

Universidade de Lisboa

Faculdade de Farmácia



**SARS-CoV-2 Serological Survey on Cascais: A Comparative Study  
Between Migrants and Natives**

Francisco José dos Santos Merca

Dissertation supervised by Professor Doctor Ana Abecasis  
and co-supervised by Doctor Sofia Seabra and Doctor Inês Bártolo

**Master in Biopharmaceutical Sciences**

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## Abstract

The outbreak of 2019-novel coronavirus disease (COVID-19), caused by SARS-CoV-2, started in late 2019. In a short time, it spread rapidly all over the world.

To assess the impact of SARS-CoV-2 pandemic, a serological survey was performed on the Cascais municipality and offered to all Cascais residents. In this serological survey, a serological test was offered by private labs in Cascais together with a questionnaire concerning socio-demographic, clinical, symptoms and COVID-19 related behavior. The aim of the study was to assess the seroprevalence of antibodies to SARS-CoV-2 and to identify sociodemographic, clinical, and behavioral factors associated with seropositivity for SARS-CoV-2 on the population of residents of Cascais. In this study we analyzed 4410 questionnaires obtained between May 21<sup>st</sup> and October 1<sup>st</sup>, 2020 for which information about country of origin was available, in order to evaluate the seroprevalence of SARS-CoV-2 in the migrants and Portuguese population residing in Cascais and to study the association between the seroprevalence and the history of chronic disease, symptoms reported and PCR positive test, among the Migrants and Portuguese population. We established the specific objectives of characterizing the socio-demographics and symptoms reported by the Migrants individuals and the Portuguese individuals, to verify a potential association between the occurrence of symptoms and the results of the serological test result and to assess the most prevalent symptoms reported and their chronic disease history on both categorized groups, migrants and Portuguese individuals, and their use of health care assistance.

The Seroprevalence among the migrants population was estimated to be 2.02% (95% CI: 1.33 – 3.07%) and the Bayesian approach corrected the seroprevalence, knowing the sensitivity and specificity of the serological test, to 1.93% (95% CI: 1.25 – 2.71%), higher seroprevalence when compared to the portuguese population.

A consistent statistically significant association was found between the seroprevalence and the household size. Regarding to the other sociodemographic factors such as sex, age group, employment status, educational level, parishes, and history of travel, no statistically significant associations were found. Concerning the history of chronic diseases of the migrants population, no statistically significant differences were found between the chronically ill and non-ill, although the population who reported to be non-chronic ill showed a higher seroprevalence compared to the chronically ill. Regarding to the COVID-19 history, and for the migrants, there was a statistically significant association between positive serological testing and having performed a previous PCR test ((odds ratio 3.43 (95% CI: 1.39 – 8.71), p-value <0.005) and having reported a previous positive PCR test ((odds ratio 24.46 (95% CI: 0.60 – 988.64), p-value <0.002).

Concerning to the COVID-19 symptoms, the seroprevalence was higher among the participants who reported to have had fever, chills, chest pain, headache, abdominal pain, anosmia (loss of smell) and ageusia (loss of taste). For the symptoms anosmia (loss of smell) and ageusia (loss of taste) there was a statistically significant association with positive serological tests (odds ratio 4.40 (95% CI: 0.62 – 16.69), p-value <0.05) and (odds ratio 9.65 (95% CI: 2.01 – 32.81), p-value <0.001), respectively. About 57.14% (95% CI: 36.55 –

75.53%) of the migrant population reported to not have had any symptoms suggestive of COVID-19 infection. Based on the relation between reported symptoms and serological tests reactivity, the symptoms headache, fever, ageusia (loss of taste), cough and nasal congestion were the ones with higher sensitivity.

Regarding to COVID-19 related implications, on the migrants population, the seroprevalence among the participants who sought medical assistance was higher compared to those who did not seek medical assistance, being 5.17% (95% CI: 2.39 – 10.83%) for those who sought. Regarding to the hospitalizations or intensive care due to COVID-19 implications, it was not possible to assess the seroprevalence due to the small sample size. On the migrant population, the seroprevalence among those who missed work or school was significantly different from those who did not miss work or school and estimated to be 5.88% (95% CI: 2.02 – 15.92), (statistically significant association, odds ratio 3.91 (95% CI: 0.83 – 12.81), p-value <0.05).

After the WHO declared the COVID-19 as a pandemic, governments across the globe mandated lockdowns on their population, limiting people's mobility during at least 3 months from the end of February to beginning of May, causing consequences not only from the health point of view, but also psychosocial. However, the measures to control the COVID-19 cannot be very efficient, due to several factors such as transmission from asymptomatic individuals. The acquisition of individual and collective protection measures are preponderant to minimize the impact of the disease on global public health, mitigating the spread of COVID-19 disease. Serological surveys are important approaches to identify previously undetected cases of SARS-CoV-2 infection and to understand the duration of antibodies to SARS-CoV-2. Furthermore, when combined with socio-demographic and clinical data, it allows to understand patterns of infection with SARS-CoV-2 in different groups. Given the large amount of data collected in Cascais, this survey is an important example of a municipality initiative that generated a large amount of scientific data that can be used to guide future prevention measures.

**Keywords:** Serological Survey; SARS-CoV-2; COVID-19; Antibodies; Migrants

## Resumo

A pandemia causada pelo SARS-CoV-2, começou no final de 2019. Em pouco tempo, espalhou-se rapidamente por todo o mundo.

Para avaliar o impacto da pandemia de SARS-CoV-2, foi realizado um inquérito serológico no concelho de Cascais e disponível para todos os residentes de Cascais. Neste inquérito serológico foi realizado um teste serológico por laboratórios privados de Cascais juntamente com um questionário sobre dados sociodemográficos, dados clínicos, sintomas e comportamentos relacionados com a COVID-19.

O objetivo do estudo foi avaliar a seroprevalência de anticorpos para SARS-CoV-2 e identificar fatores sociodemográficos, clínicos e comportamentais associados à seropositividade para SARS-CoV-2 na população de residentes em Cascais. Neste estudo analisámos 4410 questionários obtidos entre 21 de Maio e 1 de Outubro de 2020, para os quais estava disponível informação sobre o país de origem. Desta forma foi conseguido avaliar a seroprevalência do SARS-CoV-2 nos migrantes e na população portuguesa residente em Cascais e estudar a associação entre a seroprevalência e história de doença crónica, sintomas reportados e teste PCR positivo, na população migrante e portuguesa.

A seroprevalência entre a população de migrantes foi de 2,02% (IC 95%: 1,33 – 3,07%) e a abordagem Bayesiana corrigiu a seroprevalência, tendo em conta a sensibilidade e especificidade do teste serológico, corrigiu para 1,93% (IC 95%: 1,25 – 2,71%), a seroprevalência quando comparada com a população portuguesa.

Encontrou-se uma associação estatisticamente significativa entre a seroprevalência e o agregado familiar. Em relação aos fatores sociodemográficos, como sexo, faixa etária, situação profissional, escolaridade, freguesias e histórico de viagens, não foram encontradas associações estatisticamente significativas. No que diz respeito ao histórico de doenças crónicas da população migrante, não foram encontradas diferenças estatisticamente significativas entre os doentes crónicos e os não doentes, embora a população que se referiu não ser doente crónico tenha apresentado uma seroprevalência maior em relação aos doentes crónicos. Em relação ao histórico de COVID-19, para os migrantes, houve uma associação estatisticamente significativa entre teste serológico positivo e ter realizado um teste PCR anterior ((odds ratio 3,43 (IC 95%: 1,39 – 8,71), valor de  $p < 0,005$ ) e ter relatado um teste PCR anterior positivo ((odds ratio 24,46 (IC 95%: 0,60 – 988,64), valor  $p < 0,002$ ).

Em relação aos sintomas da COVID-19, a seroprevalência foi maior entre os participantes que reportaram ter tido febre, calafrios, dor no peito, cefaleia, dor abdominal, anosmia (perda do olfato) e ageusia (perda do paladar). Para os sintomas anosmia (perda do olfato) e ageusia (perda do paladar) houve associação estatisticamente significativa com testes serológicos positivos (odds ratio 4,40 (IC 95%: 0,62 – 16,69),  $p$ -valor  $< 0,05$ ) e (odds ratio 9,65 (IC 95%: 2,01 – 32,81), valor  $p < 0,001$ ), respetivamente. Cerca de 57,14% (IC 95%: 36,55 – 75,53%) da população migrante reportou não ter apresentado nenhum sintoma sugestivo de infeção por COVID-19. Com base na relação entre os sintomas reportados e a reatividade dos testes serológicos, os sintomas cefaleia, febre, ageusia (perda do paladar), tosse e congestão nasal foram os de maior sensibilidade.

Em relação às implicações relacionadas com a COVID-19, na população migrante, a seroprevalência foi maior entre os participantes que não procuraram atendimento médico 5,17% (IC 95%: 2,39 – 10,83%) em comparação com os que procuraram. Em relação aos internamentos ou cuidados intensivos por implicações da COVID-19, não foi possível avaliar a seroprevalência devido ao pequeno tamanho da amostra. Na população migrante, a seroprevalência entre aqueles que faltaram ao trabalho ou à escola foi estatisticamente significativa sendo que aqueles que não faltaram ao trabalho ou à escola, apresentando uma maior seroprevalência na população que faltou ao trabalho ou à escola, (associação estatisticamente significativa, odds ratio 3,91 (IC 95%: 0,83 – 12,81), valor  $p < 0,05$ ).

Desde que a OMS declarou a COVID-19 como uma pandemia, os governos por todo o mundo impuseram limitações à mobilidade das suas populações, limitando a mobilidade das pessoas durante pelo menos 3 meses, começando no final do mês de Fevereiro até início do mês de Maio de 2020, causando consequências não apenas do ponto de vista da saúde física, mas também psicossocial da população. Contudo, as medidas de controle do COVID-19 podem não ter sido muito eficientes, devido a diversos fatores, tais como a transmissão de indivíduos assintomáticos. A aquisição de medidas de proteção individuais e coletivas são preponderantes para minimizar o impacto da doença na saúde pública global, contendo a propagação da doença COVID-19. Os inquéritos serológicos são abordagens importantes para identificar casos de infeção por SARS-CoV-2 não detetados anteriormente e para entender a duração dos anticorpos para SARS-CoV-2. Além disso, quando combinado com dados sociodemográficos e clínicos, permite compreender padrões de infeção por SARS-CoV-2 em diferentes grupos. Dada a grande quantidade de dados recolhidos em Cascais, este inquérito é um importante exemplo de uma iniciativa de um município que gerou uma grande quantidade de dados científicos que podem ser utilizados para orientar futuras medidas de prevenção.

**Palavras-Chave:** SARS-CoV-2; Anticorpos; COVID-19; Estudo Serológico; Migrantes;

#### **Abbreviations:**

ACE2: Angiotensin-Converting Enzyme 2

ANLC: National Association of Clinical Laboratories

ARDS: Acute Respiratory Distress Syndrome

BSL: Biological Safety Level

CDC: Center for Disease Control and Prevention

CDRs: Complementary Determining Regions

CoV: Coronavirus

COVID-19: Coronavirus Disease of 2019

DGS: Directorate-General of Health

ELISA: Enzyme-Linked Immunosorbent Assay

FET: Field-effect Transistors

HCoVs: Human Coronavirus

IgA: Immunoglobulin class A

IgD: Immunoglobulin class D

IgE: Immunoglobulin class E

IgG: Immunoglobulin class G

IgM: Immunoglobulin class M

IL-6: Interleukin-6

INSA: Instituto Nacional de Saúde Doutor Ricardo Jorge

LAMP: Loop-mediated Isothermal Amplification

LFA: Lateral Flow Assay

MDA5: Melanoma Differentiation-Associated Protein 5

MERS-CoV: Middle East Respiratory Syndrome Coronavirus

ORF: Open Reading Frame

PHEIC: Public Health Emergency of International Concern

POCT: Point-of-Care Testing

R<sub>0</sub>: R naught

RDTs: Rapid Diagnosis Test

RPA: Recombinase Polymerase Amplification

RSCU: Relative Synonymous Codon Usage

RT-LAMP: Reverse Transcription Loop-mediated Isothermal Amplification

RT-PCR: Reverse Transcription Polymerase Chain Reaction

SARS: Severe Acute Respiratory Syndrome

SARS-CoV-2: Coronavirus Severe Acute Respiratory Syndrome 2

SINAVE: National Epidemiological Surveillance System

SNS: National Health Service

SPR: Surface Plasmon Resonance

TLRs: Toll like-receptors

TMPRSS2: Transmembrane Serine Protease 2

WHO: World Health Organization

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## 1 Introduction

### 1.1 Coronaviruses (CoVs)

Coronaviruses (CoVs) are part of the subfamily *Orthocoronavirinae* in the family *Coronaviridae* and the order *Nidovirales* according to the International Committee on Taxonomy of Viruses. The *Orthocoronavirinae* subfamily consists of four genera, *Alphacoronaviruses*, *Betacoronavirus*, *Gammacoronavirus* and *Deltacoronavirus*, based on their phylogenetic relationship and genomic structure (J. Cui, Li, & Shi, 2019). The *Alphacoronaviruses* and *Betacoronavirus* only infect mammals while the *Gammacoronavirus* and *Deltacoronavirus* infect birds and rarely mammals (Woo et al., 2012). In humans, the CoVs cause mainly respiratory tract infections. Currently, six human coronaviruses (HCoVs) have been identified. These include the alpha-CoVs HCoV-NL63 and HCoV-229E and the beta-CoVs HCoV-OC43, HCoV-HKU1, severe acute respiratory syndrome-CoV (SARS-CoV), the Middle East respiratory syndrome-CoV (MERS-CoV) (Y. Yin & G. Wunderink, 2018). In view of taxonomical classification, SARS-CoV-2 (COVID-19) is another among the other viruses in the species, SARS-related CoV (Umakanthan et al., 2020).

This century has seen the global spread of two previously unknown coronaviruses. In November 2002, the first known case of SARS-CoV occurred in Foshan, China. By February 2003, more than 300 cases had been reported. Individuals who were infected and subsequently travelled spread the outbreak to other countries such as Hong Kong, Vietnam, Canada, and several other countries (N. Lee et al., 2003). In March 2003, the WHO established a network of laboratories to determine the causative agent of SARS. This effort led to the identification of SARS-CoV in early April of that year. By July 2003 and after a total of 8096 reported cases, including 774 deaths in 27 countries, no more infections were detected, and the SARS pandemic was declared to be over (Wit, Doremalen, Falzarano, & Munster, 2016).

In June 2012, 10 years after the first emergence of SARS-CoV, a man in Saudi Arabia died of acute pneumonia and renal failure. A novel coronavirus, MERS-CoV was isolated from his sputum. A cluster of cases of severe respiratory disease had occurred in April 2012 in a hospital in Jordan and was retrospectively diagnosed as MERS-CoV (Hijawi et al., 2013). MERS-CoV continued to emerge and spread to countries outside of the Arabic Peninsula as a result of travel of infected persons; often, these imported MERS cases resulted in nosocomial transmission. In May 2015, a single person returning from the Middle East started a nosocomial outbreak of MERS-CoV in South Korea that involved 16 hospitals and 186 patients. As of 26 April 2016, there have been 1728 confirmed cases of MERS-CoV, including 624 deaths in 27 countries (Wit et al., 2016).

To date, there have been three major coronavirus outbreaks reported, with the most recent epidemic being the spread of the 2019 novel coronavirus (2019-nCoV, or more recently named SARS-CoV-2), which is known to cause the Coronavirus Disease-2019 (COVID-19) (Sharma, Farouk, & Lal, 2021).

#### 1.1.2 Phylogeny & Pathogenicity of SARS-CoV-2

SARS-CoV, SARS-CoV-2 and MERS-CoV belong to the *Betacoronavirus* genus in the *Coronaviridae* family and have large positive-sense RNA genome with 27.9kb and 30.1kb respectively. Similarly, to all viruses in the order *Nidovirales*, SARS-CoV and MERS-CoV

have a unique coding mechanism strategy that divide two-thirds of their viral RNA into two large polyproteins (pp1a and pp1ab), and the remaining viral genome is transcribed into a nested set of subgenomic mRNAs (Pasternak, Spaan, & Snijder, 2006)(Perlman & Netland, 2009). These two polyproteins encode for 16 non-structural proteins (nsp1 to nsp16), that make up the viral replicase-transcriptase complex, which are in the Open Reading Frame (ORF), on ORF1a and ORF1b. One unique feature function of nsp14 is providing the proofreading capability required to maintain a large RNA genome without the accumulation of detrimental mutations (Wit et al., 2016). These similarities can be observed on figure 1(a), that shows the three coronavirus genes, and their differentiations on their genes.

The phylogenetic analysis of the first genomic sequence of the 2019-nCoV genome indicated that SARS-CoV-2 belongs to the *Betacoronavirus* genus, which includes Bat SARS-like coronavirus, SARS-CoV and MERS-CoV, (Singh & V.Yi, 2021) (Figure 1(b)).

SARS-CoV-2 shares about 79% sequence similarity with SARS-CoV and encodes for a set of structural proteins (membrane protein, (M), nucleocapsid protein, (N), envelope protein, (E), and for the spike protein, (S)) and non-structural proteins (nsp) (which compose the viral replication and transcription complexes). The structural proteins, together with a lipid bilayer derived from the host, forms an enveloped virion, that delivers the viral genomic RNA into the cell (Figure 1(c))(Yang, 2021).

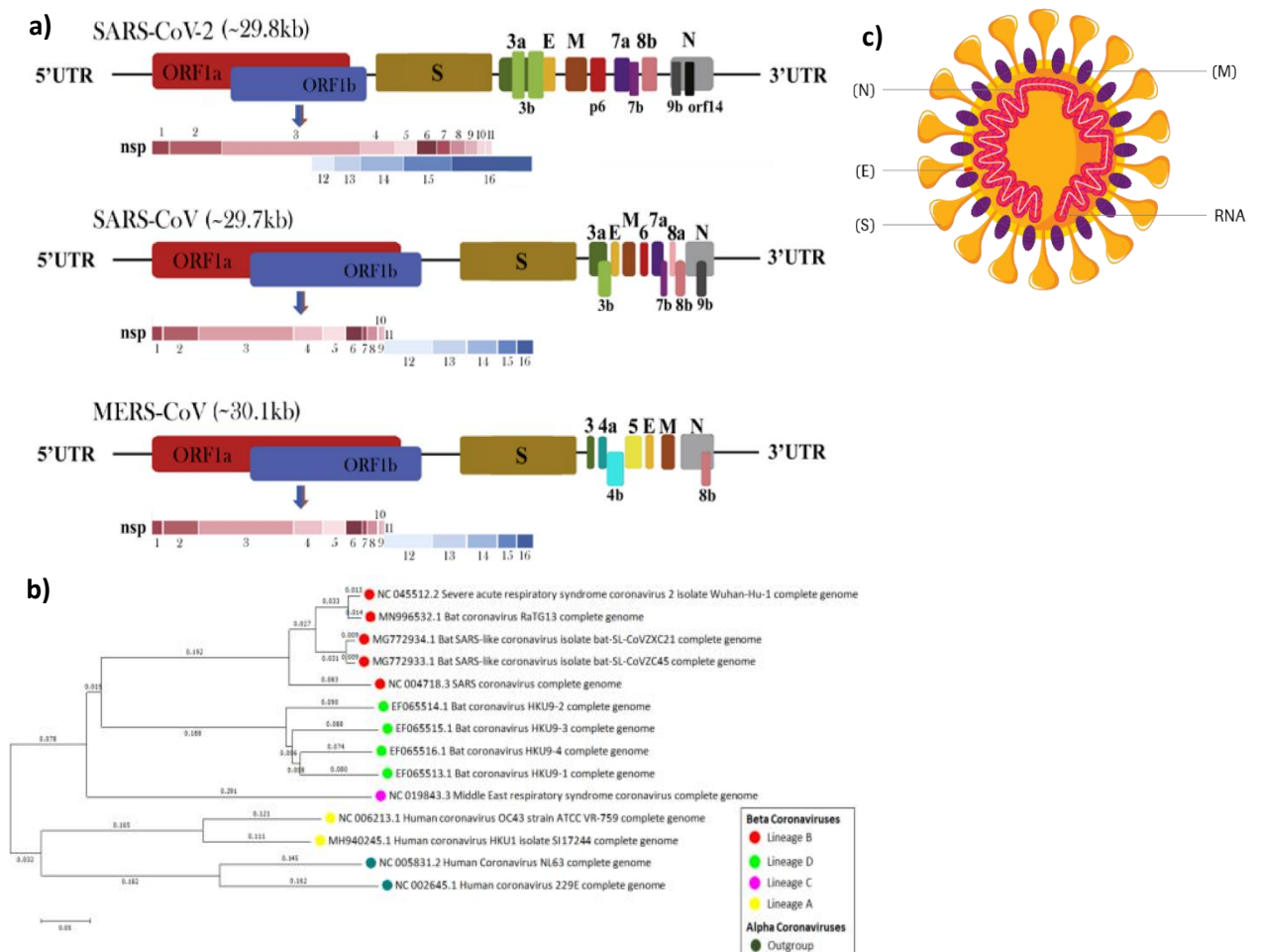


Figure 1 a) Genomic Structure of SARS-CoV, SARS-CoV-2 and MERS-CoV, b) Phylogenetic Analysis of SARS-CoV-2, *Betacoronaviruses* and *Alphacoronaviruses* and c) Viral Particle structure of SARS-

CoV-2. N- nucleocapsid, E- envelope, M -membrane protein, S-spike glycoprotein, Source: (a) Leila Mousavizadeh, Sorayya Ghasemi, (b) Goletic, T., Konjhodzic, R., Fejzic, N., et al.2021 (c) (Elecsys® Anti-SARS-CoV-2”)

The main determinant of coronaviruses tropism is the spike glycoprotein, that forms trimers on the surface of virions. The spike protein consists of two subunits: the S1 subunit, which binds to the host entry receptor angiotensin-converting enzyme 2, called (ACE2) (P. Zhou et al., 2020) and the S2 subunit, which mediates membrane fusion. These two subunits are separated by the S1-S2 site, that contains a furin cleavage motif and is cleaved in the virus-producing cell. After binding to ACE2 on the target cell, the spike protein is cleaved by the transmembrane serine protease TMPRSS2 at the S2' site (Hoffmann et al., 2020) (Lamers & Haagmans, 2022) this cleavage activates the S2 subunit trimers to fuse viral and host lipid bilayers, releasing the viral ribonucleoprotein complex into the cell. Another way to entry into the cell, is by the endosome, in which cathepsins can cleave the spike protein, but this route is not efficiently used in primary epithelial cells (Beumer et al., 2021)(Mykytyn et al., 2021).

During the SARS-CoV-2 infection, the first cells targeted are the cells in the nasopharynx or trachea, or sustentacular cells in the nasal olfactory mucosa (Ahn et al., 2021). After entry, the positive-sense SARS-CoV-2 genome directly initiates the production of viral proteins, including the replicase proteins that form replication factories on the endoplasmic reticulum membranes (Kikkert, Worm, & Zevenhoven-dobbe, 2008). These replication factories contain double-membrane vesicles in which transcription occurs, shielding the double-stranded RNA transcription from detection by the cytoplasmic pattern recognition receptors (PRRs). (Figure 2)

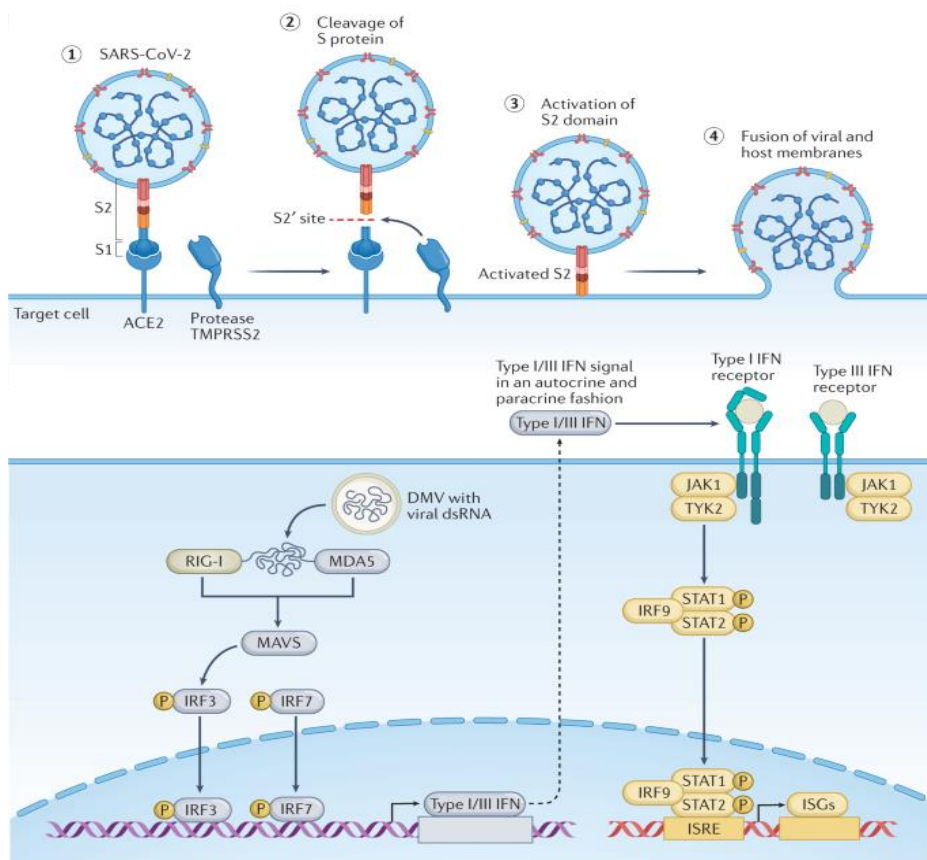


Figure 2- Cellular Pathogenesis of SARS-CoV-2, Source: (Lamers & Haagmans, 2022)

The main cytoplasmic PRRs capable of detecting SARS-CoV-2 is MDA5, (X. Yin et al., 2021) that recognizes the long double-stranded RNA and initiates a signaling cascade to promote the transcription of type I and type III interferons (Sampaio et al., 2021).

