with experience since the influenza A (H1N1) pdm09 pandemic; 2) for the re-analysis, the 7500 Fast Applied Biosystems platform from Thermo Fisher Scientific was used; 3) most samples came from individuals with severe symptoms requiring hospitalization, and the sample collection was timely.

Limitations of this study were: a) geographical distribution of cases limited to certain federative entities of the country (mainly from the center, 57.2%), b) absence of demographic and clinical characteristics of the cases. However, although the focus was on the center of the country, Mexico's first cases were in the center and north [7].

Conclusions

In conclusion, this study finds no identification of the SARS-CoV-2 virus between August 2019 and February 2020 in RT-qPCR samples in a population with social security.

Acknowledgments

The authors thank all the participants in influenza epidemiological surveillance system, including treating physicians, epidemiologists, laboratory chemists and nursing staff.

Ethical Aspects

The present research did not pose a risk according to the Regulations of the General Health Law on Research, since aliquots of samples that were previously collected were reanalyzed, hence informed consent was not required. The project was submitted for approval to the National Committee for Scientific Research (CNIC) of the IMSS with number R-2021-785-043. Once the project was completed, the samples were discarded after being inactivated with 0.5% sodium hypochlorite. This mixture was kept for at least one hour before disposal. The inactivated residues were then poured into the drain.

Conflict of Interest: None.

Ethical Approval: National Committee of Scientific Research of the IMSS was approved this study (registration number R-2021-785-043).

Competing Interests: The authors declare that there are no conflicts of interest.

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Author Contributions

JEAY: Data collection and sample processing, writing original draft preparation.

DACG: Formal analysis, writing original draft preparation, review & editing.

CEST: Project administration, resources and supervision.

BMM: Data collection and resources.

YMAH: Resources and supervision.

CGM: Conceptualization, visualization, methodology.

JEMM: Writing original draft preparation, review & editing.

JGI: Conceptualization, investigation.

AVP: Writing original draft preparation, review & editing.

PFHB: Investigation, methodology.

AMP: Translation, writing original draft preparation, review & editing.

GVA: Investigation, methodology.

LJB: Data collection and validation.

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