Interferons and chemokines are produced by the bystander epithelial cells and by local immune cells, for example, neutrophils and macrophages, in response to the presence of SARS-CoV-2 using the endosomal Toll-like receptors, TLRs, or paracrine effects of locally produced interferons (Candidate & Rezaei, 2021). Interferons signals the autocrine and paracrine effects to induce an antiviral cellular state through the production of interferon-stimulated genes, that have direct or indirect antiviral functions. During this phase, the production of cytokines also stimulates the development of adaptive B and T cells, to respond the viral infection. If the virus is not cleared through innate or adaptive responses, it can rapidly spread to the lower respiratory tract during the inhalation of virus particles from the upper respiratory tract or by gradual dissemination along the tracheobronchial tree (Figure 2).

This can lead to the infection of the alveoli, causing inflammation and limiting gas exchange. In the alveoli, the SARS-CoV-2 have shown to infect primarily alveolar type 2 cells in both *in vivo* and *in vitro* studies (Katsura et al., 2020). Whereas alveolar type 1 cells cover most of the alveolar surface and mediate gas exchanges, alveolar type 2 cells secrete pulmonary surfactants necessary for lubrication of the lungs, reducing the surface tension in the alveoli region. Additionally, the alveolar type 2 cells are the progenitors' cells of the alveolar type 1 cells in the adult human lungs, (Barkauskas et al., 2013), leading to more severe cases in older than in younger ages.

### **1.1.3 Origin, Wet markets & Natural Reservoirs**

Genomic sequencing and molecular evolutionary analyses of SARS-CoV-2 (Tabibzadeh et al., 2021), indicates that SARS-CoV-2 originated from virus reservoirs in nonhuman mammals, such as bats, through recombination and purifying selection (Singh & V. Yi, 2021).

COVID-19 was firstly identified in late December 2019, in Wuhan, China. On 30 January 2020, the WHO declared a public health emergency of international concern (PHEIC) over the novel coronavirus outbreak in Wuhan, a city located in mainland China, on the Hubei province, and that had already spread to 34 provinces across China (“International Health Regulations Emergency Committee on novel coronavirus in China”). By the 24 February 2020, the WHO acknowledged that the SARS-CoV-2 had a potential to spread rapidly and could cause a global pandemic outbreak (“COVID-19 Coronavirus Has Pandemic ‘Potential,’ Says WHO”). On 11 March 2020 WHO had declared the novel coronavirus (SARS-CoV-2) outbreak as a global pandemic (Cucinotta & Vanelli, 2020). (Figure 3)

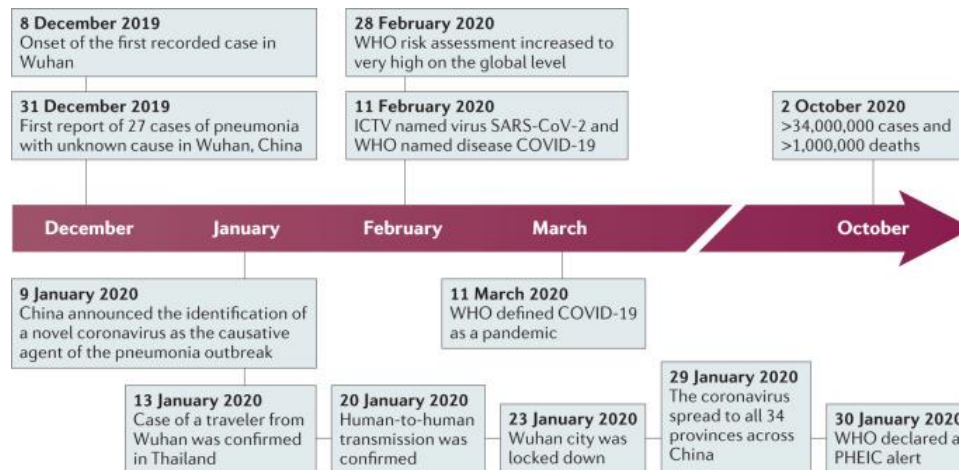


Figure 3 Timeline of events related to the COVID-19 pandemic. WHO - World Health Organization, PHEIC – Public Health Emergency of International Concern, SARS-CoV-2 – Coronavirus Severe Acute Respiratory Syndrome 2, COVID-19 – Coronavirus Disease 2019, Source: (Hu, Guo, Zhou, & Shi, 2021)

Recent studies reported that the COVID-19 pandemic started at the Huanan Seafood Wholesale Market, (Celis, Via, & Suggest, 2020). Genetic analyses of coronavirus samples collected from the market and from people infected between the months December 2019 and January 2020 was performed, as well as geolocation analyses connecting many of the samples to a section of the market where live animals were sold. These analyses add weight to original suspicions that the pandemic began at the Huanan Seafood Wholesale Market, which many of the people who were infected earliest with SARS-CoV-2 had visited. Taken those lines of evidence, it became the most likely source of the SARS-CoV-2 outbreak.

It was previously described that both SARS-CoV and MERS-CoV, had bats as the original reservoir (Sharun, Dhama, Pawde, Gort, & Rodriguez-morales, 2021). Likewise, SARS-CoV-2 virus shows high level of identity to bats coronaviruses. Its genome is 96% identical to the bat coronavirus RaTG13, detected in horseshoe bats (*Rhinopholus affinis*) in Yunnan Province in China (P. Zhou et al., 2020). Similarly, RmYN02, another bat coronavirus, has shown 97.2% similarity in the longest gene-encoding region (H. Zhou et al., 2020).

Different studies indicate that there were some intermediate species that facilitated the spillover of SARS-CoV-2 from bats to human. During the early days of the pandemic, there was a speculation that pangolins (*Manis javanica*) could have been an intermediate host (T. Zhang, Wu, & Zhang, 2020) for SARS-CoV-2. But recent Malaysian studies failed to identify any coronaviruses in pangolins rescued from the wild or confiscated from smugglers before they entered in illegal trade network. This suggests that the coronavirus found in Malayan pangolins in China may have been infected by another animal or by humans (J. Lee et al., 2020)

The intermediate hosts that played an essential role in the transmission of coronaviruses from bats to humans in the case of SARS and MERS outbreaks were palm civets (*Paradoxurus hermaphroditus*) and dromedary camels (*Camelus dromedarius*), respectively (Y. Zhang & Holmes, 2020).

Several reports of SARS-CoV-2 infection in different animals, wildlife species and experimental animal models have been published. However further detailed investigations

concerning the transmission potential of the virus from humans to animals and vice versa are needed, using an enhancing implementation of one health perspective (Prince et al., 2021).

More research studies and investigations regarding the SARS-CoV-2 circulation in animals and at the animal-human interface are needed, as well as targeted surveillance and dynamic risk assessments to aid the design and implement of effective preventive measures to limit the transmission of this or other potential pandemic viruses.

**1.1.4 Transmission, Clinical Symptoms, and Incubation Period**

CoVs are widespread among birds and mammals with cements bats forming the major evolutionary reservoir and ecological drivers of CoV diversity. These viruses can cause a large variety of diseases in pigs, cows, chicken, dogs, and cats. In humans, *Alphacoronavirus* and *Betacoronavirus* can cause a variety of illness ranging from mild-self-limiting respiratory infections to severe acute respiratory distress syndrome (ARDS).

**Clinical presentation of patients with CoVID-19**

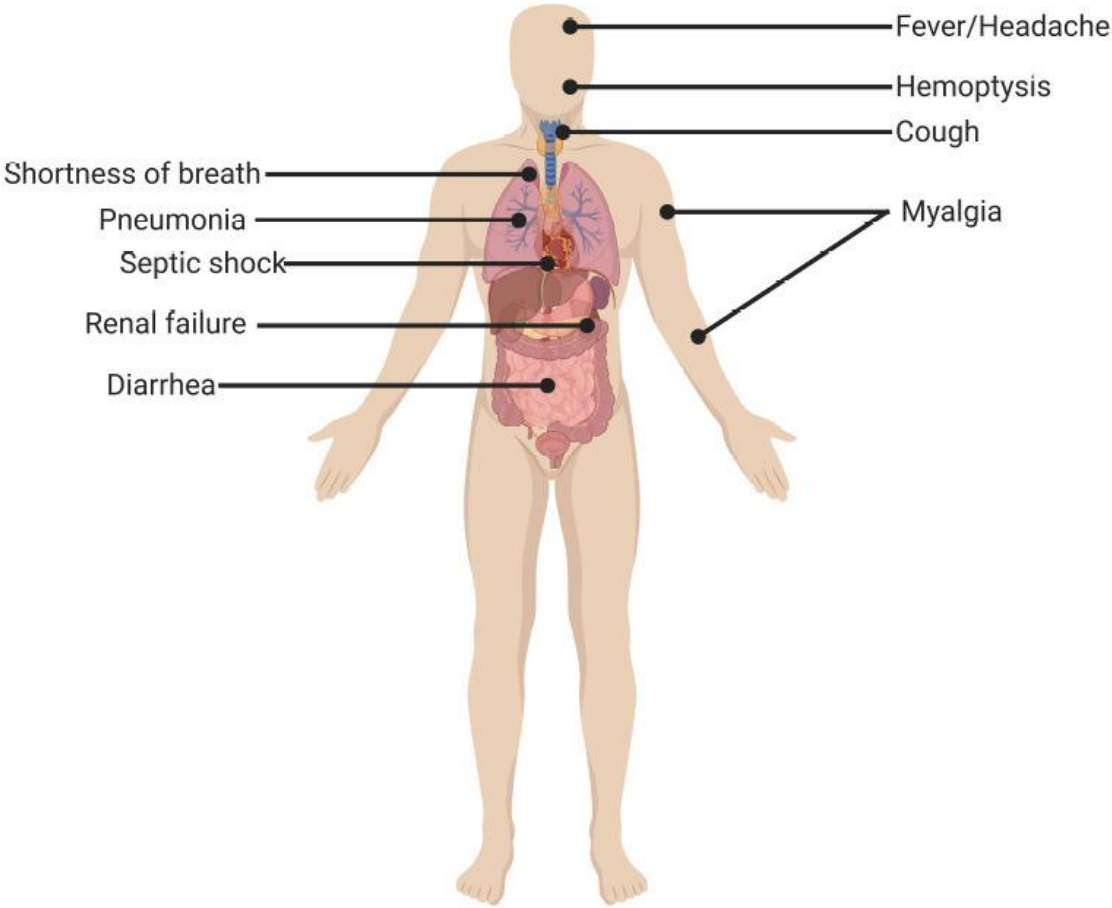


Figure 4 Clinical symptoms of COVID-19 on infected persons, Source: Cascella M, Rajnik M, Aleem A, et al. 2022

Initial cases reported in Wuhan, China, are considered to be an acquired infection from a zoonotic source from Huanan wholesale seafood market which sold poultry, snake, bats, and other farm animals. To identify the possible virus reservoir, a comprehensive genetic sequence analysis of coronaviruses was undertaken among different animal species. The results suggested that SARS-CoV-2 is a recombinant virus between the bat-CoV and an unknown

origin CoV. Recent studies reveal that based on relative synonymous codon usage (RSCU) on variety of animal species bats are the most probable wildlife reservoir of SARS-CoV-2.

Human-to-human transmission occurs through common routes such as direct transmission, transmission through aerosols and during medical procedures and have a median incubation period of 4-5 days (Lauer et al., 2020). Cough, sneeze, droplet inhalation, contact with oral, nasal and eye mucous membranes are the common modes of spread. Viral shedding occurs from respiratory tract, saliva, feces, and urine resulting in other potential less frequent sources of virus spread. The viral load is higher and of longer duration in patients with severe COVID-19. Spread of patients with COVID-19 to health workers and flight attenders who were in close contact with the infected patients were reported during the initial months of pandemic.

The clinical spectrum of symptoms of COVID-19 varies from asymptomatic to severe clinical illness characterized by acute respiratory failure requiring mechanical ventilation, septic shock, and multiple organ failure. Most symptomatic patients commonly present with fever, cough, and shortness of breath, sore throat, anosmia, dysgeusia, anorexia, nausea, malaise, myalgias and diarrhea (Figure 4) (Stokes, Zambrano, Anderson, Marder, & Raz, 2020). The spectrum of symptoms varies with the variants and variants that circulated more recently have been shown to frequently cause also rhinorrhea and nasal congestion. Based on the severity of presenting illness that includes clinical symptoms, laboratory and radiographic abnormalities, hemodynamics, and organ function. The COVID-19 infection can be categorized into five different types: (1) asymptomatic or presymptomatic infection, individuals with positive SARS-CoV-2 test without any clinical symptom consistent with COVID-19, (2) mild illness, individuals who have any symptoms of COVID-19, (3) moderate illness, individuals who have clinical symptoms or radiologic evidence of lower respiratory tract disease and who have oxygen saturation above 94% on room air, (4) severe illness, individuals who have oxygen saturation lower than 94% on room air, with a ratio of partial pressure of arterial oxygen to fraction of inspired oxygen, less than 300, with marked tachypnea respiratory frequency more than 30 breaths per min or lung infiltrates more than 50%, and (5) critical illness, individuals who have acute respiratory failure, septic shock, or multiple organ dysfunction.

To prevent COVID-19 infection it is important to assess the asymptomatic cases which are difficult to identify and propagate transmission, making transmission caused by these silent patients difficult to prevent.

Asymptomatic transmission can be defined as the transmission of SARS-CoV-2 from an asymptomatic person to a secondary patient, as ascertained by exposure and symptom onset dates, with no evidence that the secondary patient had been exposed to anyone else with COVID-19. Asymptomatic transmission is driven by two groups of patients, one with no self-perceived symptoms or clinically detectable signs throughout the 14 days of quarantine and the other transmitting the infection in the incubation period (i.e., the time period between getting infected and showing symptoms), so called presymptomatic transmission.

To assess the transmission rate among the population, an epidemiological tool can be used to verify the infection rate, which the  $R_0$  or  $R$  naught who measure the transmissibility of a determined infectious agent. Being the  $R_0$  a derivative of the followed variables – the duration of infectivity after patient gets infected, the likelihood of transmission of infection per contact between a susceptible person and an infectious individual, and the contact rate. The  $R_0$  is usually estimated retrospectively from serial epidemiological data or by theoretical mathematical models,

facilitating epidemiologists to calculate the  $R_0$ .  $R_0$  of COVID-19 was initially estimated by the WHO of being between 1.4 and 2.4, infections per person. That forecast helped governments to adopt strategies of control persons mobility quicker to avoid higher numbers of contamination right after the confirmation of COVID-19 infection (Achaiah, Subbarajasetty, & Shetty, 2020).

### **1.1.5 Asymptomatic Cases/Individuals and their role to transmit the COVID-19 disease**

Studies indicates that human-to-human transmission of SARS-CoV-2 in family settings are more likely to occur, suggesting that asymptomatic patients might serve as a possible source to propagate the COVID-19 outbreak. This evidence was mostly observed in cluster outbreaks, especially in family cluster outbreaks that occurred in China.

According to various epidemiological surveys, many source patients are asymptomatic when transmit the virus to secondary cases (Figure 5). The first study reported in January 2020 identified five patients with COVID-19 infected by a 20-years-old asymptomatic woman, who was thought to have acquired SARS-CoV-2 infection from the epidemic center of Wuhan. This patient showed no symptoms during the entire period of monitoring and isolation (Section A, Figure 5). The second study conducted by Tong et al., showed that several COVID-19 patients were infected after exposure to an asymptomatic person from Wuhan, who was later confirmed to be positive for SARS-CoV-2, after their contact with the positive case, they later transmitted the infection to their family members (Section B, Figure 5). Another study conducted by Li et al confirmed asymptomatic human-to-human transmission through close contacts in familial and hospital settings by analyzing the epidemiological, laboratory, and clinical data of 7 COVID-19 patients in a 2-family cluster. The epidemiological survey identified the source patient, a man who had had a short stop at the Hankou Station in Wuhan. (Section C, Figure 5). Another study conducted by Qian et al, evaluated a family cluster of asymptomatic COVID-19 transmission in Zhejiang, China. Two source patients were infected with SARS-CoV-2 after a visit to a spiritual attraction, who transmitted the virus to four family members before the onset of any symptoms. Later on, these four patients, infected three other relatives before knowing they're positive status. (Section D, Figure 5). A recent study conducted by Luo et al., showed that SARS-CoV-2 can be spread through sustained transmission, for at least two generations of spread, by asymptomatic/presymptomatic individuals (section E, Figure 5). On the last section, (section F, Figure 5), a source patient is highlighted, who had a travel history to the city of Huanggang, in Hubei province during the month of January 2020. During the visit through the city, he got infected by COVID-19, became an asymptomatic COVID-19 carrier, transmitting the virus to his cohabiting family members without knowing (Han, Li, Han, Zhang, & Li, 2020).

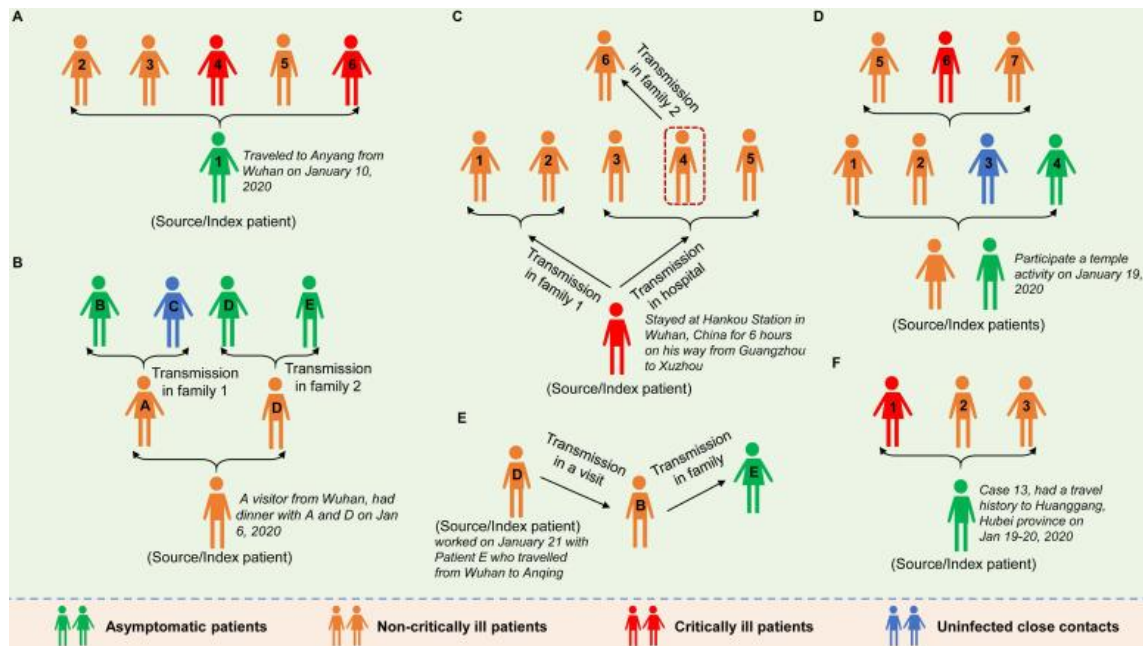


Figure 5 Transmission by the asymptomatic/presymptomatic individuals, based on studies published, Source: (Han et al., 2020)

In all the previous reports, the source patients remained either without any symptom of infection during the clinical course, (A, D and F) or developed varying degrees of pneumonia (B, C and E).

Since the asymptomatic and presymptomatic transmission is clearly established for the transmission of SARS-CoV-2, it is important to understand, the proportion of individuals that are infected through asymptomatic transmissions. This is an important question that can be responded with epidemiological tools and with the help of bioinformatics analysis that can cross information based on people contacts and mobility.

### 1.1.6 SARS-CoV-2 Comorbidities

In humans, SARS-CoV-2 infections may be asymptomatic or accompanied by clinical symptoms (Wilder-smith et al., 2005) (N. Chen et al., 2020). In certain cases, particularly in elderly and immunocompromised individuals, coronavirus infections may lead to severe pneumonia and subsequently, the death of the patient (Jarti et al., 2011).

Increased age, obesity and male sex are well-established risks factors for the development of severe COVID-19 (Williamson et al., 2020). Common co-morbidities including hypertension, heart failure, cardiac arrhythmia, diabetes, kidney failure and chronic pulmonary disease, (Karagiannidis et al., 2020) are important established risk factors for developing severe COVID-19. Such identified risk factors can also be informative in the understanding of SARS-CoV-2 pathophysiology (Lamers & Haagmans, 2022). The percentage of COVID-19 patients requiring hospitalization was six times higher in those with preexisting medical conditions than in those without medical conditions, (45.4% vs 7.6%), based on a study conducted by Stokes et al. of confirmed cases reported to the CDC during the months of January 22<sup>nd</sup> to May 30<sup>th</sup>, 2020. (Stokes et al., 2020) This study also reported that the percentage of patients who succumbed to this illness was 12 times higher in those with preexisting medical conditions than in those without medical conditions, (19.5% vs 1.6%) (Stokes et al., 2020). Data from the study,

regarding to the gender-based differences in COVID-19 severity, indicates that male patients are at higher risk of developing severe illness and creased mortality compared to female patients (Gebhard, Regitz-zagrosek, Neuhauser, Morgan, & Klein, 2020). Another study from March 1<sup>st</sup> to November 21<sup>st</sup>, 2020, evaluate the mortality rate in 209 US hospitals, showing that male patients had a higher mortality rate, (12.5%), compared to female patients (9.6%) (Finelli et al., 2021).

Regarding to the COVID-19 cases in different races and ethnic groups, disparities, also are evident (Y. Cui et al., 2020). During the COVID-19 pandemic, racial and ethnic minorities groups have reported to have a higher percentage of COVID-19 related hospitalizations, compared with White/Caucasians patients based on CDC analysis of hospitalizations from a large administrative database, including approximately 300 000 hospitalizations from March to December 2020 (Romano et al., 2021). The high percentage of COVID-19 hospitalizations among the different racial and ethnic groups was driven by a higher risk of exposure to SARS-CoV-2 and to an increased risk of developing severe COVID-19 disease (Romano et al., 2021). Several studies conducted in US and UK, shown that Black, Hispanic, and Asian ethnic minority groups had an increased risk of contracting and dying from COVID-19 infection, being the Hispanic group the one with a higher death rate (Ahmad, Cisewski, Miniño, & Anderson, 2021).

#### **1.1.7 Number of Cases and Death Toll**

Worldwide the COVID-19 cases, on 1 July 2022 are 544 million confirmed cases and 6 million deaths related to COVID-19 complications. (WHO)

In Portugal, the first confirmed case of COVID-19 was reported on March 2<sup>nd</sup>, 2020. Until March 10<sup>th</sup>, 2022, 3.3 million confirmed COVID-19 cases and 21 000 deaths were reported (“RELATÓRIO DE SITUAÇÃO - Covid 19”).

#### **1.2 Methods for diagnosis**

Since the first release the genomic sequence of SARS-CoV-2, various methods for identification of SARS-CoV-2 were created for commercial usage, separated in three types of possible tests. These can be divided in methods that diagnose active infection with SARS-CoV-2 (PCR and Antigen tests) and those that diagnose an active or prior infection with SARS-CoV-2 (Antibody/serological tests) (“The researchers taking a gamble with antibody tests for coronavirus”). The first type is a molecular diagnostic technique for genetically detection of SARS-CoV-2 infection, including reverse transcription-polymerase chain reaction (RT-PCR), real-time RT-LAMP, recombinase polymerase amplification (RPA), high-throughput sequencing of the whole genome, and cluster regularly interspersed short palindromic repeats/CRISPR associated (CRISP/Cas)-based approach (Rahimi, Mirzazadeh, & Tavakolpour, 2021). Another SARS-CoV-2 diagnostic, the antibody detection test, is a serologic tool to evaluate the antibodies produced by the immune system in response to the viral infection. If the individual were infected it will be producing IgM and IgG, two biomarkers used to evaluate the possible past-infection of the individual (Yüce, Filiztekin, & Gasia Özkaya, 2021). The third type is an antigen detection test by lateral flow assays that need to be performed by trained personnel, under specified experimental and Biological Safety Level (BSL) conditions (Yüce et al., 2021).

### **1.2.1 RT-PCR**

The RT-PCR is a version PCR method explicitly developed for RNA detection. RT-PCR is a reliable and fast technique, producing results in a few hours in a high throughput manner. RT-PCR technique is based on two consecutive reactions: a) conversion of RNA into complementary DNA (cDNA) through reverse transcription enzyme and b) amplification of the cDNA sample by polymerase chain reaction using gene-specific primers and fluorescently labeled hydrolysis probes (Yüce et al., 2021).

RT-PCR technique is currently the gold standard for SARS-CoV-2 detection tool due to its capacity to directly amplify the viral genome rather than the secondaries biomarkers such as antigens or antibodies. These kits can be used for samples from various infected parts of the body, including nasopharyngeal, oropharyngeal, or nasal swabs, upper and lower respiratory tract aspirates, bronchoalveolar lavage, and the sputum. Although the RT-PCR remains the most reliable method for SARS-CoV-2 detection so far, only a limited number of tests could be performed daily, which in periods of high incidence prevents the authorities from obtaining consistent data on the prevalence of the virus in the entire population. The sensitivity of this method usually depends on the viral RNA amount in each sample. The patients can be categorized as SARS-CoV-2 positive or negative based on the outcome of the RT-PCR result.

In this test, the patients who are in the very first days of the viral infection may not respond as “positive” with this test due to the insufficient amount of the viral load in the body and on the swab. On the other hand, a positive test may occur when the patients are no longer infectious, and pieces of viral RNA are left in the respiratory tract. This technique does not give information, about history of infection for the patients who have already recovered from the SARS-CoV-2, and the viral load has been cleared out of the body after recovery.

In this context, antibody tests are important to investigate who has already had the virus and/or developed protective antibodies due to previous infection with SARS-CoV-2.

### **1.2.2 Antigen detection test**

An antigen is a particle/fragment/ molecule that can trigger the immune system and induce antibody production to kill pathogens, protecting the host body. The antigen test unlike the PCR-based method, detects the viral component (i.e., S glycoprotein, M protein or released N protein) or the virus direct without thermal amplification. As the PCR-based methods, the antigen test reveals the active viral infection. Mainly because the antigens precede antibodies and are target-specific, they are more reliable to detect active infection compared to IgM antibody tests. On the other hand, they are less sensitive than PCR tests.

Antigen tests can be utilized on lateral flow assays, or also known as lateral flow immunochromatographic assays (LFA), who are simply immunoassays based on diagnostic devices used to detect the presence or absence of a target analyte (i.e., antibodies) in biological or other samples. This type of tests are qualitative (positive or negative) rapid diagnosis test (RDTs) used for point-of-care testing (POCT) for the detection of the presence of viral proteins (antigens) expressed by the SARS-CoV-2 virus in samples from human respiratory tract. If the targeted antigen is present in sufficient concentration in the sample, it will bind to certain antibodies embedded in a piece of paper embedded in a plastic lump and produce a clear visible signal, usually within 30 minutes after testing. As the antigens are only detected when the virus is replicating, such tests are best used to identify severe or early infection.

COVID-19 self-tests are also Antigen tests, sold in pharmacies or prepharmacies. Due to its low complexity, it can be performed by people other than health professionals or other qualified professionals to perform a self-test to diagnose infection with SARS-CoV-2. In Portugal, the Infarmed only approved the first COVID-19 self-test for the general population on 26<sup>th</sup> March 2021, by (Testes COVID-19 - INFARMED). From then on, people who bought self-tests needed to report their result, through a form available online, on the website of the DGS, or by calling to the line SNS24 (ePORTUGAL,2021).

The assays outcome depends on diverse factors such as time from the onset of the disease, the viral load in the specimen, the variant of infection, the quality of the sample collected from the individual and its processing, and the exact composition of the reagents used on test kits.

### **1.2.3 Antibody detection tests**

An antibody is a protein produced by the immune system in response to an antigen. Each antibody has sites that can bind only one specific type of antigen to remove it from the body. This specificity is determined by Complementary Determining Regions (CDRs), localized on N-terminus of the antibody. Antibodies are also called immunoglobulins (Ig), the antibodies are categorized in five classes existing IgM, IgD, IgG, IgA and IgE, which are differentiated by the C-terminus region. IgM is the first antibody produced during an infection, while the IgG is the most common and abundant one in serum. As antibodies are secreted via mucosa and blood, antibody tests can evaluate the presence or concentration of IgG and IgM levels in the blood/serum/plasma samples.

The most common antibody tests used are the lateral flow type assays (LFA) and enzyme-linked immunosorbent types of assays (ELISA). The ELISA test is the most used to evaluate the presence or concentration of antibodies. Recombinant viral antigen is coated onto the surface of plastic wells as the target molecules. Once the wells are prepared, the sample, i.e., the patient's serum, is added to the well and if the antibodies IgG or IgM against the target antigen are present in the sample, a binding process occurs. Those in excess during the first step are washed out to ensure all unbound substrate are removed. Then, a second solution with secondary anti-human antibodies are added and allowed to bind, if the antibody of interest is absent on the sample, no binding occurs. Those in excess are washed out, and the binding of target antibodies is confirmed by an enzyme-dependent color changing reaction. For the evaluation of the concentration or presence of antibodies, an imaging reading of the color change is performed. The ELISA test can only be performed in laboratories with authorization to perform moderate or high complexity tests, since the ELISA protocol requires multistep, long turnaround time, skilled personnel, and specific instrumentation.

One big issue concerning serological tests is the cross-reactivity. Cross-reactivity occurs when the antibodies bind to an antigen different from the targeted one, often due to their similarities between the molecules. The biggest issue with this cross-reactivity is that it can lead to false positives – a patient previously infected with SARS-CoV or MERS-CoV, due to their genetic similarities to SARS-CoV-2, can test positive for SARS-CoV-2, despite never having been infected by this last virus (Qi, Liu, Wang, & Zhang, 2022). Additionally, because antibodies are developed through the course of an infection and may lose their antibodies to SARS-CoV-2, a negative result on an antibody test may not confirm that the patient is/was uninfected. A majority of patients develop antibodies to SARS-CoV-2 after around 14 days in response to SARS-CoV-2 infection, while a recent study shows that both IgG and IgM

antibodies were detected five days later (Loeffelholz & Tang, 2020)(Cevik, Bamford, & Ho, 2020). This late response may lead to false negatives, where the patient is infected but has not yet produced antibodies at detectable levels, therefore it is essential to establish the sensitivity and specificity values of tests to comprehend the possibilities of false positives and false negatives (Li et al., 2020).

On the other hand, due to the late detection of antibodies, serological studies may be retroactively beneficial in epidemiologic studies, vaccine studies, and late complications, where the RT-PCR testing may yield false negatives due to cross-reactivity from previous SARS-CoV or MERS-CoV infections or low viral loads in the tested samples (Qi et al., 2022). Although the antibody test cannot tell us who is infected, as the antibodies are generated up to two weeks after the infection, it can tell us who developed an immunity to the virus or what proportion of the population has been infected by the coronavirus. Despite the homology between SARS-CoV-2 and other human coronavirus being lower, it remains immunologically relevant. The memory B cells that were produced from previous infections may contribute for the rapid antibody response, in particular the IgG and IgA (Dugan et al., 2021).

#### **1.2.4 Other methods**

The urge for testing SARS-CoV-2 infections is still a matter of emergency due to the continuous viral spreading among the population all around the globe. It would be a tremendous help to fight the viral infections if a precise and quicker test was developed due the previous methods limitations. Therefore, researchers all around the globe are seeking to develop a test that could deliver fast and accurate results, without compromising the sensitivity and selectivity. Some of the novel research-stage methods proposed for SARS-CoV-2 detection are still in development. But some methods are becoming published in various scientific journals being these methods relying on field-effect transistors (FET) and surface plasmon resonance (SPR) principles that are known to deliver fast and sensitive signals.

The SPR principle is based on the collective oscillation of electrons on a given metal surface, commonly used to monitor molecular interactions in proximity between protein-protein, protein-DNA, protein-RNA, antigen-antibody, enzyme-substrate samples in real-time, with great sensitivity (Yüce et al., 2021). The SPR method proposed by (Djaileb, Charron, & Hojjat), were based on gold chips modified by the viral nucleocapsid proteins, to detect the antibodies in human serum samples in 15 minutes after the biological sample collection with nanomolar sensitivity, but no clinical testing was performed. Other SPR method in development is based on plasmonic photothermal effect (PPT) and localized surface plasmon resonance (LSPR), published by Qui et al., which uses gold nanoparticles decorated with oligonucleotides that are complementary to the selected viral gene regions. This dual-function system successfully detects the RdRp gene, E gene, ORF1a gene regions with picomolar sensitivity. This system works in real-time and gives reliable results in minutes on the antigen or antibody-modified surfaces. For that reason, SPR can be considered as an alternative testing station to laboratory-based antigen and antibody kits for faster SARS-CoV-2 diagnosis of the clinical samples.

The FET principle is based on graphene key properties, such as optical transparency, electrical conductivity, robust pi-interactions with macromolecules and tuneability, competing with similar materials in the field. In FET-based biosensors, biological molecules change the charge distribution of the surface, or induce a surface potential by binding to the surface, which

is then measured as a conductance value with high sensitivity. On a study conducted by Seo et al., the graphene sheets are coated with SARS-CoV-2 spike protein-specific antibodies used as active surface for monitoring the signal produced by the viral protein binding. Another study conducted by (Zhang et al.), used an electrical probe where the FET surface was modified with the receptor binding domain to be able to detect viral S glycoprotein. This platform showed an excellent sensitivity down to the picomolar level in real-time without a specific label. Graphene-based electrodes are commonly used in many laboratories specialized in electrochemical techniques. Mainly due to their simple surface modification of the graphene electrodes it can be converted as a sensor for many viral antigens, being a portable detection system compared with those in “classical methods” for SARS-CoV-2 detection.

Another method was developed, a Loop-mediated isothermal amplification (LAMP), as a rapid SARS-CoV-2 detection tool. This method is a cost-effective alternative to standard PCR given that here the expensive thermocycler instrument that operate for the amplification step/process, is not needed. This method can synthesize up to  $10^9$  copies of the targeted gene in less than an hour, and the amplification reaction is processed in one single tube at 60-65°C by using two to three different gene-specific primers sets and strand-displacement polymerase enzymes. Magnesium pyrophosphate is formed during this process, which can be measured by the naked eye or by a simple analytical device, such as turbidimetry or photometry, for an increased sensitivity. Dyed molecules can be employed in the reaction for selective quantitative detection of the target genes. A Reverse Transcription LAMP (RT-LAMP) applies an additional transcription step for the detection of RNA targets. At the moment, only one RT-LAMP-based kit, “Sherlock™ CRISPR SARS-CoV-2” kit, was developed and received emergency use authorization (EUA) from the FDA. This kit was designed to target ORF1ab, N and RP genes, being the third gene an internal extraction control gene. The RT-LAMP assay has showed 95% accuracy in clinically validated positive samples (n=45), and no cross-reactivity was observed in any of the clinical validated negative's samples (n=25). The analytical tools that rely on the LAMP principle for SARS-CoV-2 detection may give “false negatives” results for the patients who passed the infection similar to RT-PCR. Researchers are still tuning the current model to improve the test sensitivity as well the specificity, by decreasing the viral load required for more reliable test result.

Despite the unprecedented researchers' efforts to develop sensitive, selective, and rapid test kits for the general population, only a small portion of the world population had been tested by April 2020, and the testing priority was given to those who shown severe clinical symptoms for the COVID-19 disease or frontline healthcare providers, such as nurses, medics, and health assistances (Maxmen, 2020). For that reason, it was necessary to evaluate the presence of the SARS-CoV-2 virus among the population, conducting serological surveys to evaluate the presence of antibodies against the novel SARS-CoV-2 virus.

### **1.3 Innate and Adaptive Immunity**

The immune system is a collection of cells, chemicals and processes that function to protect the skin, respiratory passages, intestinal tract and other areas from viruses, cancer cells, toxins, microbes (such as bacteria, fungi, and parasites) and foreign antigens (Marshall, Warrington, Watson, & Kim, 2018). Besides the structural and chemical barriers that protect us from infections, the immune system can be simplistically viewed into the innate immune system and into adaptive immune system. Even though the adaptive and innate immune systems are connected in important and powerful ways, each one consists of different cell types performing

different roles. The adaptive immune system is composed of T and B lymphocytes and the innate immune system with all other immune cell types of which granulocytes, monocytes, macrophages, and NK cells, are the more common cells, but many other innate immune cells also exist including different dendritic cells, innate lymphoid cells, or mast cells.

Most immune responses involve both the innate immune system and the adaptive immune system, evoking innate and adaptive immunity, respectively. The extent to which both arms are recruited to an immune response differs between stimuli, being the innate immune system the first line of host defense, being an antigen-independent (non-specific) mechanism, against pathogens. Its response limits the viral entry, translation, replication, and assembly and it helps identifying and removing the infected cells, that coordinate and accelerate the development of adaptive immunity (Qi et al., 2022)(Marshall et al., 2018). The innate immune response has no immunologic memory, therefore, it is unable to recognize or “memorize” the same pathogen when exposed to it in the future. Although, adaptive immunity is antigen-dependent and antigen-specific, therefore involves a lag time between exposure to a foreign antigen and his response. The keynote of the adaptive immunity it is their capability for memorize where to bind on foreigners’ antigens/ pathogens, leading to a quicker and efficient immune response (Marshall et al., 2018).

Therefore, the innate and adaptive immunity are not mutually exclusive mechanism of host defense, but rather complementary in either system resulting in host vulnerability or inappropriate responses.

### **1.3.1 Innate immune response to SARS-CoV-2**

Recent reports concerning patients with SARS-CoV-2 pneumonia identified interleukin (IL)-6 as a potential pathogen factor in the initiation of acute respiratory distress syndrome (ARDS). IL-6 is a pleiotropic cytokine which functions as a mediator for both innate and adaptive immune functions. It has diverse immune and biological actions, including direction of immune cell differentiation, sentinel responses to invading pathogens and ischemic injury. It is also critical for plasma cell growth and immunoglobulin production. Excessive and unregulated IL-6 transcription is commonly seen in patients with autoimmune or inflammatory disorders. Data from patients with SARS-CoV-2 suggest that IL-6 transcription is initiated and sustained after respiratory epithelium is infected.

The virus has a tendency for the activation of alveolar and circulating macrophages, resulting in copious and sustained IL-6 production resulting in the cytokine storm, endothelial cell damage, capillary leak, and clinical and pathological features of ARDS. The inhibition of IL-6 production or blockage binding receptor could be an important therapeutic option for limiting morbidity and mortality (Jordan, 2021).

### **1.3.2 Adaptive immune response to SARS-CoV-2**

Adaptive immunity involves the co-ordination of T and B cell immune responses to the SARS-CoV-2 virus. Adaptive immunity is responsible for long-lasting and possible sterilizing immunity to the virus, and it is very important in the long term to ascertain the nature of the B and T cell immune events and whether they result in long-lasting immunity with B or T cell development or dissipating over time, resulting in a risk for re-infection.

Humans make SARS-CoV-2 specific antibodies, in this case CD4<sup>+</sup> and CD8<sup>+</sup> T cells in response to SARS-CoV-2 infection. Therefore, antibodies and T cells, (CD4<sup>+</sup> and CD8<sup>+</sup>), each

have a protective role in control viral infections, but the role and importance of each component depends on the viral infection. For that reason, is important to measure antigen-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells and antibodies on the individual.

T cells are detected after almost all SARS-CoV-2 infections (Sette & Crotty, 2021), being the CD4<sup>+</sup> T cell more prominent, due that, it was being associated of primary control of SARS-CoV-2 infection. Nevertheless, is particularly important to evaluate the T cell responses against SARS-CoV-2 Spike protein, due the fact that is the binding site of SARS-CoV-2 virus to the host ACE2 receptor (Jordan, 2021; Sette & Crotty, 2021)

#### **1.4 Serological studies**

Serological studies are important to understanding the real incidence of SARS-CoV-2 virus infection, given that many patients are asymptomatic and remain undiagnosed. COVID-19 epidemics, involve imported cases, clusters of community transmission linked to workplaces, social gatherings and nursing homes and outbreaks among the migrants workers. These are several factors that facilitate propagation of the SARS-CoV-2 virus in the population (Clapham et al., 2020) and (Tee et al., 2020). To mitigate the active cases of COVID-19, public health policies were implemented involving a comprehensive set of control measures, including isolation of COVID-19 cases in hospitals or dedicated government facilities, extensive manual or digital contact tracing, strict quarantine rules, testing of incoming travelers and of exposed individuals. Gradual border closures were implemented worldwide from the end of January 2020, with border closures to all but essential services at the end of March 2020. At early stages of the pandemic, the success of public health measures was particularly dependent on their timely implementation, and on comprehensive diagnosis/surveillance systems to be able to efficiently trace where the virus was being introduced or circulating.

##### **1.4.1 Worldwide**

Across the world, several sero-epidemiological surveys were conducted to evaluate and estimate the seroprevalence among different populations. Several serological investigations across the world have been published during the 12 months of the COVID-19 pandemic. Several systematic reviews and meta-analyses of SARS-CoV-2 seroprevalence were carried out to access the seroprevalence among different populations and sub-populations (Chen et al.,) conducted a systematic review and meta-analysis from the serological studies available from PubMed, Embase, Web of Science and other five preprint servers. 16 506 studies were identified from the initial search, but only 404 serological studies were included in the meta-analysis. The meta-analysis (Chen et al.,), divided five different groups of exposure, within each WHO region. Close contacts, high-risk health-care workers, low-risk health-care workers, general population, and poorly defined populations. It was concluded that close contacts (18.0%, 95%CI 15.7 – 20.3) and high-risk health-care workers (17.1%, 95%CI 9.9 – 24.4) had a higher seroprevalence than did low-risk health-care workers (4.2%, 95% CI 1.5 – 6.9) and the general population (8.0%, 95%CI 6.8 – 9.2). The seroprevalence also varied across WHO regions, with the lowest seroprevalence of general populations in the Western Pacific region (6.9% 95%CI 0.0—5.0).

From the several serological surveys conducted, only a few were focusing on the migrants population. Herein, we highlight two of these studies the first one being conducted in Oman, and the other one in Singapore.

The Oman's serological survey was conducted during the first 11 months of the COVID-19 pandemic. This sero-survey used a multi-stage stratified sampling method from July to November 2020. A questionnaire was used to gather demographics, history of acute respiratory infection and symptoms, COVID-19 contacts, previous diagnosis or admission, travel history and risk factors information. During this survey 17457 participants were evaluated, 30% of which were female and 66,3% were Omani. Each Cycle (Cycle 1- Cycle 4) represented 5 to 10 working days, with an interval of 2-4 weeks between each of them. Starting the Cycle 1 from 12 July 2020, Cycle 2 from 16 August 2020, Cycle 3 from 13 September 2020 and Cycle 4 from 8 November 2020 (Al-abri et al., 2021).

The seroprevalence ranged from 5.5% (4.8 – 6.2%) in Cycle 1 to 22% (19.6 – 24.6%) in Cycle 4. There was no difference in seroprevalence between the different genders but significant differences between age groups. The seroprevalence in non-Omani was higher compared to Omans in Cycle 1 (9.1% (7.6 – 10.9%) vs 3.2% (2.6 – 3.9%)) and in Cycle 4 (24.3% (21.0 – 27.9%) vs 16.8% (14.9 – 18.9%)). The participants who had close contact or contacted persons with COVID-19 had a 96% risk to be infected, with adjusted odds ratio (AOR) of 1.96 (95% CI: 1.64 – 2.34). Among the different working classes, laborer's had a 58% higher risk to be infected compared to office workers, with AOR of 1.58% (95% IC: 1.04 – 2.35) (Al-abri et al., 2021).

The serological study conducted in Singapore was divided in two cohorts, the first one was in a migrants worker dormitory and the second was in general population living in Singapore. The study was conducted during the months May to July 2020, collecting blood samples on recruitment and 2 to 6 weeks after the first collection. The incidence of SARS-CoV-2 infection in migrants workers and in the general population was determined by the detection of neutralizing antibodies against SARS-CoV-2 and adjusting assay for sensitivity and specificity through a Bayesian model framework. No evidence of community exposure to SARS-CoV-2 was found prior to September 2019. The data from the study estimate that < 2 per 1000 adult's residents in the community were infected with SARS-CoV-2 in May to July 2020, with a cumulative seroprevalence of 0.16% (95% IC: 0.008 – 0.72%), a comparison with a comprehensive national case which suggested that 1 in 4 infections in the general population had symptoms associated with the COVID-19 disease. In contrast with the migrant worker cohort, where two-thirds had been infected by July 2020, presenting a cumulative seroprevalence of 63.8% (95% IC: 57.9 – 70.3%), with no symptoms reported among these infections.

#### **1.4.2 Portugal**

In Portugal the first COVID-19 case was reported on 2 March 2020. Until 8<sup>th</sup> June 2020, approximately 45477 cases and 1644 deaths had been reported by the Direção-Geral da Saúde 2020. With the aim of studying the epidemiology of SARS-CoV-2, Portugal conducted the first national sero-epidemiological survey for COVID-19, coordinated by the Instituto Nacional de Saúde Doutor Ricardo Jorge (INSA), in conjugation with the National Association of Clinical Laboratories (ANLC) and the Hospitals from the National Health Service (SNS).

The objective of the National Serological Survey was to characterize the distribution of specific antibodies to SARS-CoV-2 and to evaluate the extension of infection by SaRS-CoV-2 on the resident population in Portugal. The seroprevalence of specific antibodies to SARS-CoV-2 was compared between age groups and between parts of the country. Another objective was

to ascertain the fraction of asymptomatic infections by SARS-CoV-2 in the resident population in Portugal.

During the first serological study 2301 samples from residents in Portugal were analyzed, aged 1 year or more, stratified by age group. Socio-demographic, epidemiological, and clinical data were collected, through the application of a questionnaire. A blood sample was collected from each of the participants, in 96 collection points from 7 clinical pathology laboratories of the National Association of Clinical Laboratories, and in 18 hospitals of the National Health Service, between May 21 to 8 July 2020. According to the case definition selected in this study (presence of IgM or IgG specific against SARS-CoV-2), at the national level, a seroprevalence of 2.9% (95% IC: 2.0 – 4.2 %) was estimated. This value was much higher than the cumulative incidence of the reported infection, at the end of the study period, by the National epidemiological Surveillance System (SINAVE) (0.44%), and also higher than the proportion of participants who, in this study, reported previous SARS-CoV-2 infection (0.8%). Regarding the distribution by sex, the estimated seroprevalence was higher in males (4.1% 95% IC: 2.6 – 6.6%) compared with females (1.8% 95% IC: 0.9 – 3.4%). Similar seroprevalence values were observed in the studied age groups. Ranging from 2.2% (95% IC: 0.8 – 5.5%) in the 10 to 19 age group and 3.2% (95% IC: 1.5 – 6.7%) in the 40 to 59 age group. At the regional level the seroprevalence varied between 1.2% (95% IC: 0.3 – 4.0%) in Alentejo and 3.5% (95% IC: 1.9 – 6.3%) in Lisbon and Vale do Tejo, yet these differences were not statistically significant. Concerning education level, statistically significant differences were found between the higher seroprevalence for the individuals who have secondary education (6.4% (95% IC: 3.2 – 12.5%)) and those with a higher education who had a lower seroprevalence (1.4% (95% IC: 0.6 – 3.4%)) (DGS, 2020).

The seroprevalence in individuals who had previous contact with a suspected or confirmed case of COVID-19 was higher compared to those who did not have contact with suspect or confirmed cases of COVID-19, (22.3% vs 2.0%). Those who reported anosmia or three or more symptoms including: fever, chills, asthenia, odynophagia, cough, dyspnea, headache, nausea/vomiting, and diarrhea had a higher seroprevalence of 6.5% (95% IC: 2.6 – 15.3%).

The global value of seroprevalence of specific IgM and IgG against SARS-CoV-2 estimated in the ISN COVID-19 is close to the values obtained in sero-epidemiological studies carried out in other countries. The estimated value is compatible with a limited extent of infections in the Portuguese population, between March and June 2020, and far from the value thought necessary to achieve herd immunity (projected in the literature at that time to be between 43 to 67%). The differences observed between seroprevalence, and cumulative incidence of infection reported by SINAVE and the proportion of participants who, in this study, reported previous SARS-CoV-2 infection are consistent with evidence of a lower capacity to capture mild or asymptomatic cases by routine surveillance systems. About 44% of individuals with specific antibodies against SARS-CoV-2 reported no previous symptoms associated to COVID-19.

The results of this National survey supported the maintenance of individual and collective protection recommendations for all individuals, regardless of their level of specific antibodies against SARS-CoV-2 and supported the need to monitor the evolution of its seroprevalence in the Portuguese population. After the first report released by the DGS during the month of July

2020, two reports updating the general seroprevalence among the population were released by the DGS, on 7<sup>th</sup> May 2021 and on 17<sup>th</sup> December, 2021, respectively. After the first report that identified the general seroprevalence of 2.9% (95% CI: 2.0 – 4.2%), the second phase of the nationwide serological survey, reported a seroprevalence of about 15.5% (95%: 14.6 – 16.5%). This increase in the seroprevalence is thought to be due to the increased epidemic activity of COVID-19 since October 2020. The latest phase of the national serological survey indicated a seroprevalence of 86.4% (95% CI: 85.2 – 87.6%). This high seroprevalence is due to the vaccination program, established by the DGS. During the vaccination process it was established that older people and health workers were the first to get a vaccine shot for the COVID-19, increasing gradually the levels of seroprevalence on the general population according to age group (DGS, 2021) (DGS, 2021).

#### **1.4.2.1 Cascais Municipality**

The municipality of Cascais belongs to the district of Lisbon and consists of 4 parishes called Alcabideche, São Domingos de Rana, Union of parishes of Carcavelos and Parede and Union of parishes of Cascais and Estoril. From the national census of 2021, the preliminary data indicates circa 214 158 inhabitants, with 46.59% of the population being male and 53.41% female. According to the age pyramid, most inhabitants of Cascais were aged between 25- 64 years. Concerning to the household number of inhabitants 23 433 persons respond that lived by themselves, 27 801 who had a household of 2 persons, 17 045 who had a household of 3 persons, 12 691 who have a household of 4 persons, and 5 527 who have a household of 5 or more persons living in the same house (Censos 2021). Regarding to the migrant population living in Cascais, the (PORDATA) database indicates that 15.9% were migrants, being circa 16.6% male and 15.3% woman. This corresponds to an increase of almost 5% compared to the previous data available in (PORDATA) 2008, the nationwide (Censos 2021) database indicates that in the total Portuguese population, 10 344 802 habitants, 555 299 persons are migrants, 103 068 originating from the European Union and 452 231 from outside the European Union.

In the municipality of Cascais, the school network in (PORDATA) accounted for a total of 274 schools, its area of action covered from pre-school to secondary education. Regarding to the school levels, of the population residing in Cascais municipality, 9 767 individuals do not have any school level, 30 426 have completed the first basic level of education (1<sup>o</sup>-4<sup>o</sup> grade), 15 484 had completed the second level of basic education (5<sup>o</sup>-6<sup>o</sup> grade), 34 083 had completed the third basic level of education (7<sup>o</sup>-9<sup>o</sup> grade), 37 171 reported to have completed the secondary level, 2 709 reported to have completed the medium school level and 44 184 to have the superior/university level of education, in 2011. From (Censos 2021) reveals that 27 947 (13.05%) had no schooling, 27 647 (12.91%) had the first cycle completed, 16 385 (7.65%) had the second cycle completed, 30 692 (14.33%) had the third cycle completed, 51 131 (23.88%) had the secondary or post-secondary completed and 60 356 (28.18%) had finished University education.

Regarding to the health care services available, six public hospitals, non-public and private hospitals respond to the population health care needs, with an annual occupation rate of 77.8% (PORDATA). There is a ratio of 173 persons per health care worker (medical doctor, nurse, health care assistant or technician of diagnosis in hospital units) being one primary healthcare facility per 69 062 habitants 42 pharmacies exist on the Cascais municipality, with

about 5 101 habitants per pharmacy. The gross mortality rate in the municipality of Cascais, in 2021 was estimated to be around 11.2% per 1 000 habitants. From the deaths reported in the year 2020, 27.8% were related to be related to cardiovascular disease, 25.8% related to oncological diseases, 0.1% related to injuries and aging, 1.7% related to complications of diabetes, 7.3% related to diseases in the respiratory system, 3.2% related to diseases in the digestive system and 0.5% related to suicide (PORDATA) Male habitants living in Cascais had a higher incidence of mortality compared to the woman.

#### **1.4.2.1.1 Cascais Serological Study**

Following the initiative of the Municipality of Cascais in partnership with Roche, the clinical laboratories Joaquim Chaves and Germano de Sousa, and the Global Health and Tropical Medicine – IHMT, NOVA Lisbon University, a seroprevalence study was carried out in the municipality of Cascais with the objective of assessing the prevalence of antibodies to SARS-CoV-2 among the population residing in Cascais.

## **2 Objectives of the study**

### **2.1 General Objectives**

In the context of the Cascais SARS-CoV-2 Serological Survey, this study aims:

- To evaluate the putative relationship between sociodemographic and clinical characteristic related to SARS-CoV-2 positivity among Migrants and Portuguese population;

### **2.2 Specific Objectives**

- To characterize the sociodemographics of Migrants and Portuguese individuals that participated in Cascais Serological Study;
- To verify association between the occurrence of symptoms and the results of the SARS-CoV-2 serological test among Migrants and Portuguese individuals that participated in Cascais Serological Study;
- To assess the symptoms and chronic disease related to SARS-CoV-2 infection among Migrants and Portuguese individuals that participated in Cascais Serological Study;

### **3 Methods**

#### **3.1 Study design and participants**

This study is an observational and cross-sectional study conducted by the Cascais Municipality to evaluate the impact of SARS-CoV-2 on the municipality. The study combined two data collection components. The first one through a blood serological test provided by the Roche Laboratories to evaluate the levels of antibodies IgM and IgG presented by those who participated on the survey. The second by fulfilling a questionnaire divided in three parts with questions related to socio-demographic, history of chronic disease and COVID-19 related questions. The survey was conducted between 21<sup>st</sup> May 2020 and 12<sup>th</sup> February 2021. The timeframe of the data analyzed in the present study was between 21<sup>st</sup> May 2020 and 1<sup>st</sup> October 2020 that corresponds to the period after the phase of lock down in the first wave of the pandemic.

#### **3.2 Data collection**

##### **3.2.1 Detection of SARS-CoV-2 antibodies**

For the detection of SARS-CoV-2 specific antibodies (IgG and IgM), the serologic test used was the Elecsys Anti-SARS-CoV-2 from Roche Diagnostics, Switzerland. This test is an immunoassay for the qualitative detection of antibodies to SARS-CoV-2 in human serum and plasma. This assay uses a recombinant protein representing the nucleocapsid antigen in a double-antigen sandwich assay format, for the detection of high affinity antibodies against SARS-CoV-2. This test has a sensitivity after 14 days of 99.5% and a specificity of 99.8% to identify immune response to SARS-CoV-2.

##### **3.2.2 Questionnaires**

During the blood collection for the detection of specific antibodies against SARS-CoV-2, the participants responded to a questionnaire concerning sociodemographic, chronic diseases, covid-19 contact, and symptoms (Annex I).

The first section of the questionnaire included questions about the socio-demographic information, such as, age, country of origin, household, education level, professional status, locality. The second section included questions about medical history: chronic disease, such as Diabetes, Chronic Lung Disease, Asthma, Cardiovascular Disease, Obesity, Autoimmune Disease and Renal Disease. The third section referred to contact with COVID-19 confirmed or suspected cases history of SARS-CoV-2 testing, and if so the PCR result of the COVID-19 test. The fourth section referred to COVID-19 related symptoms. The fifth section addressed health care support needs and work/school abstentism due to COVID infection.

The first distributed version of the questionnaire did not include some of the questions, such as Home Country, Household, Chronic Kidney Disease, Travel History during the months of January and February 2020 and Intensive Care Hospitalization due to COVID infection (Anex I). An updated version of the inquiry with these questions was introduced at the latest months of the survey ( Anex II).

### 3.3 Data analysis

The variables under analysis were divided into five categories: Sociodemographic, (gender, age, country of birth, locality, level of education, employment status and size of household), Chronic diseases (diagnosed with diabetes, chronic lung disease, asthma, cardiovascular disease including hypertension, obesity, autoimmune disease, kidney disease and other disease), COVID-19 related risk (contact with suspected or confirmed cases, date of contact, travelled abroad during the month of January and February 2020 and where, and PCR test and result), COVID-19 symptoms (fever, chills, fatigue, muscle pain, joint pain, sore throat, cough, congested nose/runny nose, shortness of breath, wheezing, chest pain, other respiratory complaints, conjunctivitis, headache, changes in consciousness, seizures, other neurological complaints, nausea or vomiting, diarrhea, abdominal pain, loss of smell, loss of taste, loss of appetite, nose bleeding), and questions regarding the impact of COVID-19 on daily lives (sought medical assistance due to symptoms, missed work, stayed hospitalized and, if hospitalized, stayed in intensive care).

During the data analysis a new variable was created with the combination of symptoms suggestive of COVID-19, according to Pollan et al,2020 , being them, anosmia (loss of smell) or at least 3 symptoms (fever, chills, asthenia (fatigue)), odynophagia (sore throat), cough, dyspnea (shortness of breath), headache, nausea/vomiting, and diarrhea.

The sociodemographic variables were compared with the sociodemographic census data of the municipality (Censos 2021) available in (PORDATA), to assess the representativeness of the different categories of respondents in the sample.

Seroprevalence was estimated and stratified by sociodemographic and clinical variables. The posterior distribution of seroprevalence of each group was also obtained from a Bayesian model (Larremore et al., 2021) that incorporated uncertainty from test sensitivity and specificity. The comparison of seroprevalence estimates between groups was performed by calculating the odds ratio and using the Chi-square test, or Fisher's exact test (in the cases of expected frequencies lower than 5).

The statistical analyses were performed using RStudio version 4.1.2, with RScripts developed specifically for this purpose.

**4 Results**

**4.1 Characterization of the sample**

A total of 21373 individuals participated in the Cascais Serological survey, from 21<sup>st</sup> May 2020 to 12<sup>th</sup> February 2021. From the 21373 individuals, only 19608 individuals had serological test result. The 1765 individuals without serological test result, can be due to missing informed consent (Anex III). The timeframe of the study was stipulated to be from the beginning of Serological survey, in May 2020 till the beginning of October 2020. This period ranged from after the government’s lockdown policies having been lifted to before the second pandemic wave start. From the total 19608 individuals who had a serological test result, 17309 individuals were included in the survey in these months. From these, only 4410 individuals respond to the question regarding to their origin country, since this question was only included in the updated version of the questionnaire.

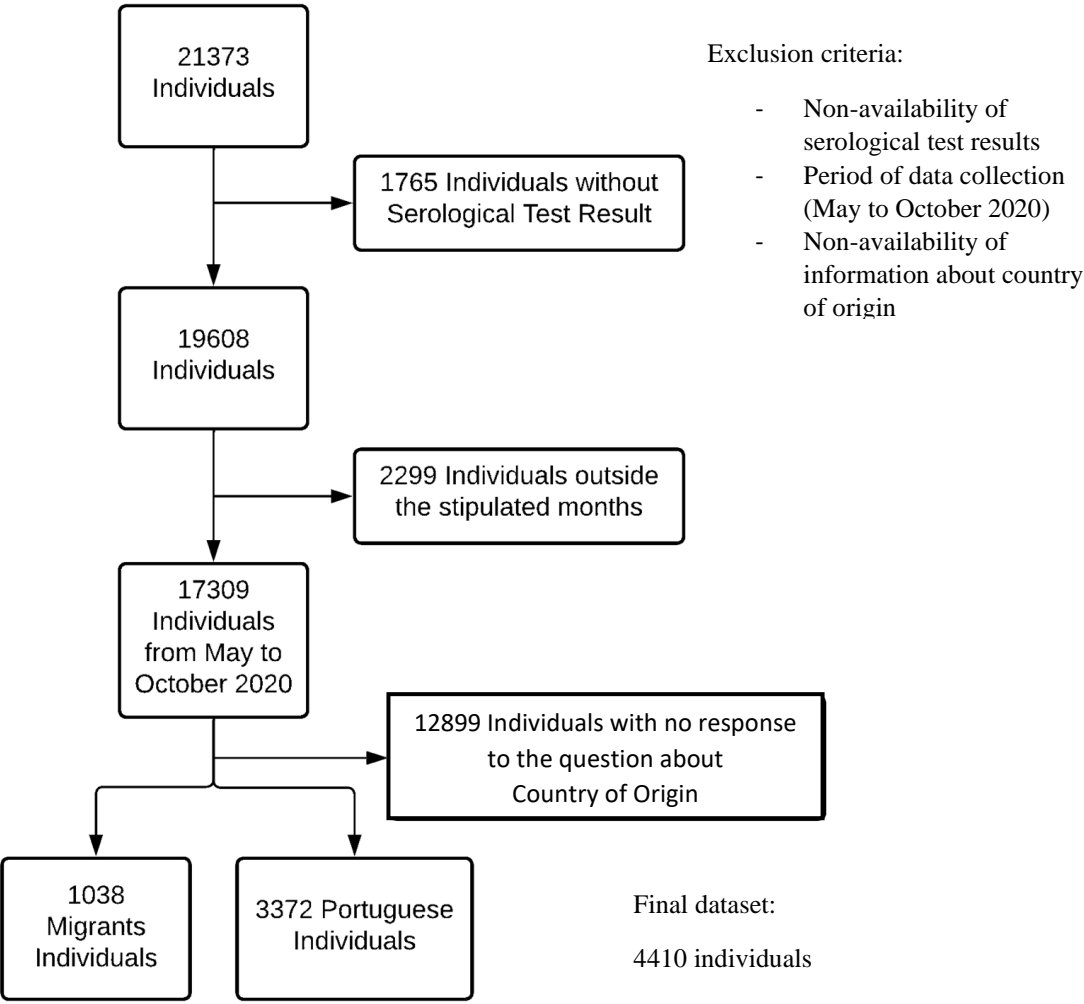


Figure 6 Flowchart summarizing the criteria used for defining the final analysed dataset

## 4.2 Sociodemographic Characterization of the Cascais Population

The total of 4410 individuals that responded to the question about country of origin, from May 21<sup>st</sup> to October 1<sup>st</sup>, 2020, corresponds to a coverage of 2.06% of the resident population of Cascais municipality (214158 residents lived in Cascais, according to the national (CENSOS 2021) (PORDATA - Estatísticas, gráficos e indicadores de Municípios, Portugal e Europa). 2902 (66.06%) of the participants were women compared with, 1491 (33.94%) male participants. The median age of the participants was 47 years old. 46.61% have University education level and 60.25% were employed. From the parishes in the municipality of Cascais, “Cascais and Estoril” had a higher percentage of participants, (30.16%). From the 4410 inquiries that responded to the question about the country of birth, 76,46% were Portuguese and 23.6% were born outside Portugal. From the different countries reported, the individuals from Brazil (43.2%), followed by Angola (16.4%) and Mozambique (11.6%) were the most represented. Afterwards, countries of origin were categorized according to the geographic regions of World Health Organization (WHO) (World Health Organization). (Table 1)

1038 migrants individuals, (23.54%), responded to the serological survey and 3372 Portuguese individuals, (76.46%), respond to the serological survey, during the time period between May 21<sup>st</sup> to October 1<sup>st</sup>,2020. This corresponds to a coverage of 0.48% of the migrant resident population living in Cascais municipality, and 1.58% of the Portuguese resident population living in Cascais. (Table 2)

From the 1038 migrant individuals, 693 (67.09%) participants were women and 340 (32.91%) were male. The median age of the participants was 47 years old. Regarding to the school level, 55.80% had University education level. 58.29% are employed. A vast majority of the migrants origin was from CPLP, such as Brazil, Angola, Mozambique, Cape Verde and Sao Tome and Principe, representing 75.53% of the migrants origins, 37.87% of migrants lived in Cascais and Estoril parish. As for household, 46.92% had a household of 2-3 members (Table 2).

From the 3372 portuguese individuals, 2209 (65.74%) were women and 1151 (34.26%) were male. The median age of the participants was 47 years old. According to the schooling level 43.78% have University education level. 60.85% are employed. 32.10% live in São Domingos de Rana parish, and 50.89% have a household of 2-3 people living in together. (Table 2)

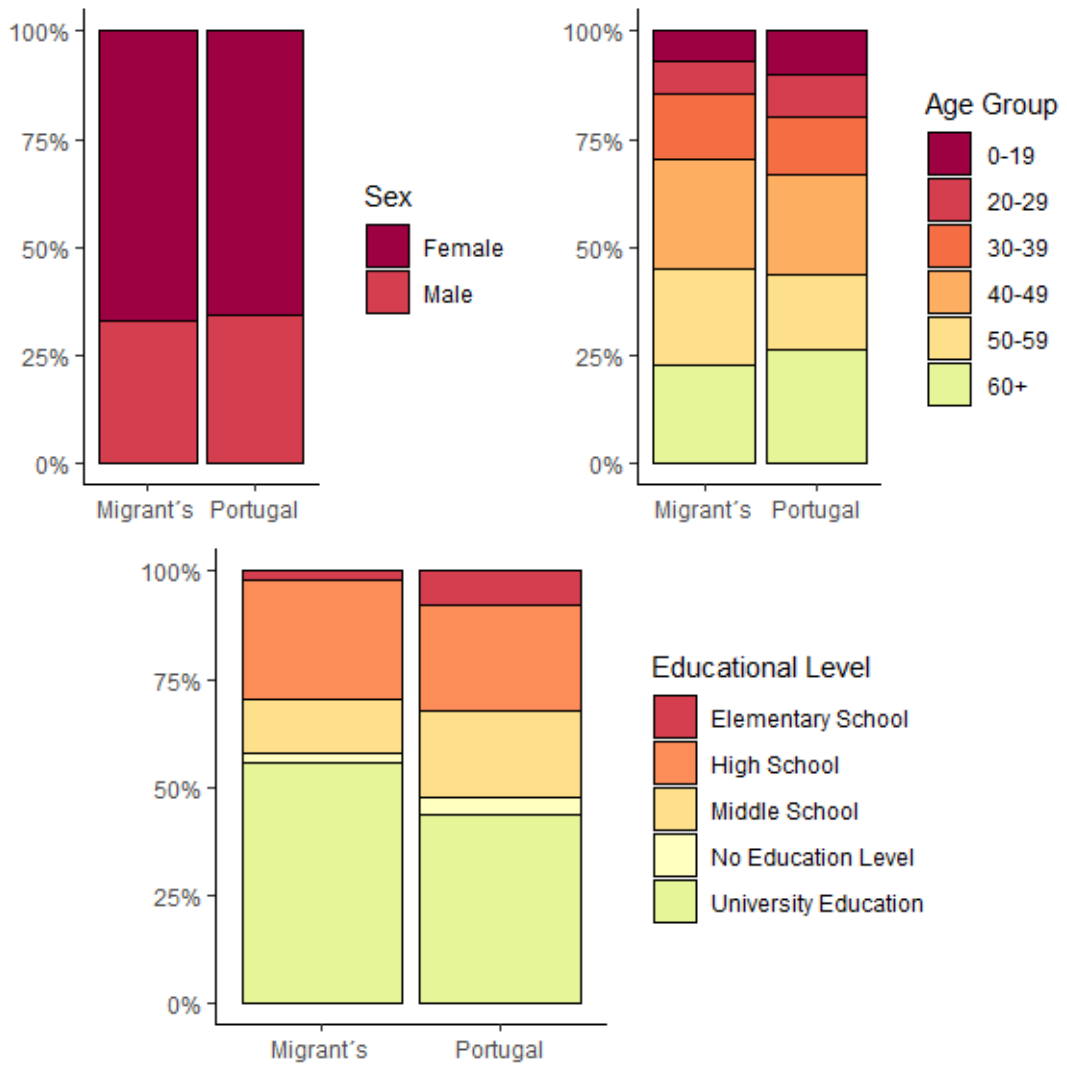


Figure 7 Descriptive sociodemographic information of the studied population regarding sex, age group and educational level, comparing migrants and Portuguese

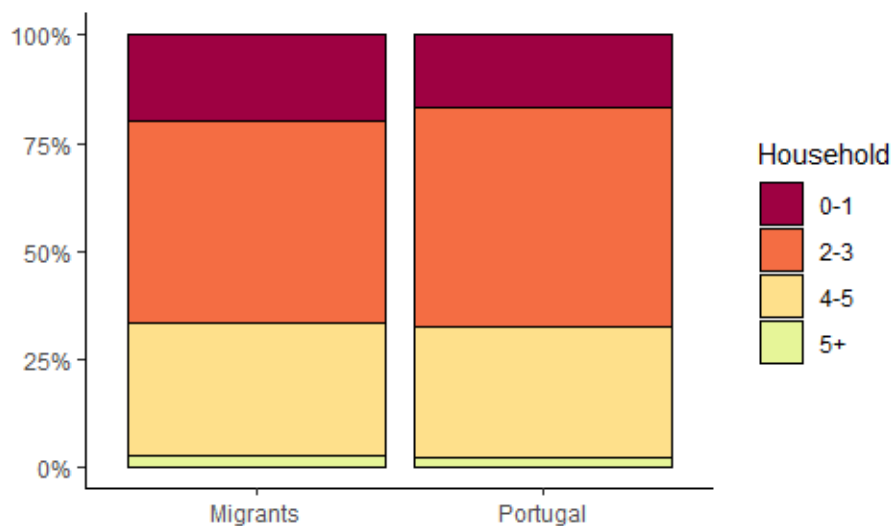
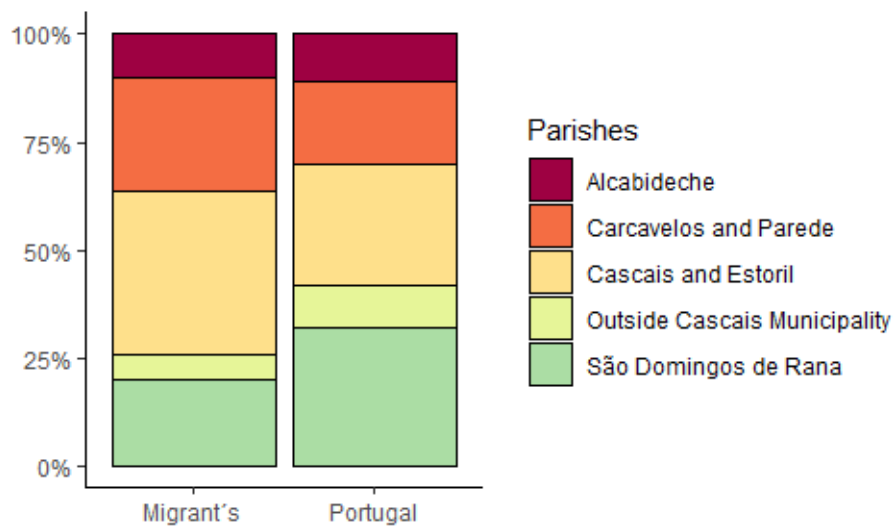
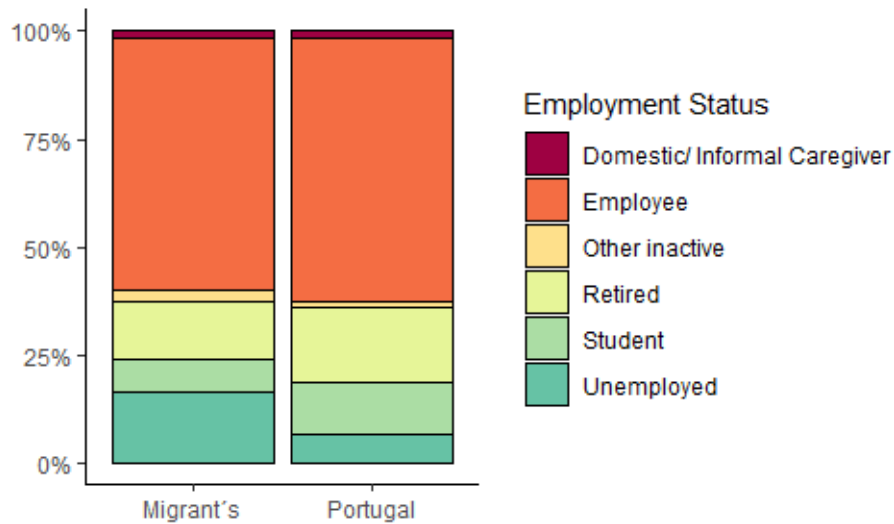


Figure 8 Descriptive sociodemographic information of the studied population regarding employment status, parish of residence and household size, comparing migrants and Portuguese

### 4.3 Seroprevalence for SARS-CoV-2 according to Sociodemographic data among Migrants and Portuguese population

Of the 4410 individuals that responded to the country of origin question, 72 individuals had a reactive serological test, corresponding to an overall seroprevalence of 1.63% (95% CI: 1.29 – 2.05%). Considering the known sensitivity and specificity of the serological test, the Bayesian approach estimated a corrected seroprevalence of 1.47% (95% CI: 1.16 – 1.79%) (Prevalence calculator (known sensitivity and specificity)).

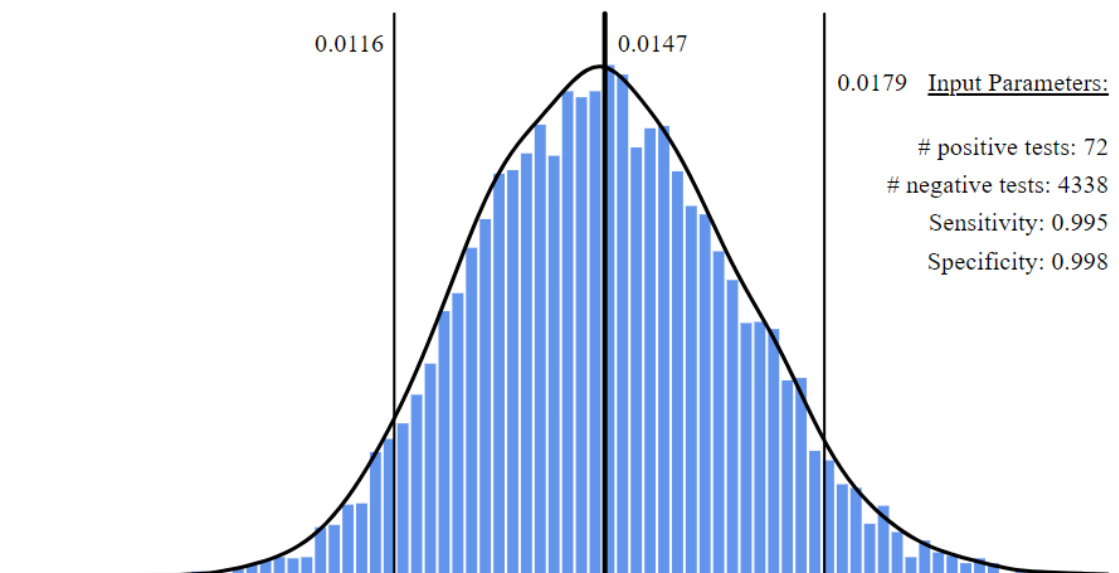


Figure 9 Corrected seroprevalence calculated by the Bayesian test, by (Larremore et al., 2021), among the Overall population (migrants + native population). The values for the estimated seroprevalence and the 95% credible interval are shown on top of the graph.

When testing for association between the serological test results and each of the sociodemographic variables, no statistically significant associations were found except for when a household is bigger than 5 persons (6.90% (95% CI: 3.20 – 14.24%); odds ratio: 4.06 (95% CI: 1.37 – 10.70), p-value <0.05 (Table 1).

Some categories of the variables have small sample size and consequently large confidence intervals, which may limit the detection of statistical significance. Although not statistically significant, we report here the groups with highest mean seroprevalence values, as an indication for future studies. Men had a higher mean seroprevalence of 2.08% (95% CI: 1.47 – 2.94%), the age group with highest mean seroprevalence were the age group 30-39 years old with 2.36% (95% CI: 1.41 – 3.92%). The education group with a higher mean seroprevalence was the High School education level with 2.29% (95% CI: 1.55 – 3.35%). The seroprevalence among the employment status were higher on individuals unemployed 2.84% (95% CI: 1.59 – 5.02%). Concerning the different regions of origins, the individuals from CPLP had a higher mean seroprevalence 2.42% (95% CI: 1.56 – 3.75%). Individuals living in the parishes of Cascais and Estoril presented a higher seroprevalence 2.26% (95% CI: 1.58 – 3.23%), compared to those living in the other parishes. The seroprevalence in household of 5+ people were higher (odds ratio 4.06 (95% CI: 1.37 – 10.70%), p-value <0.05) with a seroprevalence value of 6.90% (95% CI: 3.20 – 14.24%), compared to smaller households, the large confidence

interval due to the small sample size of the groups (n=6). The seroprevalence in the group of individuals that traveled during the months of January and February of 2020 had slightly higher mean seroprevalence of 1.70% (95% CI: 0.93 – 3.11%) (Table 1).

Overall								
Sociodemographic	N	%	Reactive	Seroprevalence (95% CI) (%)	Odds ratio (95% CI)	Chi-square or Fisher test (*) (p-value)	Wald test (Pr(> z ))	Wald test (Pr(> z )) Adjusted
<b>Participants</b>	4410		72	1.63 (1.29-2.05)				
<b>Sex</b>								
Feminine	2902	66,06	41	1.41 (1.04-1.91)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Masculine	1491	33,94	31	2.08 (1.47-2.94)	<i>1.48 (0.92-2.37)</i>	0.100	0.102	0.118
<b>Age Groups</b>								
0-19	414	9,47	8	1.93 (0.98-3.77)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
20-29	403	9,22	9	2.23 (1.18-4.19)	<i>1.16 (0.43-3.16)</i>	0.763	0.763	0.691
30-39	594	13,58	14	2.36 (1.41-3.92)	<i>1.21 (0.51-3.10)</i>	0.650	0.650	0.559
40-49	1048	23,97	18	1.72 (1.09-2.70)	<i>0.88 (0.39-2.18)</i>	0.779	0.780	0.914
50-59	803	18,36	9	1.12 (0.59-2.12)	<i>0.57 (0.21-1.56)</i>	0.253	0.259	0.332
60+	1111	25,41	12	1.08 (0.62-1.88)	<i>0.55 (0.22-1.43)</i>	0.193	0.199	0.235
<b>Educational Level</b>								
Elementary School	283	6,49	4	1.41 (0.55-3.58)	<i>0.93 (0.27 - 2.37)</i>	1.00*	0.837	0.651
High School	1094	25,09	25	2.29 (1.55-3.35)	<i>1.46 (0.85 - 2.48)</i>	0.157	0.159	0.186
Middle School	803	18,42	8	1.00 (0.51-1.95)	<i>0.64 (0.27 - 1.33)</i>	0.239	0.243	0.378
No Education Level	148	3,39	2	1.35 (0.37-4.79)	<i>0.92 (0.14 - 3.07)</i>	1.00*	0.832	0.890
University Education	2032	46,61	32	1.57 (1.12-2.21)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
<b>Employment Status</b>								
Domestic/ Informal Caregiver	79	1,82	0	0.00 (0.00-4.64)			0.976	0.977
Employee	2621	60,25	42	1.60 (1.19-2.16)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Other inactive	68	1,56	1	1.47 (0.08-7.87)	<i>1.04 (0.04 - 4.85)</i>	1.00*	0.932	0.921
Retired	714	16,41	9	1.26 (0.66-2.38)	<i>0.79 (0.36 - 1.57)</i>	0.509	0.510	0.958
Student	481	11,06	8	1.66 (0.85-3.25)	<i>1.06 (0.45 - 2.15)</i>	0.923	0.923	0.349
Unemployed	387	8,90	11	2.84 (1.59-5.02)	<i>1.82 (0.88 - 3.44)</i>	0.084	0.088	0.084
<b>Regions</b>								
African Region	31	0.70	0	0.00 (0.00 - 11.03)				
CPLP <sup>1</sup>	784	17.78	19	2.42 (1.56 - 3.75)	<i>1.63 (0.93 - 2.73)</i>	0.074		
European Region	175	3.97	2	1.14 (0.31 - 4.07)	<i>0.81 (0.12 - 2.63)</i>	1.00*		
Portugal	3372	76.46	51	1.51 (1.15 - 1.98)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Region of the Americas	33	0.75	0	0.00 (0.00 - 10.43)				
South-East Asia Region	5	0.11	0	0.00 (0.00 - 43.45)				
Western Pacific Region	10	0.23	0	0.00 (0.00 - 27.75)				
<b>Parishes</b>								
Alcabideche	454	10,69	5	1.10 (0.47-2.55)	<i>0.49 (0.16 – 1.18)</i>	0.125	0.133	0.134

Carcavelos and Parede	887	20,88	8	0.90 (0.46-1.77)	0.40 (0.17 – 0.84)	<b>0.020</b>	0.020	0.023
Cascais and Estoril	1281	30,16	29	2.26 (1.58-3.23)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Outside Cascais Municipality	379	8,92	7	1.85 (0.90-3.76)	0.83 (0.33 – 1.81)	0.625	0.625	0.574
São Domingos de Rana	1247	29,35	21	1.68 (1.10-2.56)	0.74 (0.41 – 1.30)	0.295	0.297	0.190
<b>Household</b>								
1	718	17,51	13	1.81 (1.06-3.07)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
2-3	2049	49,96	36	1.76 (1.27-2.42)	0.96 (0.52-1.90)	0.925	0.925	0.981
4-5	1247	30,41	17	1.36 (0.85-2.17)	0.75 (0.36-1.59)	0.436	0.438	0.338
5+	87	2,12	6	6.90 (3.20-14.24)	4.06 (1.37-10.70)	<b>0.011*</b>	0.006	0.012
<b>Traveler</b>								
No	3620	86,05	57	1.57 (1.22-2.03)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Yes	587	13,95	10	1.70 (0.93-3.11)	1.10 (0.52-2.07)	0.817	0.817	0.796

From the migrants population, 21 individuals had a reactive serological test, with an overall seroprevalence of 2.02% (95% CI: 1.33 – 3.07%). Considering the known sensitivity and specificity of the serological test, the Bayesian test estimate a seroprevalence of 1.93% (95% CI: 1.25 – 2.71%) (Prevalence calculator (known sensitivity and specificity)).

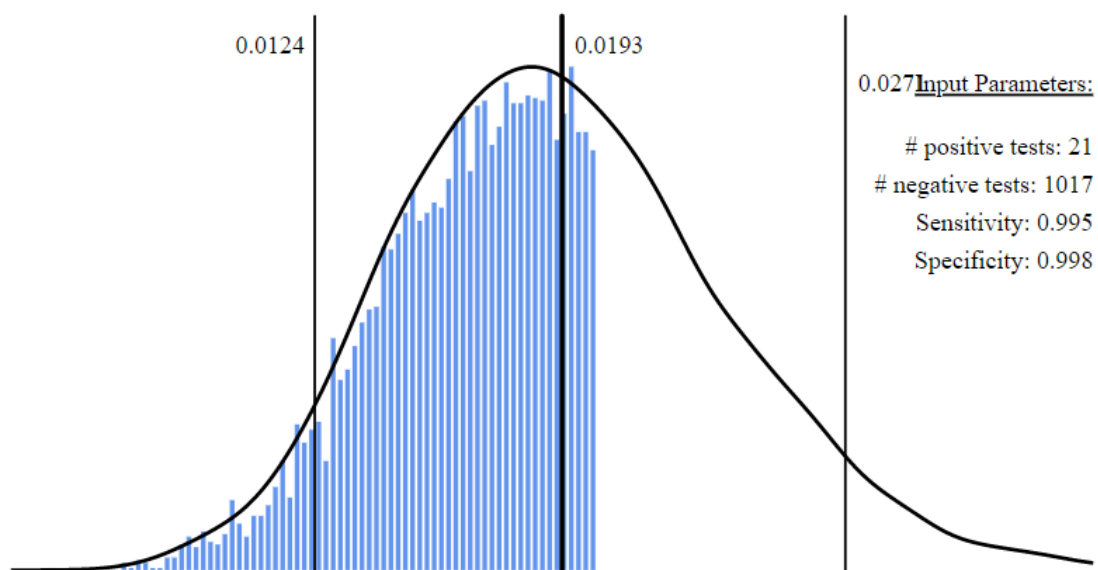


Figure 10 Seroprevalence estimated by the Bayesian approach, by (Larremore et al., 2021), among migrant population. The values for the estimated seroprevalence and the 95% credible interval are shown on top of the graph.

In the migrants population, from the sociodemographic variables analysed, significant differences in seroprevalence were observed between different educational levels more particularly between University education with Elementary School (odds ratio 9.30% (95% CI: 1.18 – 44.89%) and with University education with Middle School (odds ratio 3.84% (95% CI: 3.84 – 13.32%), and on households of different sizes, with a seroprevalence of 12.50% (95% CI: 4.34 – 31.00%) in individuals living in households of 5+ people, (odds ratio 4.43 (95% CI: 0.83 – 18.77%; p-value<0.05). Men showed a seroprevalence of 2.35% (95% CI: 1.20 – 4.57%),

higher than women 1.88% (95% CI: 1.10 – 3.18%). The age group that presented the highest mean seroprevalence was the age group of 20-29 with 5.19% (95% CI: 2.04 – 12.61%). Among groups of different education levels, the elementary school education level group had a higher seroprevalence with 8.70% (95% CI: 2.42 – 26.80%). For the employment status variable, unemployed individuals shown a higher seroprevalence of 3.59% (95% CI: 1.66 – 7.62%). Individuals originating from CPLP countries had a higher seroprevalence 2.42% (95% CI: 1.56 – 3.75%). When comparing migrants living in the different parishes, the seroprevalence was higher in the parish of São Domingos de Rana 4.06% (95% CI: 2.07 – 7.81%). Interesting for the people who did not travel the seroprevalence was higher, compared to those who had travelled, 2.37% (95% CI: 1.51 – 3.72%) (Table 2).

For the portuguese population, 51 individuals presented a reactive serological test, with an overall seroprevalence of 1.51% (95% CI: 1.15 – 1.98%). Considering the known sensitivity and specificity of the serological test, the Bayesian estimate of seroprevalence was 1.35% (95% CI: 1.02 – 1.72%) (Prevalence calculator (known sensitivity and specificity)).

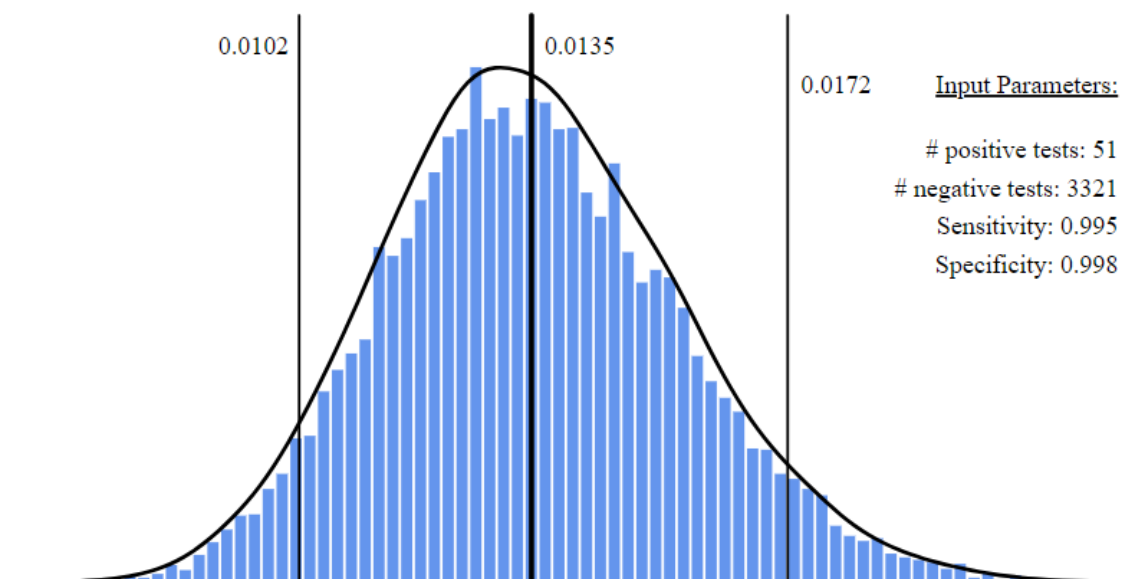


Figure 11 Seroprevalence estimated by the Bayesian approach, by (Larremore et al., 2021), among native population. The values for the estimated seroprevalence and the 95% credible interval are shown on top of the graph.

Significant differences in seroprevalence were only observed between different Educational sizes, the Middle School with the reference, University Level, (odds ratio 0.26 (95% CI: 0.06 – 0.74%), p-value<0.05. Between sexes, the seroprevalence was higher in men showing a seroprevalence of 2.00% (95% CI: 1.34 – 2.98%) compared to the women that have a seroprevalence of 1.27% (95% CI: 0.88 – 1.83%). The age group that presented a higher seroprevalence was the age group 0-19, with a value of 2.06% (95% CI: 1.00 – 4.19%). The educational level that presented a higher seroprevalence was the High School education level, with a seroprevalence of 2.09% (95% CI: 1.31 – 3.32%). Regarding the employment status, the group that shown a higher seroprevalence was the “other inactive” with a seroprevalence of 2.50% (95% CI: 0.13 – 12.88%), but with large confidence interval due to small sample size. From the different parishes, the Portuguese individuals that live on the parish Cascais and

Estoril showed a higher seroprevalence, of 2.20% (95% CI: 1.43 – 3.37%). The household of 5+ shown an seroprevalence of 4.76% (95% CI: 1.36 – 13.09%), higher from the others households. The persons who travelled shown a seroprevalence of 2.59% (95% CI: 1.37 – 4.86%) higher than those that did not travelled (Table 2; Figures 12 & 13).

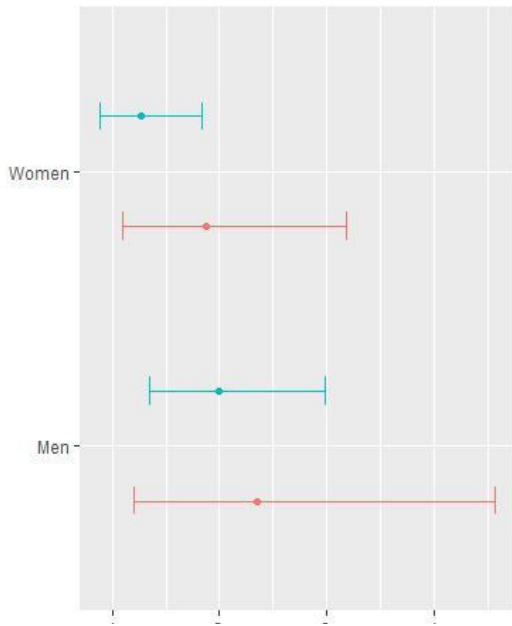
Table 1 Seroprevalence information regarding the different sociodemographic factors among the migrant and Portuguese population

Sociodemographic	Migrants								Portuguese								Migrants VS Portuguese
	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	Chi-square or Fisher test (*) (p-value)	Wald test (Pr(> z ))	Wald test (Pr(> z )) Adjusted	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	Chi-square or Fisher test (*) (p-value)	Wald test (Pr(> z ))	Wald test (Pr(> z )) Adjusted	
<b>Participants</b>	1038		21	2.02 (1.33-3.07)					3372		51	1.51 (1.15-1.98)					0.265
<b>Sex</b>																	
Female	693	67,09	13	1.88 (1.10-3.18)	ref	ref	ref	ref	2209	65,74	28	1.27 (0.88-1.83)	ref	ref	ref	ref	0.244
Male	340	32,91	8	2.35 (1.20-4.57)	1.27 (0.49-3.07)	0.610	0.61	0.593	1151	34,26	23	2.00 (1.34-2.98)	1.59 (0.90-2.78)	0.100	0.103	0.124	0.694
<b>Age Groups</b>																	
0-19	74	7,18	1	1.35 (0.07-7.27)	ref	ref	ref	ref	340	10,17	7	2.06 (1.00-4.19)	ref	ref	ref	ref	1*
20-29	77	7,48	4	5.19 (2.04-12.61)	3.61 (0.49-100.22)	0.367*	0.220	0.209	326	9,75	5	1.53 (0.66-3.54)	0.75 (0.21-2.42)	0.611	0.612	0.680	0.079*
30-39	153	14,85	6	3.92 (1.81-8.29)	2.66 (0.43-69.90)	0.432*	0.316	0.314	441	13,19	8	1.81 (0.92-3.54)	0.88 (0.31-2.57)	0.805	0.805	0.933	0.213*
40-49	264	25,63	5	1.89 (0.81-4.36)	1.27 (0.19-33.97)	1.00*	0.756	0.744	784	23,45	13	1.66 (0.97-2.82)	0.79 (0.32-2.16)	0.641	0.641	0.800	0.787*
50-59	226	21,94	2	0.88 (0.24-3.17)	0.61 (0.05-19.48)	0.574*	0.728	0.741	577	17,26	7	1.21 (0.59-2.48)	0.58 (0.19-1.76)	0.313	0.319	0.421	1*
60+	236	22,91	3	1.27 (0.43-3.67)	0.86 (0.10-25.07)	1.00*	0.957	0.963	875	26,17	9	1.03 (0.54-1.94)	0.49 (0.18-1.41)	0.167*	0.166	0.205	0.726*
<b>Educational Level</b>																	
No Education Level	21	2,05	0	0.00 (0.00-15.46)								1.57 (0.43-5.56)	0.94 (0.14 – 3.22)	1.00*	0.866	0.531	
Elementary School	23	2,24	2	8.70 (2.42-26.80)	9.30 (1.18 – 44.89)	0.035*	0.001	0.002	260	7,8	2	0.77 (0.21-2.76)	0.46 (0.07 – 1.55)	0.298*	0.249	0.307	0.039*
High School	281	27,41	8	2.85 (1.45-5.52)	2.74 (0.93 – 8.59)	0.081*	0.062	0.064	813	24,38	17	2.09 (1.31-3.32)	1.18 (0.62 – 2.18)	0.603	0.603	0.924	0.476

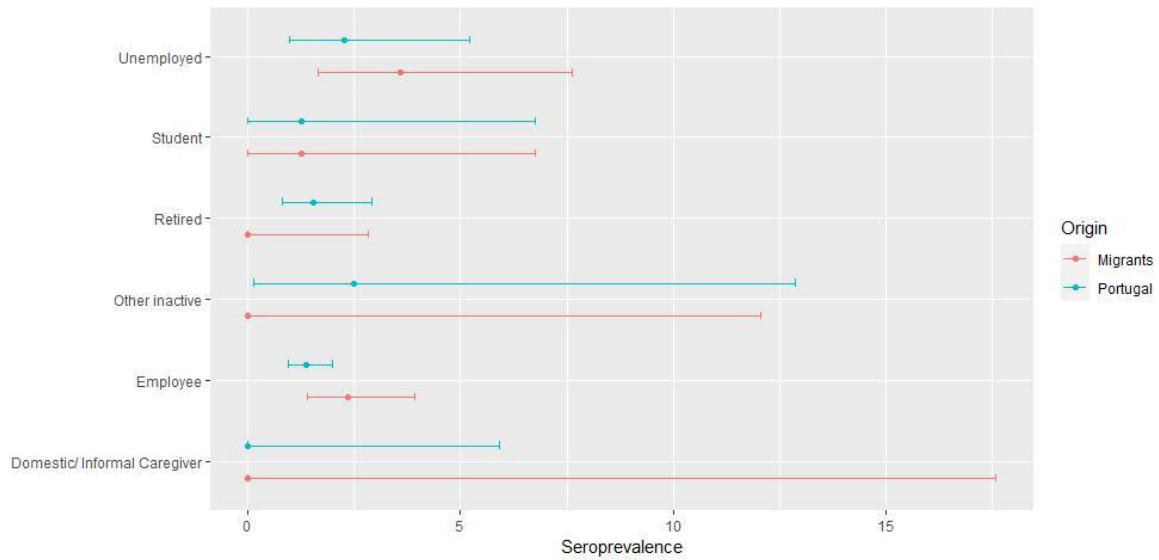
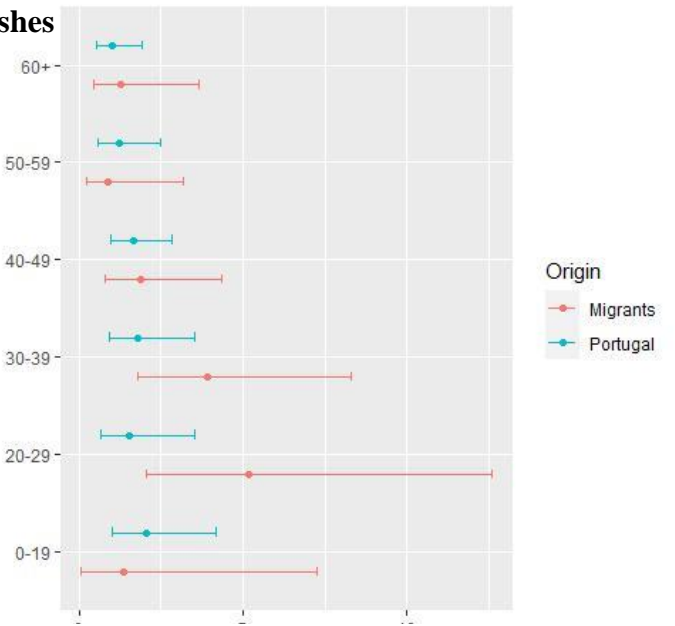
Sociodemographic	Migrants									Portuguese									Migrants VS Portuguese
	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	Chi-square or Fisher test (*) (p-value)	Wald test (Pr(> z ))	Wald test (Pr(> z )) Adjusted	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	Chi-square or Fisher test (*) (p-value)	Wald test (Pr(> z ))	Wald test (Pr(> z )) Adjusted	Chi-square or Fisher test (*) (p-value)		
Middle School	128	12,49	5	3.91 (1.68-8.82)	3.84 (1.06 – 13.32)	0.034*	0.01	0.006	675	20,24	3	0.44 (0.15-1.30)	0.26 (0.06 – 0.74)	0.013	0.022	0.013	0.004*		
University Education	572	55,8	6	1.05 (0.48-2.27)	ref	ref	ref	ref	1460	43,78	26	1.78 (1.22-2.60)	ref	ref	ref	ref	0.240		
<b>Employment Status</b>																			
Domestic/ Informal Caregiver	18	1,77	0	0.00 (0.00-17.59)			0.995	0.997	61	1,83	0	0.00 (0.00-5.92)		0.979	0.987				
Employee	580	2,75	14	2.36 (1.41-3.92)	ref	ref	ref	ref	2027	60,85	28	1.38 (0.96-1.99)	ref	ref	ref	ref	0.087		
Other inactive	28	12,95	0	0.00 (0.00-12.06)			0.994	0.996	40	1,20	1	2.50 (0.13-12.88)	2.08 (0.09 - 10.09)	0.435*	0.557	0.362			
Retired	132	58,29	0	0.00 (0.00-2.83)			0.986	0.991	582	17,47	9	1.55 (0.82-2.91)	1.13 (0.50 - 2.34)	0.767	0.767	0.219			
Student	79	7,85	1	1.25 (0.00-6.75)	0.59 (0.02 - 3.03)	1.000*	0.536	0.480	401	12,04	7	1.75 (0.85-3.56)	1.29 (0.51 - 2.83)	0.576	0.577	0.577	1*		
Unemployed	161	16,39	6	3.59 (1.66-7.62)	1.57 (0.54 - 4.01)	0.411*	0.381	0.495	220	6,60	5	2.27 (0.97-5.21)	1.70 (0.56 - 4.12)	0.367*	0.302	0.237	0.539*		
<b>Regions</b>																			
African Region	31	2,99	0	0.00 (0.00-11.03)															
CPLP <sup>1</sup>	784	75,53	19	2.42 (1.56-3.75)	ref	ref	ref	ref											
European Region	175	16,86	2	1.14 (0.31-4.07)	0.50 (0.07 - 1.75)	0.400*													
Portugal																			
Region of the Americas	33	3,18	0	0.00 (0.00-1.04)															
South-East Asia Region	5	0,48	0	0.00 (0.00-43.45)															
Western Pacific Region	10	0,96	0	0.00 (0.00-27.75)															

Sociodemographic	Migrants									Portuguese									Migrants VS Portuguese
	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	Chi-square or Fisher test (*) (p-value)	Wald test (Pr(> z ))	Wald test (Pr(> z ) Adjusted)	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	Chi-square or Fisher test (*) (p-value)	Wald test (Pr(> z ))	Wald test (Pr(> z ) Adjusted)	Chi-square or Fisher test (*) (p-value)		
<b>Parishes</b>																			
Alcabideche	98	10.03	1	1.02 (0.05-5.56)	0.47 (0.02 - 2.57)	0.696*	0.405	0.463	356	10.88	4	1.12 (0.44-2.85)	0.52 (0.15 - 1.40)	0.208	0.217	0.220	1*		
Carcavelos and Parede	257	26.31	2	0.78 (0.21-2.79)	0.33 (0.05 - 1.34)	0.214*	0.141	0.114	630	19.26	6	0.95 (0.44-2.06)	0.44 (0.16 - 1.04)	0.063	0.070	0.091	1*		
Cascais and Estoril	370	37.87	9	2.43 (1.28-4.56)	ref	ref	ref	ref	911	27.85	20	2.20 (1.43-3.37)	ref	ref	ref	ref	0.801		
Outside Cascais Municipality	55	5.93	0	0.00 (0.00-6.53)			0.987	0.986	324	9.90	7	2.16 (1.05-4.39)	1.00 (0.38 - 2.29)	0.971	0.971	0.953			
São Domingos de Rana	197	20.16	8	4.06 (2.07-7.81)	1.70 (0.62 - 4.58)	0.279	0.284	0.307	1050	32.10	13	1.24 (0.72-2.11)	0.56 (0.27 - 1.13)	0.100	0.105	0.068	0.012*		
<b>Household</b>																			
1	190	19.81	6	3.16 (1.46-6.72)	ref	ref	ref	ref	528	16.80	7	1.33 (0.64-2.71)	ref	ref	ref	ref	0.177*		
2-3	450	46.92	8	1.78 (0.90-3.47)	0.55 (0.19 - 1.74)	0.373*	0.282	0.323	1599	50.89	28	1.75 (1.21-2.52)	1.30 (0.60 - 3.29)	0.505	0.507	0.449	0.970		
4-5	295	30.76	4	1.36 (0.53-3.43)	0.43 (0.10 - 1.56)	0.200*	0.185	0.225	952	30.30	13	1.37 (0.80-2.32)	1.02 (0.41 - 2.77)	0.949	0.949	0.938	1*		
5+	24	2.50	3	12.50 (4.34-31.00)	4.43 (0.83 - 18.77)	0.066*	0.047	0.123	63	2.01	3	4.76 (1.36-13.09)	3.81 (0.76 - 14.51)	0.080*	0.062	0.085	0.352*		
<b>Traveler</b>																			
No	759	75.98	18	2.37 (1.51-3.72)	ref	ref	ref	ref	2861	89.18	39	1.36 (1.00-1.86)	ref	ref	ref	ref	0.052		
Yes	240	24.02	1	0.42 (0.02-2.32)	0.20 (0.01 - 0.95)	0.058*	0.088	0.064	347	10.82	9	2.59 (1.37-4.86)	1.95 (0.87 - 3.90)	0.075	0.080	0.219	0.055*		

## Age group



## Parishes



## Educational level

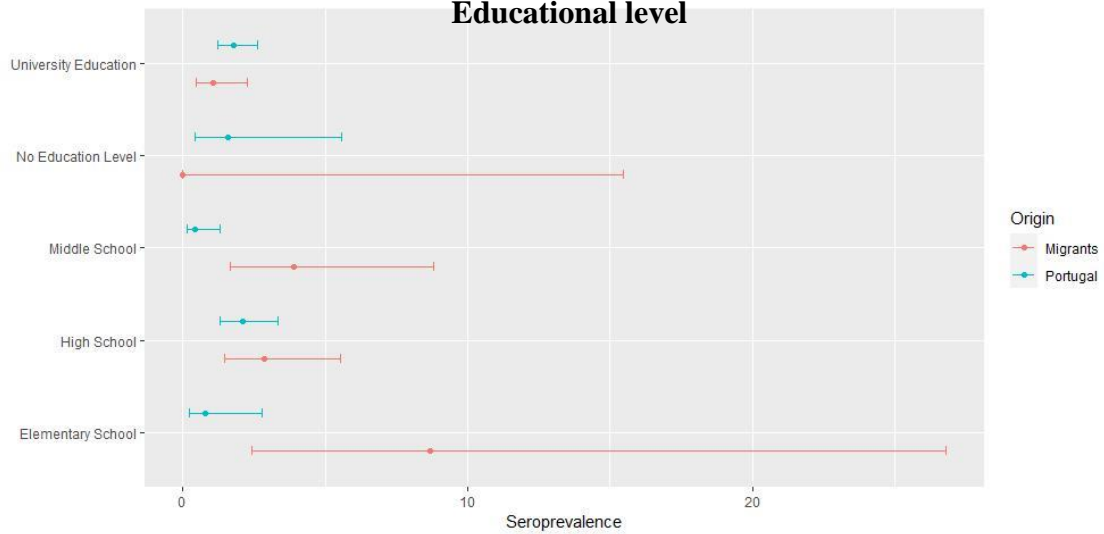


Figure 12 Seroprevalence (%) according to the Sex Age group, Employment status and Educational level, on the migrants and Portuguese population

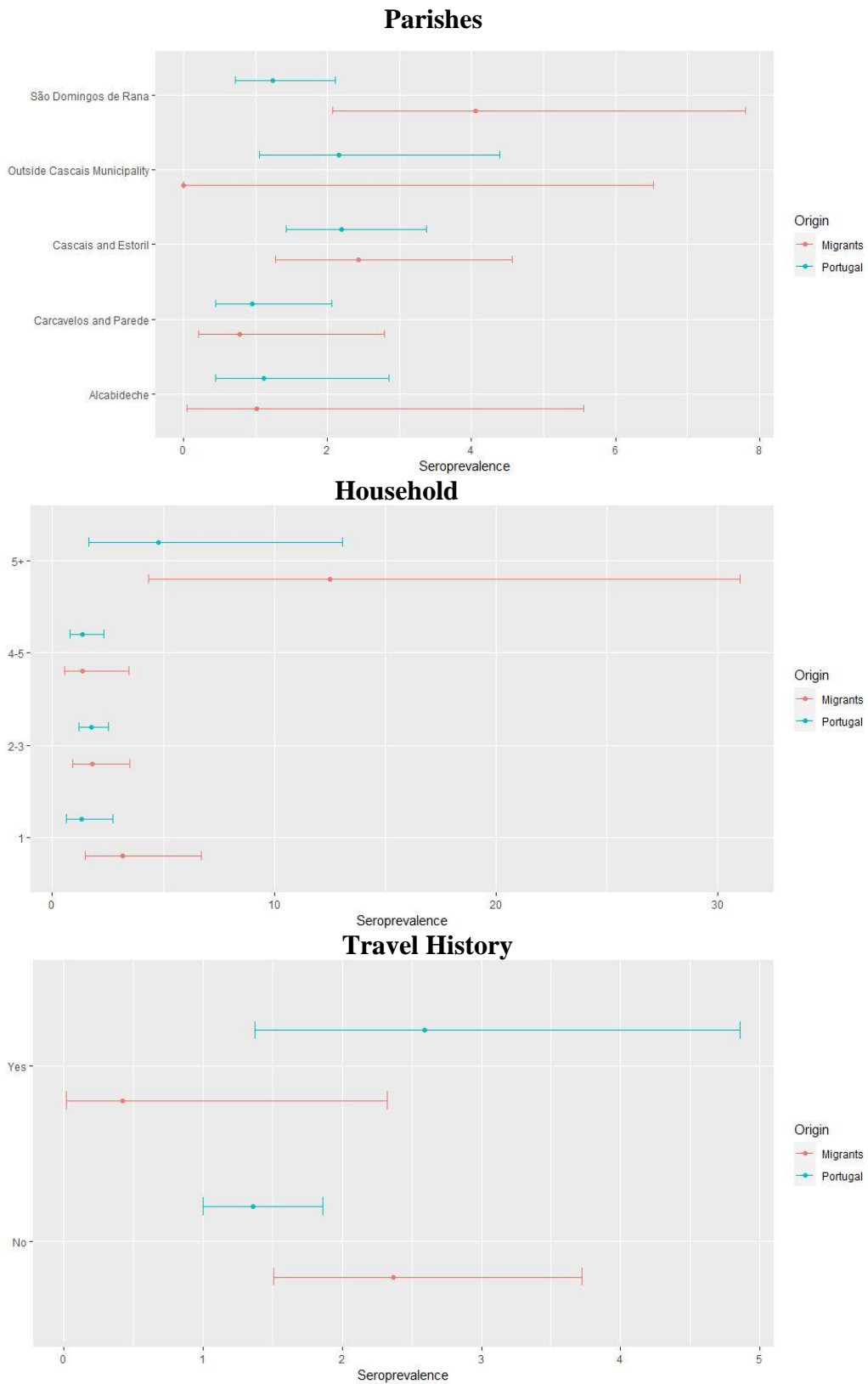


Figure 13 Seroprevalence (%) according to the Parishes, Household and Travel History, on the migrants and Portuguese population

#### 4.4. Seroprevalence for SARS-CoV-2 according to history of chronic diseases among the Migrants and Portuguese population

From the 4410 individuals, 1311 (29.73%) participants reported having at least one chronic disease, while 350 (7.94%) participants reported to have at least two chronic diseases. Regarding the seroprevalence among the patients with chronic diseases the individuals with at least one showed a seroprevalence of 1.22% (95% CI: 0.75 – 1.97%) and in the individuals that reported to have at least two chronic diseases the seroprevalence was calculated at 0.86% (95% CI: 0.29 – 2.49%). For each of the chronic diseases, the seroprevalence did not differ significantly between patients with or without chronic illness. (Table 3) The seroprevalence was generally lower in the patients chronically ill than in those without, with the exception of cardiovascular disease and obesity (Table 3).

Table 2 Seroprevalence information regarding the history different of chronic diseases among the overall population (migrant and Portuguese population)

Chronic Historic				Overall				
At least 1 Chronic Disease	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	Chi-square or Fisher test (*) (p- value)	Wald test (Pr(> z ))	Wald test (Pr(> z ) Adjusted)
No	3099	70,27	56	1.81 (1.39-2.34)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Yes	1311	29,73	16	1.22 (0.75-1.97)	<i>0.68 (0.37-1.16)</i>	0.160	0.163	0.336
<b>At least 2 Chronic Disease</b>								
No	4060	92,06	69	1.70 (1.35-2.15)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Yes	350	7,94	3	0.86 (0.29-2.49)	<i>0.53 (0.12-1.42)</i>	0.233	0.242	0.437
<b>Diabetes</b>								
No	3543	93,93	61	1.72 (1.34-2.21)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Yes	229	6,07	1	0.44 (0.02-2.43)	<i>0.29 (0.01-1.28)</i>	0.181*	0.171	0.218
<b>Chronic Lung Disease</b>								
No	3635	97,32	60	1.65 (1.29-2.12)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Yes	100	2,68	1	1.00 (0.05-5.45)	<i>0.69 (0.03-3.12)</i>	1.00*	0.616	0.730
<b>Asthma</b>								
No	3430	90,22	56	1.63 (1.26-2.11)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Yes	372	9,78	5	1.34 (0.58-3.11)	<i>0.84 (0.29-1.93)</i>	0.674	0.674	0.683
<b>Cardiovascular Disease</b>								
No	3208	83,89	51	1.59 (1.21-2.08)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Yes	616	16,11	10	1.62 (0.88-2.96)	<i>1.03 (0.49-1.97)</i>	0.951	0.951	0.617
<b>Obesity</b>								
No	3462	93,69	57	1.65 (1.27-2.13)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Yes	233	6,31	4	1.72 (0.67-4.33)	<i>1.08 (0.32-2.67)</i>	0.793*	0.935	0.756
<b>Autoimmune Disease</b>								
No	3417	94,55	59	1.73 (1.34-2.22)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Yes	197	5,45	0	0.00 (0.00-1.91)			0.975	0.984
<b>Kidney Disease</b>								
No	3574	98,7	59	1.65 (1.29-2.12)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Yes	47	1,3	0	0.00 (0.00-7.56)			0.981	0.982

Table 3 Seroprevalence information regarding to the COVID-19 History factors among the overall population (migrant and Portuguese population)

COVID-19 PCR Test				Overall				
Previous Contact with Suspected or Confirmed Cases	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	Chi-square or Fisher test (*) (p-value)	Wald test (Pr(> z ))	Wald test (Pr(> z ) Adjusted)
No	2695	90,13	31	1.15 (0.81-1.63)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Yes	295	9,87	25	8.47 (5.81-12.21)	7.96 (4.58-13.68)	<b>0.001</b>	<b>0</b>	
<b>Did a previous RT-PCR test</b>								
No	2993	71,62	30	1.00 (0.70-1.43)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Yes	1186	28,38	41	3.46 (2.56-4.66)	3.53 (2.20-5.74)	<b>0.001</b>	<b>0</b>	
<b>RT-PCR Test Result</b>								
Negative	1132	98,26	28	2.47 (1.72-3.55)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Positive	20	1,74	12	60.00 (38.66-78.12)	57.72 (21.96-161.13)	<b>0.001*</b>	<b>0</b>	

Among the migrants population about 299 (28.81%) participants reported to have at least one chronic disease, while 76 (7.32%) participants reported to have at least two chronic diseases. The seroprevalence on the patients with at least one or two chronic diseases was similar, 1.34% (95% CI: 0.52 – 3.39%) and 1.32% (95% IC: 0.07 – 7.08%), respectively. For some of the chronic diseases, the seroprevalence of the chronically ill was zero, but the sample size was small. For the other diseases, the seroprevalence did not differ significantly between ill versus non-ill (Table 5).

### History of Chronic Diseases

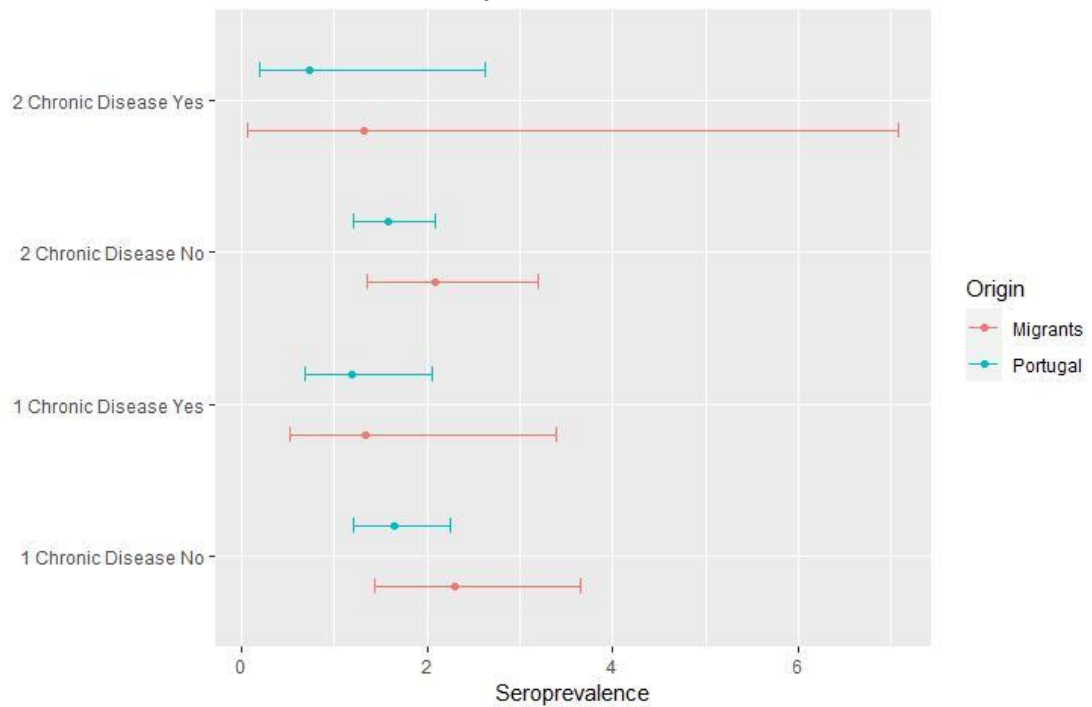


Figure 14 Seroprevalence (%) according to the Chronic Diseases Historic, among the migrants vs Portuguese population

Although among to the portuguese population, only 1012 (30.01%) participants reported to have at least one self-reported chronic disease and about 274 (8.13%) participants reported to have at least two or more self-reported disease. The levels of seroprevalence between participants with at least one chronic disease were lower, 1.19% (95% CI: 0.68 – 2.06%), compared to the participants that did not have any chronic disease. The seroprevalence in participants that have at least two chronic diseases was lower, of about 0.73% (95% CI: 0.20 – 2.62%) compared to those that did not report any chronic disease. For each of the chronic disease, the seroprevalence did not differ significantly between patients chronic ill and without chronic disease (Table 5). The seroprevalence was generally lower in the patients chronic ill, with exception of patients with asthma, that had a higher seroprevalence of 1.76% (95% CI: 0.75 – 4.05%) and on the patients with cardiovascular diseases, presented a similar seroprevalence.

Table 4 Seroprevalence information regarding to the history of chronic diseases among the migrant and Portuguese population

Chronic Historic		Migrants							Portuguese							Migrants VS Portuguese
At least 1 Chronic Disease	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	Chi-square or Fisher test (*) (p-value)	Wald test (Pr(> z ) Adjusted)	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	Chi-square or Fisher test (*) (p-value)	Wald test (Pr(> z ) Adjusted)	Chi-square or Fisher test (*) (p-value)	
						ref	ref						ref	ref		
No	739	71,19	17	2.30 (1.44-3.65)	ref	ref	ref	2360	69,99	39	1.65 (1.21-2.25)	ref	ref	ref	0.258	
Yes	299	28,81	4	1.34 (0.52-3.39)	0.59 (0.17-1.63)	0.318	0.324	1012	30,01	12	1.19 (0.68-2.06)	0.72 (0.36-1.34)	0.309	0.311	0.432	0.770*
<b>At least 2 Chronic Disease</b>																
No	962	92,68	20	2.08 (1.35-3.19)	ref	ref	ref	3098	91,87	49	1.58 (1.20-2.08)	ref	ref	ref	0.306	
Yes	762	7,32	1	1.32 (0.07-7.08)	0.71 (0.03-3.48)	0.736*	0.652	274	8,13	2	0.73 (0.20-2.62)	0.49 (0.07-1.59)	0.434*	0.28 (0****)	0.429	0.523*
<b>Diabetes</b>																
No	866	95,48	18	2.08 (1.32-3.26)	ref	ref	ref	2677	93,44	43	1.60 (1.19-2.16)	ref	ref	ref	0.362	
Yes	412	4,52	0	0.00 (0.00-8.57)				188	6,56	1	0.53 (0.03-2.95)	0.37 (0.02-1.71)	0.363*	0.271	0.329	
<b>Chronic Lung Disease</b>																
No	878	97,45	18	2.05 (1.30-3.22)	ref	ref	ref	2757	97,28	42	1.52 (1.13-2.05)	ref	ref	ref	0.295	
Yes	235	2,55	0	0.00 (0.00-14.31)				77	2,72	1	1.30 (0.07-7.00)	0.97 (0.04-4.49)	1.00*	0.874	0.999	
<b>Asthma</b>																

Chronic Historic		Migrants								Portuguese								Migrants VS Portuguese	
At least 1 Chronic Disease	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	Chi-square or Fisher test (*) (p-value)	Wald test (Pr(> z ))	Wald test (Pr(> z )) Adjusted	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	Chi-square or Fisher test (*) (p-value)	Wald test (Pr(> z ))	Wald test (Pr(> z )) Adjusted	Chi-square or Fisher test (*) (p-value)		
						ref	ref	ref						ref	ref	ref			
No	824	90,35	18	2.18 (1.39-3.43)	ref	ref	ref	ref	2606	90,17	38	1.46 (1.06-2.00)	ref	ref	ref	ref	0.159		
Yes	885	9,65	0	0.00 (0.00-4.18)					284	9,83	5	1.76 (0.75-4.05)	1.24 (0.42-2.92)	0.689	0.690	0.692			
<b>Cardiovascular Disease</b>																			
No	779	84,86	15	1.93 (1.17-3.15)	ref	ref	ref	ref	2429	83,59	36	1.48 (1.07-2.04)	ref	ref	ref	ref	0.397		
Yes	139	15,14	3	2.16 (0.74-6.15)	1.17 (0.26-3.64)	0.745*	0.855	0.526	477	16,41	7	1.47 (0.71-3.00)	1.01 (0.41-2.15)	0.981	0.981	0.855			
<b>Obesity</b>																			
No	826	92,81	16	1.94 (1.20-3.12)	ref	ref	ref	ref	2636	93,98	41	1.56 (1.15-2.10)	ref	ref	ref	ref	0.460		
Yes	649	7,19	2	3.13 (0.86-10.70)	1.74 (0.25-6.36)	0.376*	0.52	0.400	169	6,02	2	1.18 (0.33-4.21)	0.81 (0.12-2.67)	1.00*	0.704	0.839	0.310*		
<b>Autoimmune Disease</b>																			
No	833	95,2	18	2.16 (1.37-3.39)	ref	ref	ref	ref	2584	94,34	41	1.59 (1.17-2.15)	ref	ref	ref	ref	0.278		
Yes	42	4,8	0	0.00 (0.00-8.38)					155	5,66	0	0.00 (0.00-2.42)							
<b>Kidney Disease</b>																			
No	864	99,08	18	2.08 (1.32-3.27)	ref	ref	ref	ref	2710	98,58	41	1.51 (1.12-2.05)	ref	ref	ref	ref	0.260		
Yes	82	0,92	0	0.00 (0.00-32.44)					39	1,42	0	0.00 (0.00-8.97)							

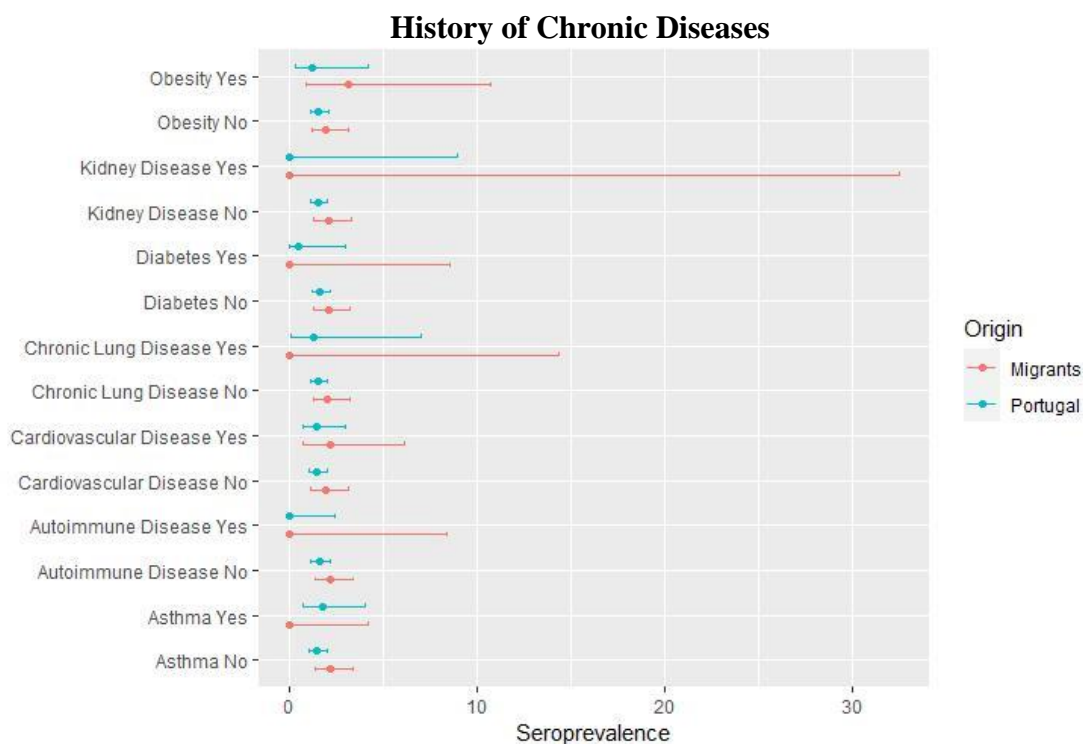


Figure 15 Seroprevalence (%) according to the different chronic diseases on the migrants and Portuguese population

#### 4.5. Seroprevalence of SARS-CoV-2 according to COVID-19 History on the Migrants and Portuguese population

On the migrants and portuguese population, about 295 respondents, (9.87%), had reported to have had a previous contact with a suspected or confirmed case, of these, the seroprevalence was calculated to be 8.47% (95% CI: 5.81 – 12.21%), a higher and statistically significant different value compared to those who did not contact (odds ratio 7.96 (95% CI: 4.58 – 13.68), p-value<0.0001) (Table 4). From the 1186 participants, 28.38% responded to have had performed previously a PCR test to detect the presence of the SARS-CoV-2 virus. The estimated seroprevalence among these participants was 3.46% (95% CI: 2.56 – 4.66%), a value higher than for those that did not perform a previous RT-PCR test (statistically significant association: odds ratio 3.53 (2.20 – 5.74), p-value <0.0001). From the 1186 participants that performed a previous RT-PCR test, 20 individuals reported to have had positive RT-PCR for COVID-19 disease and 12 of those had a positive serological test, corresponding to a seroprevalence of 60.00% (95% CI: 38.66 – 78.12%), a value much higher than for those who had a negative RT-PCR tests result (statistically significant association: odds ratio 57.72% (95% CI: 21.96 – 161.13), p-value <0.0001) (Table 4).

On the migrants population, about 63 respondents, (8.67%), reported to have had a previous contact with a suspected or confirmed case. The seroprevalence among them was estimated to be 6.35% (95% CI: 2.50 – 15.22%), but the seroprevalence did not differ significantly between the individuals that were in previous contact with a suspected or confirmed case (Table 5). About 267 (26,75%) participants reported having had performed a RT-PCR test previously, with an estimated seroprevalence of 4.12% (95% CI: 2.32 – 7.23%), higher than in those that did not perform the test (statistically significant association: odds ratio (3.43 (95% CI: 1.39 -8.71), p-value <0.005). From the 267 participants, that performed a PCR test, 2 (0.74%) reported to have had a positive PCR test, and one of them had a reactive serological test, corresponding to a seroprevalence of 50.00% (95% CI: 2.56 – 97.44), higher than for those who reported a negative test (statistically significant association: odds ratio 24.46 (95% CI: 0.60 – 988.64), p-value <0.001) (Table 5) (Figure 16).

Among the Portuguese population, about 232 respondents, circa 10.25%, reported a previous contact with a suspected or confirmed case. The seroprevalence in this group was estimated to be 9.05% (95% CI: 6.00 – 13.44%), higher than in those that did not have contact with suspected or confirmed cases (statistically significant association - odds ratio (13.30 (95% CI: 6.77 – 26.82%), p-value <0.0001). From the 919 (28.89%) individuals that reported to have performed a previous RT-PCR test, 30 had a reactive serological test, corresponding to a seroprevalence of 3.26% (95% CI: 2.30 – 4.62%). This value was higher than for those that did not perform a previous RT-PCR test (significant association; odds ratio (3.59% (95% CI: 2.05 – 6.41), p-value <0.0001). From the 919 individuals that performed a RT-PCR test, about 18 participants reported to have had a positive RT-PCR test, and from those 11 participants were reactive for the presence of antibodies, with an estimated seroprevalence of 61.11% (95% CI: 38.62 – 79.69), higher than for those that reported a negative RT-PCR test (statistically significant association: odds ratio 71.48 (95% CI: 25.05 – 218.65), p-value <0.0001) (Table 5) (Figure 16).

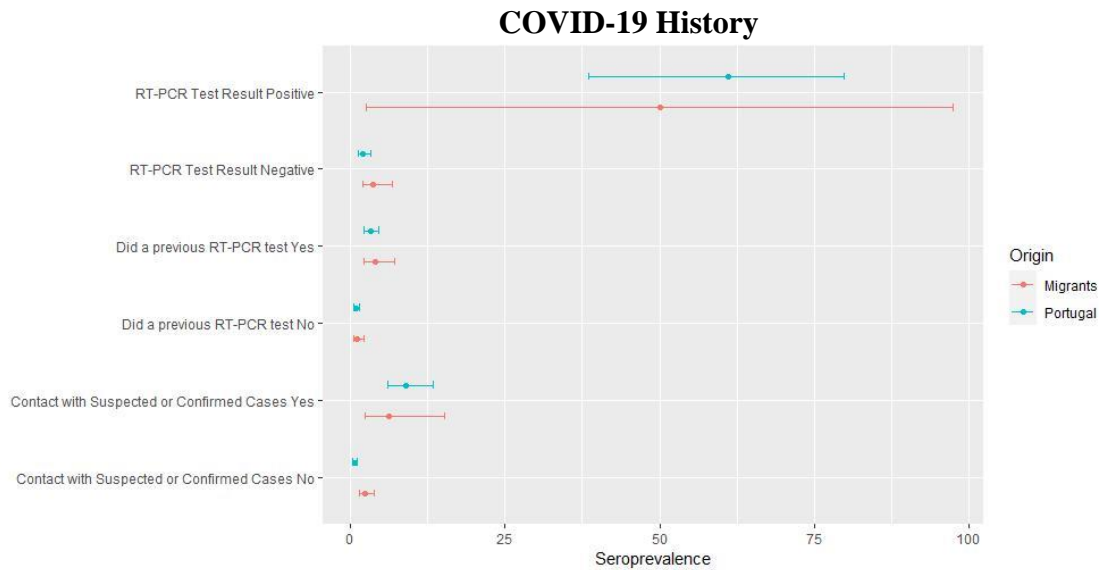


Figure 16 Seroprevalence (%) according to the COVID-19 history, among the migrants and Portuguese population 4.6.

### Seroprevalence for SARS-CoV-2 according to reported symptoms on the Migrants and Portuguese population

A total of 2106 participants, (47.76%), reported to have had at least one symptom from March 21<sup>st</sup> to October 1<sup>st</sup>, 2020. About 543 participants (14,48%), reported to have had a symptom suggestive of COVID-19 infection, such as, anosmia (loss of smell) or at least 3 symptoms among them being, fever, chills, asthenia (fatigue), odynophagia (sore throat), cough, dyspnea (shortness of breath), headache, nausea/vomiting, and diarrhea.

For most of each of the symptoms suggestive of COVID-19 infection analyzed, the seroprevalence was significantly higher among those participants reporting the symptoms, except for diarrhea, nose bleeding, altered consciousness, convulsions, and other neurological symptoms. Those reporting these symptoms had a lower seroprevalence compared to those who reported not having had it, from those who reported symptoms, being the seroprevalence higher (odds ratio ranged from 1.45 to 20.31 in the symptoms,  $p$ -value $<0.05$ ) The highest seroprevalence was registered in those who reported ageusia (loss of taste) (20.31% (95% CI: 12.27 – 31.71%)), anosmia (loss of smell) (16.28% (95% CI: 9.95 – 25.49%)), fever (7.69% (95% CI: 4.86 – 11.97%)) and anorexia (loss of appetite) (6.00% (95% CI: 2.78 – 12.48)). For the remaining symptoms that showed a significant difference, the seroprevalence ranged between 2.62% to 4.49% (Table 6).

Table 5 Seroprevalence information regarding the different reported COVID-19 symptoms among the overall population (migrant and Portuguese population)

Symptoms	N	%	Reactive	Seroprevalence (%) (95% CI)	Overall			
					Odds ratio (95% CI)	p-value	Sensitivity (%)	Specificity (%)
None	2304	52,24	28	1.22 (0.84-1.75)	ref	ref		
At least 1	2106	47,76	44	2.09 (1.56-2.79)	1.73 (1.08-2.82)	<b>0.022</b>	61.1	52.47
<b>Symptoms suggesting COVID-19 infection</b>								
No	3206	85,52	37	1.15 (0.84-1.59)	ref	ref		
Yes	543	14,48	22	4.05 (2.69-6.06)	3.63 (2.09-6.16)	<b>0.001</b>	37.29	85.88
<b>Fever</b>								
No	3743	94,42	49	1.31 (0.99-1.73)	ref	ref		
Yes	221	5,58	17	7.69 (4.86-11.97)	6.31 (3.47-10.97)	<b>0.001</b>	25.76	94.77
<b>Chills</b>								
No	3741	94,80	56	1.50 (1.15-1.94)	ref	ref		
Yes	205	5,20	8	3.90 (1.99-7.51)	2.72 (1.17-5.47)	<b>0.008</b>	12.5	94.93
<b>Asthenia (Fatigue)</b>								
No	3296	82,61	45	1.37 (1.02-1.82)	ref	ref		
Yes	694	17,39	22	3.17 (2.10-4.75)	2.37 (1.39-3.93)	<b>0.001</b>	32.84	82.87
<b>Myalgia (Muscle ache)</b>								
No	3440	86,52	51	1.48 (1.13-1.94)	ref	ref		
Yes	536	13,48	17	3.17 (1.99-5.02)	2.19 (1.22-3.75)	<b>0.005</b>	25	86.72
<b>Joint pain</b>								

Overall								
Symptoms	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	p-value	Sensitivity (%)	Specificity (%)
No	3527	89,22	58	1.64 (1.27-2.12)	ref	ref		
Yes	426	10,78	10	2.35 (1.28-4.27)	1.46 (0.69-2.75)	0.292	14.71	89.29
<b>Odynophagia (Sore Throat)</b>								
No	3355	84,06	56	1.67 (1.29-2.16)	ref	ref		
Yes	636	15,94	11	1.73 (0.97-3.07)	1.05 (0.52-1.94)	0.913	16.42	84.07
<b>Cough</b>								
No	3405	85,60	52	1.53 (1.17-2.00)	ref	ref		
Yes	573	14,40	15	2.62 (1.59-4.27)	1.75 (0.94-3.05)	0.061	22.39	85.73
<b>Nasal Congestion</b>								
No	3234	80,83	52	1.61 (1.23-2.10)	ref	ref		
Yes	767	19,17	16	2.09 (1.29-3.36)	1.31 (0.72-2.26)	0.357	23.53	80.91
<b>Dyspnea (Shortness of Breath)</b>								
No	3742	94,95	58	1.55 (1.20-2.00)	ref	ref		
Yes	199	5,05	5	2.51 (1.08-5.75)	1.68 (0.57-3.86)	0.291	7.94	95.00
<b>Wheezing</b>								
No	3796	96,00	64	1.69 (1.32-2.15)	ref	ref		
Yes	158	4,00	3	1.90 (0.65-5.43)	1.18 (0.28-3.24)	0.839	4.48	96.01
<b>Chest Pain</b>								
No	3879	97,76	63	1.62 (1.27-2.07)	ref	ref		
Yes	89	2,24	4	4.49 (1.76-10.99)	2.95 (0.86-7.38)	0.038	5.97	97.82

Overall								
Symptoms	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	p-value	Sensitivity (%)	Specificity (%)
<b>Other Respiratory Symptoms</b>								
No	3900	98,63	64	1.64 (1.29-2.09)	ref	ref		
Yes	54	1,37	2	3.70 (1.02-12.54)	2.47 (0.37-8.20)	0.240	3.03	98.66
<b>Conjunctivitis</b>								
No	3872	97,70	65	1.68 (1.32-2.13)	ref	ref		
Yes	91	2,30	2	2.20 (0.60-7.66)	1.41 (0.21-4.60)	0.704	2.99	97.72
<b>Headache</b>								
No	2929	73,24	42	1.43 (1.06-1.93)	ref	ref		
Yes	1070	26,76	24	2.24 (1.51-3.32)	1.58 (0.94-2.61)	0.075	36.36	73.40
<b>Altered Consciousness</b>								
No	3923	99,04	67	1.71 (1.35-2.16)	ref	ref		
Yes	38	0,96	0	0.00 (0.00-9.18)			0	99.02
<b>Convulsions</b>								
No	3959	99,80	67	1.69 (1.33-2.14)	ref	ref		
Yes	8	0,20	0	0.00 (0.00-32.44)			0	99.79
<b>Other Neurological Symptoms</b>								
No	3929	99,34	66	1.68 (1.32-2.13)	ref	ref		
Yes	26	0,66	0	0.00 (0.00-12.87)			0	99.33
<b>Nausea / Vomiting</b>								
No	3811	95,59	64	1.68 (1.32-2.14)	ref	ref		

Overall								
Symptoms	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	p-value	Sensitivity (%)	Specificity (%)
Yes	176	4,41	4	2.27 (0.89-5.70)	1.41 (0.42-3.47)	0.552	5.88	95.61
<b>Diarrhea</b>								
No	3580	89,63	61	1.70 (1.33-2.18)	ref	ref		
Yes	414	10,37	6	1.45 (0.67-3.13)	0.87 (0.33-1.87)	0.703	8.96	89.61
<b>Abdominal Pain</b>								
No	3823	96,18	63	1.65 (1.29-2.10)	ref	ref		
Yes	152	3,82	4	2.63 (1.03-6.57)	1.67 (0.49-4.13)	0.356	5.97	96.21
<b>Anosmia (Loss of Smell)</b>								
No	3887	97,84	53	1.36 (1.04-1.78)	ref	ref		
Yes	86	2,16	14	16.28 (9.95-25.49)	14.13 (7.22-26.07)	<b>0.001</b>	20.90	98.16
<b>Ageusia (Loss of Taste)</b>								
No	3908	98,39	54	1.38 (1.06-1.80)	ref	ref		
Yes	64	1,61	13	20.31 (12.27-31.71)	18.27 (9.03-34.80)	<b>0.001</b>	19.40	98.69
<b>Anorexia (Loss of Appetite)</b>								
No	3872	97,48	61	1.58 (1.23-2.02)	ref	ref		
Yes	100	2,52	6	6.00 (2.78-12.48)	4.08 (1.53-8.98)	<b>0.001</b>	8.96	97.59
<b>Nose Bleeding</b>								
No	3925	98,72	67	1.71 (1.35-2.16)	ref	ref		
Yes	51	1,28	0	0.00 (0.00-7.00)			0	98.70
<b>Other Symptoms</b>								

Symptoms	N	%	Reactive	Seroprevalence (%) (95% CI)	Overall			
					Odds ratio (95% CI)	p-value	Sensitivity (%)	Specificity (%)
No	3879	99,08	63	1.62 (1.27-2.07)	ref	ref		
Yes	36	0,92	2	5.56 (1.54-18.14)	3.81 (0.56-12.94)	0.066	3.08	99.12

From those who had reactive serological test in this study, 38.89% (95% CI: 28.47 – 50.44%) reported having had no symptoms, i.e., they were asymptomatic. The symptoms with higher sensitivity (proportion of reactive cases that are symptomatic) were Headache (36.36%), Asthenia (Fatigue) (32.84%), Fever (25.76%), Myalgia (Muscle Ache), Nasal Congestion (23.53%), Cough (22.39%) and Anosmia (Loss of Smell) (20.90%), among the Migrants and Portuguese Population (Table 6).

Among the Migrants population, a total of 532 participants, (51.25%), reported to have had at least one symptom and about 160 participants (17.94%), reported to have had a symptom suggestive of COVID-19 infection.

For most of each of the symptoms analyzed, the seroprevalence was higher in those who reported fever, chills, chest pain, headache, abdominal pain, anosmia (loss of smell), ageusia (loss of taste) and other symptoms. Those reporting these symptoms had a higher seroprevalence compared with non-reactive. From the symptoms analyzed, anosmia (loss of smell) (7.69% (95% CI: 2.14 – 24.14%) ageusia (loss of taste) (15.00% (95% CI: 5.24 – 36.04%) had a statistically significant association with the result of the the serological test. For the remaining symptoms with a higher seroprevalence in those reporting the symptom than in those reporting not having had it, such as fever (4.69% (95% CI: 1.61 – 12.90%), chills (3.17% (95% CI: 0.87 – 10.86%), chest pain (3.57% (95% CI: 0.18 – 17.71%), headache (2.46% (95% CI: 1.20 – 5.00%), abdominal pain (4.35% (95% CI: 1.20 – 14.53%) and other symptoms (10.00% (95% CI: 0.51 – 40.42%), there was no statistically association (Table 6) (Figure 17).

From those migrants who had a reactive serological test in this study, 57.14% (95% CI: 36.55 – 75.53%) reported having had no symptoms. The symptoms with higher sensitivity (proportion of reactive cases that are symptomatic) were Headache (35%), Fever (15%), Ageusia (Loss of Taste) (15%), Cough (14.29%) and Nasal Congestion (14.29%) (Table 6).

## COVID-19 Symptoms

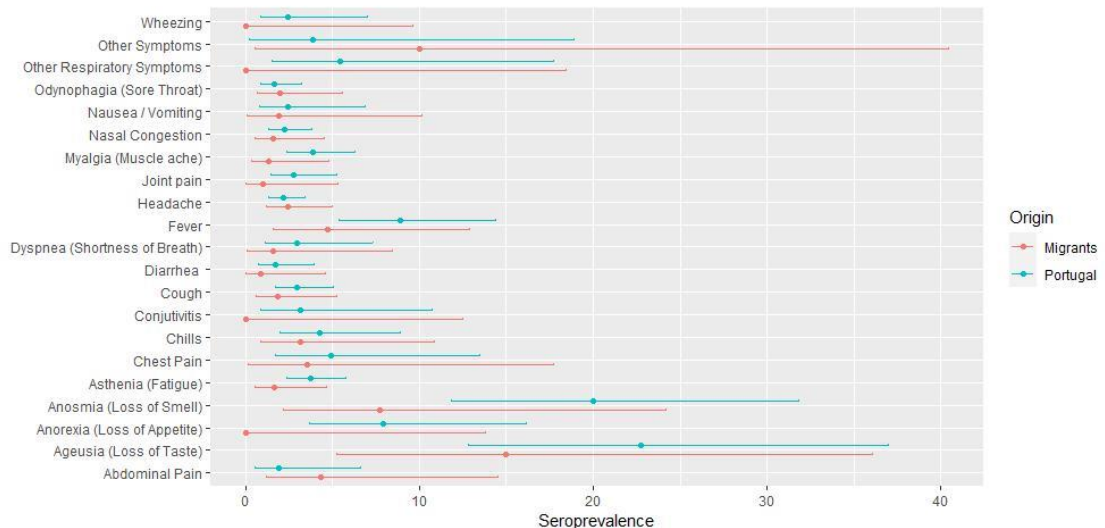


Figure 17 Seroprevalence (%) according to the reported COVID-19 symptoms, among the migrants and Portuguese population

Concerning the Portuguese population, a total of 1574 participants, (46,68%), had reported to have at least one symptom. And about 383 participants, (13.41%), reported to have a symptom suggestive of COVID-19 infection. For the symptoms analyzed, the seroprevalence were higher in the symptomatic group, for most of the symptoms categorized. For the reported symptoms altered consciousness, convulsions, other neurological symptoms, and nose bleeding, there were no reactive cases. Only the symptoms anosmia (loss of smell), ageusia (loss of taste), anorexia (loss of appetite), fever, chills, asthenia (fatigue), myalgia (muscle ache) and chest pain showed a higher seroprevalence when compared to those that reported not having had those symptoms, with statistically significant association (odds ratio ranging from 3.21 to 23.54, p-value <0.05). The highest values of seroprevalence were among those reporting ageusia (loss of taste) (22.73% (95% CI: 12.84 – 36.99%) and anosmia (loss of smell) (20.00% (95% CI: 11.83 – 31.78%). For the other symptoms there were no statistically significant association between occurrence of symptoms and result of the serological test. (Table 7) (Figure 17)

From those migrants who had a reactive serological test in this study, 31.37% (95% CI: 20.33 – 45.03%) reported having had no symptoms. The symptoms with higher sensitivity (proportion of reactive cases that are symptomatic) were Asthenia (Fatigue) (40.43%), Headache (36.96%), Nasal Congestion (27.66%), Cough (26.09%) and Anosmia (Loss of Smell) (25.53%) (Table 7).

Table 6 Seroprevalence information regarding the different reported symptoms among the migrant and Portuguese population

Symptomology		Migrants							Portuguese							
Symptoms	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	p-value	Sensitivity (%)	Specificity (%)	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	p-value	Sensitivity (%)	Specificity (%)
None	506	48,75	12	2.37 (1.36-4.10)	ref	ref			1798	53,32	16	0.89 (0.55-1.44)	ref	ref		
At least 1	532	51,25	9	1.69 (0.89-3.18)	0.71 (0.28-1.71)	0.437	42.86	48.57	1574	46,68	35	2.22 (1.60-3.08)	2.52 (1.41-4.71)	<b>0.002</b>	68.63	53.66
<b>Symptoms suggesting COVID-19 infection</b>																
No	732	82,06	13	1.78 (1.04-3.01)	ref	ref			2474	86,59	24	0.97 (0.65-1.44)	ref	ref		
Yes	160	17,94	5	3.13 (1.34-7.11)	1.82 (0.56-4.95)	0.272	27.78	82.27	383	13,41	17	4.44 (2.79-6.99)	4.75 (2.48-8.91)	<b>0.001</b>	41.46	87.00
<b>Fever</b>																
No	876	93,19	17	1.94 (1.22-3.09)	ref	ref			2867	94,81	32	1.12 (0.79-1.57)	ref	ref		
Yes	64	6,81	3	4.69 (1.61-12.90)	2.58 (0.57-8.04)	0.142	15	93.37	157	5,19	14	8.92 (5.39-14.41)	8.71 (4.40-16.43)	<b>0.001</b>	30.43	95.20
<b>Chills</b>																
No	877	93,30	18	2.05 (1.30-3.22)	ref	ref			2864	95,28	38	1.33 (0.97-1.82)	ref	ref		

Symptomology		Migrants							Portuguese							
Symptoms	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	p-value	Sensitivity (%)	Specificity (%)	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	p-value	Sensitivity (%)	Specificity (%)
Yes	63	6,70	2	3.17 (0.87-10.86)	1.67 (0.24-6.01)	0.551	10	93.37	142	4,72	6	4.23 (1.95-8.91)	3.35 (1.24-7.53)	<b>0.005</b>	13.64	95.41
<b>Asthenia (Fatigue)</b>																
No	762	80,46	17	2.23 (1.40-3.54)	ref	ref			2534	83,27	28	1.10 (0.77-1.59)	ref	ref		
Yes	185	19,54	3	1.62 (0.55-4.66)	0.75 (0.17-2.29)	0.605	15	80.37	509	16,73	19	3.73 (2.40-5.76)	3.48 (1.89-6.25)	<b>0.001</b>	40.43	83.64
<b>Myalgia (Muscle ache)</b>																
No	790	84,22	18	2.28 (1.45-3.57)	ref	ref			2650	87,23	33	1.25 (0.89-1.74)	ref	ref		
Yes	148	15,78	2	1.35 (0.37-4.79)	0.63 (0.09-2.22)	0.474	10	84.10	388	12,77	15	3.87 (2.36-6.28)	3.21 (1.67-5.87)	<b>0.001</b>	31.25	87.53
<b>Joint pain</b>																
No	833	89,00	20	2.40 (1.56-3.68)	ref	ref			2694	89,29	38	1.41 (1.03-1.93)	ref	ref		
Yes	103	11,00	1	0.97 (0.05-5.30)	0.45 (0.02-2.20)	0.355	4.76	88.85	323	10,71	9	2.79 (1.47-5.21)	2.03 (0.91-4.06)	0.059	19.15	89.43
<b>Odynophagia (Sore Throat)</b>																
No	792	83,72	17	2.15 (1.34-3.41)	ref	ref			2563	84,17	39	1.52 (1.12-2.07)	ref	ref		

Symptomology		Migrants							Portuguese							
Symptoms	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	p-value	Sensitivity (%)	Specificity (%)	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	p-value	Sensitivity (%)	Specificity (%)
<b>Cough</b>																
Yes	154	16,28	3	1.95 (0.66-5.57)	0.94 (0.21-2.88)	0.876	15	83.69	482	15,83	8	1.66 (0.84-3.24)	1.11 (0.47-2.27)	0.821	17.02	84.19
No	782	82,58	18	2.30 (1.46-3.61)	ref	ref			2623	86,54	34	1.30 (0.93-1.81)	ref	ref		
<b>Nasal Congestion</b>																
Yes	165	17,42	3	1.82 (0.62-5.21)	0.82 (0.18-2.48)	0.701	14.29	83.69	408	13,46	12	2.94 (1.69-5.07)	2.33 (1.14-4.42)	<b>0.011</b>	26.09	86.73
No	758	79,79	18	2.37 (1.51-3.72)	ref	ref			2476	81,15	34	1.37 (0.98-1.91)	ref	ref		
<b>Dyspnea (Shortness of Breath)</b>																
Yes	192	20,21	3	1.56 (0.53-4.49)	0.68 (0.15-2.06)	0.494	14.29	79.65	575	18,85	13	2.26 (1.33-3.83)	1.67 (0.84-3.12)	0.119	27.66	81.29
No	877	93,30	19	2.17 (1.39-3.36)	ref	ref			2865	95,47	39	1.36 (1.00-1.86)	ref	ref		
<b>Wheezing</b>																
Yes	63	6,70	1	1.59 (0.08-8.46)	0.83 (0.03-4.08)	0.758	5	93.26	136	4,53	4	2.94 (1.15-7.32)	2.27 (0.66-5.77)	0.130	9.30	95.54
No	893	96,12	20	2.24 (1.45-3.43)	ref	ref			2903	95,97	44	1.52 (1.13-2.03)	ref	ref		

Symptomology		Migrants							Portuguese							
Symptoms	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	p-value	Sensitivity (%)	Specificity (%)	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	p-value	Sensitivity (%)	Specificity (%)
Yes	36	3,88	0	0.00 (0.00-9.64)			0	96.04	122	4,03	3	2.46 (0.84-6.98)	1.72 (0.40-4.80)	0.409	6.38	96.00
<b>Chest Pain</b>																
No	910	97,01	19	2.09 (1.34-3.24)	ref	ref			2969	97,99	44	1.48 (1.11-1.98)	ref	ref		
Yes	28	2,99	1	3.57 (0.18-17.71)	1.96 (0.08-10.05)	0.592	5	97.06	61	2,01	3	4.92 (1.69-13.49)	3.60 (0.82-10.27)	<b>0.032</b>	6.38	98.06
<b>Other Respiratory Symptoms</b>																
No	919	98,18	20	2.18 (1.41-3.34)	ref	ref			2981	98,77	44	1.48 (1.10-1.98)	ref	ref		
Yes	17	1,82	0	0.00 (0.00-18.43)			0	98.14	37	1,23	2	5.41 (1.50-17.70)	4.08 (0.60-14.02)	0.053	4.35	98.82
<b>Conjunctivitis</b>																
No	912	97,12	20	2.19 (1.42-3.36)	ref	ref			2960	97,88	45	1.52 (1.14-2.03)	ref	ref		
Yes	27	2,88	0	0.00 (0.00-12.46)			0	97.06	64	2,12	2	3.13 (0.86-10.70)	2.24 (0.33-7.49)	0.305	4.26	97.92
<b>Headache</b>																
No	665	70,07	13	1.95 (1.15-3.32)	ref	ref			2264	74,23	29	1.28 (0.89-1.83)	ref	ref		

<b>Syptomology</b>																
<b>Migrants</b>																
<b>Portuguese</b>																
<b>Symptoms</b>	<b>N</b>	<b>%</b>	<b>Reactive</b>	<b>Seroprevalence (%) (95% CI)</b>	<b>Odds ratio (95% CI)</b>	<b>p-value</b>	<b>Sensitivity (%)</b>	<b>Specificity (%)</b>	<b>N</b>	<b>%</b>	<b>Reactive</b>	<b>Seroprevalence (%) (95% CI)</b>	<b>Odds ratio (95% CI)</b>	<b>p-value</b>	<b>Sensitivity (%)</b>	<b>Specificity (%)</b>
Yes	284	29,93	7	2.46 (1.20-5.00)	1.28 (0.47-3.19)	0.617	35	70.18	786	25,77	17	2.16 (1.35-3.44)	1.71 (0.91-3.11)	0.080	36.96	74.40
<b>Altered Consciousness</b>																
No	931	99,04	20	2.15 (1.39-3.29)	ref	ref			2992	99,04	47	1.57 (1.18-2.08)	ref	ref		
Yes	9	0,96	0	0.00 (0.00-29.91)			0	99.02	29	0,96	0	0.00 (0.00-11.70)			0	99.02
<b>Convulsions</b>																
No					ref	ref			3019	99,74	47	1.56 (1.17-2.06)	ref	ref		
Yes									8	0,26	0	0.00 (0.00-32.44)			0	99.73
<b>Other Neurological Symptoms</b>																
No	930	99,15	20	2.15 (1.40-3.30)	ref	ref			2999	99,40	46	1.53 (1.15-2.04)	ref	ref		
Yes	8	0,85	0	0.00 (0.00-32.44)			0	99.13	18	0,60	0	0.00 (0.00-17.59)			0	99.39
<b>Nausea / Vomiting</b>																
No	897	94,52	19	2.12 (1.36-3.28)	ref	ref			2914	95,92	45	1.54 (1.16-2.06)	ref	ref		

Symptomology		Migrants							Portuguese							
Symptoms	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	p-value	Sensitivity (%)	Specificity (%)	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	p-value	Sensitivity (%)	Specificity (%)
<b>Diarrhea</b>																
Yes	52	5,48	1	1.92 (0.10-10.12)	1.03 (0.04-5.10)	0.924	5	94.81	124	4,08	3	2.42 (0.83-6.87)	1.66 (0.38-4.63)	0.444	6.25	95.95
No	827	87,42	19	2.30 (1.48-3.56)	ref	ref			2753	90,32	42	1.53 (1.13-2.06)	ref	ref		
Yes	119	12,58	1	0.84 (0.04-4.61)	0.41 (0.02-1.99)	0.302	5	87.26	295	9,68	5	1.69 (0.73-3.91)	1.14 (0.39-2.66)	0.823	10.64	90.34
<b>Abdominal Pain</b>																
No	898	95,13	18	2.00 (1.27-3.15)	ref	ref			2925	96,50	45	1.54 (1.15-2.05)	ref	ref		
Yes	46	4,87	2	4.35 (1.20-14.53)	2.36 (0.34-8.63)	0.282	10	95.24	106	3,50	2	1.89 (0.52-6.62)	1.32 (0.20-4.35)	0.776	4.26	96.51
<b>Anosmia (Loss of Smell)</b>																
No	916	97,24	18	1.97 (1.25-3.08)	ref	ref			2971	98,02	35	1.18 (0.85-1.63)	ref	ref		
Yes	26	2,76	2	7.69 (2.14-24.14)	4.40 (0.62-16.69)	<b>0.046</b>	10	97.40	60	1,98	12	20.00 (11.83-31.78)	21.03 (9.89-42.21)	<b>0.001</b>	25.53	98.39
<b>Ageusia (Loss of Taste)</b>																
No	921	97,87	17	1.85 (1.16-2.94)	ref	ref			2987	98,55	37	1.24 (0.90-1.70)	ref	ref		

Symptomology		Migrants							Portuguese							
Symptoms	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	p-value	Sensitivity (%)	Specificity (%)	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	p-value	Sensitivity (%)	Specificity (%)
Yes	20	2,13	3	15.00 (5.24-36.04)	9.65 (2.01-32.81)	<b>0.001</b>	15	98.15	44	1,45	10	22.73 (12.84-36.99)	23.54 (10.30-49.99)	<b>0.001</b>	21.28	98.86
<b>Anorexia (Loss of Appetite)</b>																
No	920	97,46	20	2.17 (1.41-3.33)	ref	ref			2952	97,49	41	1.39 (1.03-1.88)	ref	ref		
Yes	24	2,54	0	0.00 (0.00-13.80)			0	97.40	76	2,51	6	7.89 (3.67-16.17)	6.21 (2.28-14.14)	<b>0.001</b>	12.77	97.65
<b>Nose Bleeding</b>																
No	926	98,30	20	2.16 (1.40-3.31)	ref	ref			2999	98,85	47	1.57 (1.18-2.08)	ref	ref		
Yes	16	1,70	0	0.00 (0.00-19.36)			0	98.26	35	1,15	0	0.00 (0.00-9.89)			0	98.83
<b>Other Symptoms</b>																
No	922	98,93	19	2.06 (1.32-3.20)	ref	ref			2957	99,13	44	1.49 (1.11-1.99)	ref	ref		
Yes	10	1,07	1	10.00 (0.51-40.42)	5.88 (0.23-34.52)	0.085	5	99.01	26	0,87	1	3.85 (0.20-18.90)	3.00 (0.12-14.63)	0.326	2.22	99.15

#### 4.7. Seroprevalence for SARS-CoV-2 according to COVID-19 implications on the Migrants and Portuguese population

On the migrants and portuguese population, about 420 (11.35%) participants sought medical assistance due to COVID-19 implications or symptoms. The seroprevalence among these participants was estimated to be 5.95% (95% CI: 4.06 – 8.64%), higher than for those who did not seek medical support (statistically significant association: (odds ratio 5.14 (3.04 – 8.64), p-value <0.0001). For those who were hospitalized, 38 (1.03%) individuals, none had a reactive serological test. Among the hospitalized cases, those who needed intensive care support were 15 individuals and none had a reactive test. Regarding to the impact of COVID-19, those who miss work or school were about 144 (3.99%) individuals and their, seroprevalence was estimated to be 9.03% (95% CI: 5.35 – 14.83%) (odds ratio 6.97 (95% CI: 3.53 – 12.82), p-value <0.001) (Table 8).

Table 7 Seroprevalence information regarding the COVID-19 impact among the overall population (migrant and Portuguese population)

COVID-19 Impact Overall									
Seek Medical Assistance	N	%	Reactive	Seroprevalence (95% CI)	Odds ratio (95% CI)	Chi-square or Fisher test (*) (p-value)	Wald test (Pr(> z ))	Wald test (Pr(> z )) Adjusted	
No	3282	88,65	40	1.22 (0.90-1.66)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Yes	420	11,35	25	5.95 (4.06-8.64)	5.14 (3.04-8.52)	<b>0.001</b>	<b>0</b>		
<b>Miss Work / School</b>									
No	3464	96,01	49	1.41 (1.07-1.87)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Yes	144	3,99	13	9.03 (5.35-14.83)	6.97 (3.53-12.82)	<b>0.001*</b>	<b>0</b>		
<b>Hospitalized</b>									
No	3636	98,97	65	1.79 (1.41-2.27)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Yes	38	1,03	0	0.00 (0.00-9.18)			0.983		
<b>Intensive Care</b>									
No	2147	99,31	39	1.82 (1.33-2.47)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Yes	15	0,69	0	0.00 (0.00-2.04)			0.984		

On the migrants population, about 116 (13.29%) participants sought medical assistance due to COVID-19 implications or symptoms. The seroprevalence among the participants who sought medical assistance was estimated to be 5.17% (95% CI: 2.39 – 10.83%), statistically significant difference between the participants who sought medical assistance and those who did not (odds ratio 4.11 (95% CI: 1.35 – 11.45), p-value <0.005). Only 14 (1.63%) participants reported to be hospitalized, and 6 (1.23%) participants reported to need intensive care due to health complications. In these participants the seroprevalence was zero (with a very small sample size). Only 51 (6.01%) participants, reported to have missed work or school due to COVID-19 symptoms or complications. The seroprevalence among these participants was estimated to be 5.88% (95% CI: 2.02 – 15.92%), those who reported not having missed work

or school (statistically significant association: odds ratio 3.91 (95% CI: 0.83 – 12.81), p-value <0.05) (Table 9) (Figure 18).

On the portuguese population, about 304 (10.75%) of participants sought medical assistance. Among these participants the estimated seroprevalence was established at 6.25% (95% CI: 4.04 – 9.55%), higher than in those who reported not having sought medical assistance (statistically significant association: odds ratio 5.56 (95% CI: 3.03 – 9.95), p-value <0.0001). Only 24 (0.85%) participants, reported having needed hospital support and 9 participants needed intensive care support. The seroprevalence among them was zero, with a small sample size. 93 (3.37%) participants reported to have missed work or school due to COVID-19 symptoms or complications. The assessed seroprevalence among them was 10.75% (95% CI: 5.95 – 18.67%), higher than in those reporting not having missed work or school (statistically significant association: odds ratio 8.88 (95% CI: 4.03 – 17.93), p-value <0.0001) (Table 9) (Figure 18).

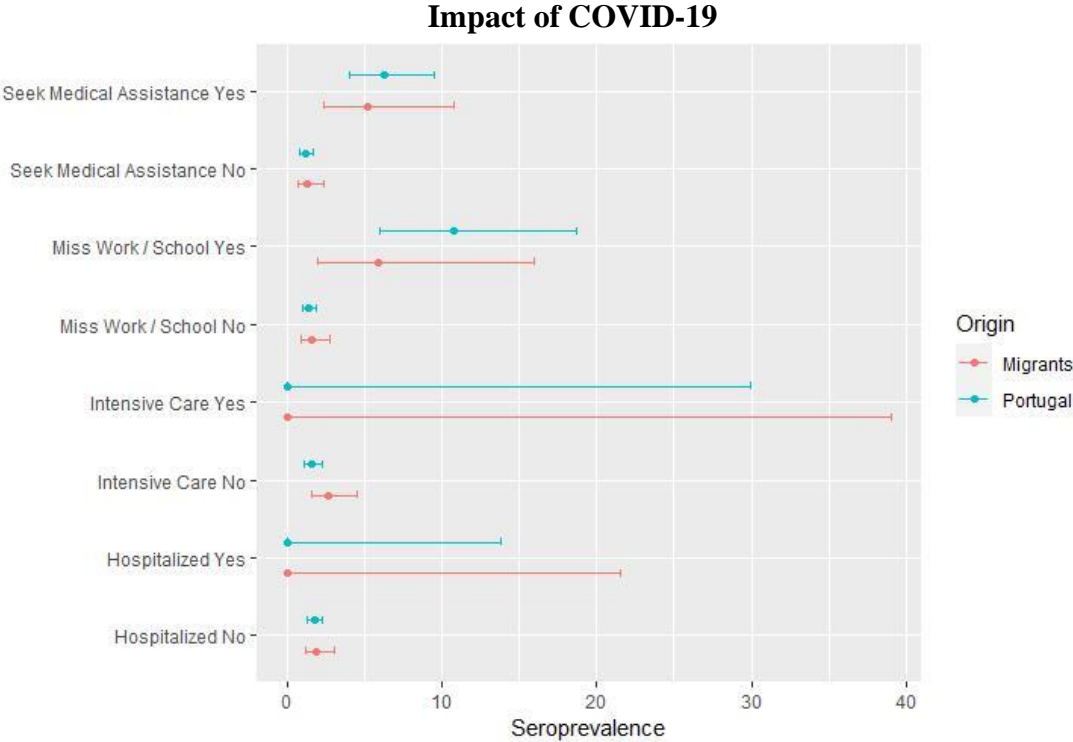


Figure 18 Seroprevalence (%) according to the impact of COVID-19, among the migrants and Portuguese population

Table 8 Seroprevalence information regarding to COVID-19 impact among the migrant and Portuguese population

COVID-19 Impact			Migrants						Portuguese							
Seeked Medical Assistance	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	Chi-square or Fisher test (*) (p-value)	Wald test (Pr(> z ))	Wald test (Pr(> z )) Adjusted	N	%	Reactive	Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	Chi-square or Fisher test (*) (p-value)	Wald test (Pr(> z ))	Wald test (Pr(> z )) Adjusted
No	757	86,71	10	1.32 (0.72-2.41)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>	2525	89,25	30	1.19 (0.83-1.69)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Yes	116	13,29	6	5.17 (2.39-10.83)	4.11 (1.35-11.45)	<b>0.013*</b>	0.008	0.006	304	10,75	19	6.25 (4.04-9.55)	5.56 (3.03-9.95)	<b>0.001</b>	0.001	0.001
<b>Missed Work / School</b>																
No	798	93,99	13	1.63 (0.95-2.77)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>	2666	96,63	36	1.35 (0.98-1.86)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Yes	51	6,01	3	5.88 (2.02-15.92)	3.91 (0.83-12.81)	0.066*	0.043	0.041	93	3,37	10	10.75 (5.95-18.67)	8.88 (4.03-17.93)	<b>0.001*</b>	0.001	0.001
<b>Hospitalized</b>																
No	847	98,37	16	1.89 (1.17-3.05)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>	2789	99,15	49	1.76 (1.33-2.32)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Yes	14	1,63	0	0.00 (0.00-21.53)					24	0,85	0	0.00 (0.00-13.80)				
<b>Intensive Care</b>																
No	483	98,77	13	2.69 (1.58-4.55)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>	1664	99,46	26	1.56 (1.07-2.28)	<i>ref</i>	<i>ref</i>	<i>ref</i>	<i>ref</i>
Yes	6	1,23	0	0.00 (0.00-39.03)					9	0,54	0	0.00 (0.00-29.91)				

## 5 Discussion

The serological survey carried out by the Municipality of Cascais allowed the collection of sociodemographic, clinical and exposure data from a large number of participants and to associate them with the results of the serological screening to detect antibodies against the SARS-CoV-2 virus. In this study the results analyzed corresponded to the data collection period between 21<sup>st</sup> May and 1<sup>st</sup> October 2020, corresponding to the period after the first wave of the COVID-19 pandemic. The overall seroprevalence among the migrants and portuguese population was calculated to be 1.63% (95% CI: 1.29 – 2.05%), and posterior Bayesian estimate was of 1.47% (95% Confidence Interval: 1.16 – 1.79%), considering the known sensitivity and specificity of the serological test. For the migrants population, the overall seroprevalence was slightly higher, 2.02% (95% CI: 1.33 – 3.07%), when compared with the overall seroprevalence of the migrants and portuguese population. These values are lower than the 2.9% (95% CI: 2.0 – 4.2%) obtained from the 1<sup>st</sup> national serological study (RELATÓRIO DE SITUAÇÃO - Covid 19) conducted by the INSA. The differences in the seroprevalence can be related to lower risks of exposure factors in Cascais compared to the national situation. However, the national serological survey was carried out for people who went to a collection point to carry out a blood collection with a medical prescription, for reasons other than COVID-19. As a different sampling method from the one adopted in the Cascais serological survey, the comparison of results is only indicative.

The seroprevalence estimated in this migrants population, 2.02% (95% CI: 1.33 – 3.07%), is lower compared with the Oman's study that among the migrants population estimated it to be 9.1% (95% CI: 7.6 – 10.9%) (Al-abri et al., 2021) during the months of May to July of 2020, compared during the duration of our study conducted between May to October of 2020.

Similar to other Serological surveys performed worldwide, this study found no differences in SARS-CoV-2 seroprevalence, between genders, age groups, different educational levels, different parishes and between travelers and non-travelers (Alsuwaidi et al., 2021)(Al-abri et al., 2021)(Rostami et al., 2021)(Wilder-smith et al., 2005)(Clapham et al., 2020). Only the household size was significantly associated with the serological test result, with the groups of households larger than five persons, presenting higher seroprevalence when compared to the households constituted by only one person. This finding reinforces the hypothesis that people living in bigger households have greater chances of being infected with SARS-CoV-2 when comparing with those living alone. On the other hand, it goes in contrast with a study conducted in Spain, that indicated that those with a household size of 2 persons had a higher seroprevalence, 5.7% (95% CI: 5.1 – 6.5%). (Ramanathan et al., 2020).

Although the other socio-demographic variables showed no statistically significant association with the serological test result, we identified the groups with higher mean seroprevalence as a guidance for future studies. The mean seroprevalence values were higher among the male population in the different population's groups, is in line with the national serological survey. Regarding to the different age groups, on the Migrants and Portuguese population combined, the age group 30-39 has shown a higher seroprevalence. For the migrants population, a higher mean seroprevalence was found among the age group 20-29. And finally,

in the Portuguese population the age group 0-19 showed a higher seroprevalence. These results point to a different scenario compared to the national serological survey that indicates the age group of 40-59 with a higher seroprevalence, compared with the others age groups. In general, other studies indicated the younger generation with a higher seroprevalence, due to higher social contacts (Al-abri et al., 2021) (Canto e Castro et al., 2021).

Regarding the seroprevalence in the different educational levels for the different populations, the national serological survey indicated that the individuals with high school education level have a higher seroprevalence, 6.4% (95% CI: 3.2 – 12.5%), consistent with our findings in the Portuguese population, that also indicate high school education level as the one with a higher value of seroprevalence. On the other hand, in the migrants population individuals with elementary School were found to have a higher seroprevalence.

When analysing employment status, unemployed and “other inactive” had a higher seroprevalence in the complete study population, as well as on the migrants population and on the Portuguese population. These findings are in contrast with the results of the national serological survey, in which the employed participants had the highest seroprevalence of 3.8% (95% CI: 2.2 – 6.3%)(INSA/DGS), compared to the other occupational groups.

Regarding to the seroprevalence among the travelers and non-travelers, we did not find any significant association, although the mean seroprevalence was slightly higher in the migrants that reported to have not travelled and in the Portuguese who reported to have travelled. The question about travel history included in this questionnaire was only referring to the period between January and February of 2020, which limits the interpretation of these results. Epidemiological studies, conducted by (Borges et al., 2022) indicated that persons who flown from Italy, United Kingdom, Spain and France to Portugal were the ones with a higher number of infections, during the first months of COVID-19 pandemic. While information about countries of travel was present in our sero-epidemiological questionnaires, we did not analyze that information yet.

Chronic patients from the different population groups showed generally a lower seroprevalence, although not significantly different from the non-ill. In general, the chronically-ill patients may have adopted more protective measures to avoid infection given that the higher risk of aggravated outcomes of COVID-19 in the chronically-ill was widely announced since the start of the pandemic. Only the obese showed a higher seroprevalence, but not significantly different from the ones who reported to not have obesity and this was consistent both for migrants and Portuguese. Supporting these results, a US study that evaluated this same topic showed that people with chronic diseases had a lower seropositivity rate when compared to the non-chronically ill. (Bajema et al., 2021).

For the more severe cases of COVID-19, those who needed hospitalization or intensive care were not present in this study, as revealed by the absence of reactive cases in the participants that reported having needed hospitalization or intensive care.

The symptoms fever, chills, asthenia (fatigue), myalgia (muscle ache), chest pain, anosmia (loss of smell), ageusia (loss of taste), anorexia (loss of appetite) were the ones that showed a statistically significant association with the serological test results in the migrants and

Portuguese population. As the pandemic developed, the symptoms reported suffered some changes, as the SARS-CoV-2 virus mutated to generate other variants (Harvey et al., 2021).

This study had some limitations, such as the design of the study that implied that inclusion of individuals in the study was strongly conditioned by the willingness of people residing in the Cascais municipality to travel to the laboratory centers to carry out the serological test. The results are limited to the population residing in Cascais, a single municipality, with its own different characteristics distinct from other municipalities. For example, it is a municipality with a high number of inhabitants with a higher education level, with high salaries, and a younger population. Cascais inhabitants also have disseminated access to health services, with an extensive network of places dedicated to primary and secondary health care available.

With these data, it was not possible to establish a temporal sequence between exposure to the risk factor and the consequent development of COVID-19 disease. The quantification of the immunoassay for the SARS-CoV-2 virus antibodies, as well as the durability of the serum levels of these same antibodies was not measured in this study. Also, the serological tests done here only assess previous exposure and not immunity, as no neutralizing antibodies were measured. During the study, the participants that were residents of nursing homes, people who were hospitalized or population groups that would not have a way to travel to places to perform the PCR and serological tests, were not included. Finally, some symptoms reported as having high specificity also have a small number of participants with that symptom, that is a very high percentage without symptoms, which influences the value of specificity.

## **6 Conclusion**

The pandemic caused by the disease COVID-19 has had serious consequences not only from a health point of view, but also for the psychosocial sphere of the world population. As such, it is essential to understand the main characteristics of the dissemination of the disease within the population, to stop its spread. In this perspective, serological studies are fundamental not only to measure the immunity of the population, but also to identify the sociodemographic, behavioral and symptomatologic factors associated to infection with SARS-CoV-2.

The present seroprevalence study was carried out in the population residing in the municipality of Cascais between 21<sup>st</sup> May 2020 to 1<sup>st</sup> October 2020. Male participants had a higher seroprevalence and age also showed to be an important factor, as older people had a lower chance of obtaining a reactive serological test. Regarding to the chronic diseases, in general, chronic-ill had a lower chance of obtaining a reactive serological test, that can be explained by adopting more preventive measures and due to being categorized as risk populations to developing severe disease or even death.

Regarding to the symptoms reported, fever, chills, asthenia (fatigue), myalgia (muscle ache), chest pain, anosmia (loss of smell), ageusia (loss of taste), anorexia (loss of appetite) were the ones associated with positive serological testing both among the migrants and Portuguese population. The smaller sample size of the migrants population limited more definitive conclusion about symptoms reported by seropositive individuals. On the other hand, as the pandemic developed and variants causing the epidemic in Portugal changed, symptoms associated with COVID-19 also changed.

The control of the COVID-19 pandemic spread can be difficult due to the asymptomatic individuals. Identification of previously infected asymptomatic individuals is only possible through serological surveys. Such initiatives can help guide more effective individual and collective preventive measures to minimize the impact of the disease on global public health.

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## **8 ANNEX**

**8.1 I: First Version of Serological Questionnaire**

**8.2 II: Updated Version of Serological Questionnaire**

**8.3 III: Informed Consent Germano Sousa**

## ANNEX I: First Version of Serological Questionnaire

# COVID-19

## INQUÉRITO SEROLÓGICO

Código de Identificação do laboratório:   
Código de Identificação do Teste:

### CARACTERIZAÇÃO SOCIOECONÓMICA

1. Sexo  Masculino  Feminino  Outro

2. Ano de Nascimento

3. Código Postal

4. Qual o seu nível de escolaridade (ano mais elevado de escolaridade que completou ou ao qual obteve equivalência)?

Menos que a 4ª Classe

1º Ciclo Completo (4º ano)

2º Ciclo Completo (6º ano)

3º Ciclo Completo (9º ano)

Ensino Secundário (12º ano - cursos gerais, tecnológicos ou profissionais)

Ensino Pós-Secundário Não Superior (Curso Especialização Tecnológica)

Frequência Universitária ou Bacharelato/ Curso Médio

Ensino Superior (Licenciatura, Mestrado, Doutoramento)

Não Sabe/ Não Responde

5. Em que situação se encontra, quanto à ocupação ou atividade profissional?

Empregado/a

Desempregado/a

(inclui estágios pagos, licenças de maternidade/paternidade, baixas médicas por doença, lay-off ou bolsas de investigação)

Estudante /Em Formação  Reformado/a

Prestador/a de Trabalho Doméstico  Prestador/a de Serviço Comunitário OU Cuidador Informal

Incapacitado/a permanentemente  Outra situação de inatividade

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# COVID-19

## INQUÉRITO SEROLÓGICO

Código de Identificação do laboratório:

Código de Identificação do Teste:

6. Caso tenha respondido que se encontra empregado/a, qual a sua atividade profissional principal?

### ANTECEDENTES | DOENÇAS CRÓNICAS

7. Está diagnosticado para alguma das seguintes doenças crónicas?

Diabetes  Sim  Não  Não sabe

Doença Pulmonar Crónica (exclui asma)  Sim  Não  Não sabe

Asma  Sim  Não  Não sabe

Doença Cardiovascular (inclui hipertensão)  Sim  Não  Não sabe

Obesidade  Sim  Não  Não sabe

Doença Autoimune  Sim  Não  Não sabe

Outras Doenças. Quais?

### ANTECEDENTES - COVID 19

8. Esteve em contacto com algum caso suspeito ou confirmado de COVID-19?

Sim  Não  Não sabe/Não responde

9. Se respondeu "Sim", indique a última data de contacto:

10. Já realizou algum teste para deteção de COVID-19?  Sim  Não  Não sabe

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# CASCAIS

Tudo começa nas pessoas

# COVID-19

## INQUÉRITO SEROLÓGICO

Código de Identificação do laboratório:   
Código de Identificação do Teste:

11. Caso tenha respondido "Sim", qual foi o resultado?

- Positivo. Em caso afirmativo, indique a data do primeiro teste positivo:  
//
- Negativo
- Inconclusivo
- Não sabe/ Não Responde

12. Desde o início de março, já teve algum dos seguintes sintomas, mesmo que passageiros?

Em caso afirmativo, identificar se os primeiros sintomas ocorreram:

A) Última Semana B) Há 2 Semanas C) Há 3 Semanas D) Há 1 Mês E) Há mais de 1 mês

- |   |  |                  |
|---|--|------------------|
| 12.1 Febre ( $\geq 37,5^{\circ}\text{C}$ )  | Sim <input type="checkbox"/> Não <input type="checkbox"/> NS/NR <input type="checkbox"/> | Há quanto tempo: |
| 12.2 Arrepios                               | Sim <input type="checkbox"/> Não <input type="checkbox"/> NS/NR <input type="checkbox"/> | Há quanto tempo: |
| 12.3 Fadiga (Cansaço)                       | Sim <input type="checkbox"/> Não <input type="checkbox"/> NS/NR <input type="checkbox"/> | Há quanto tempo: |
| 12.4 Dores Musculares                       | Sim <input type="checkbox"/> Não <input type="checkbox"/> NS/NR <input type="checkbox"/> | Há quanto tempo: |
| 12.5 Dores Articulares                      | Sim <input type="checkbox"/> Não <input type="checkbox"/> NS/NR <input type="checkbox"/> | Há quanto tempo: |
| 12.6 Dor de Garganta                        | Sim <input type="checkbox"/> Não <input type="checkbox"/> NS/NR <input type="checkbox"/> | Há quanto tempo: |
| 12.7 Tosse                                  | Sim <input type="checkbox"/> Não <input type="checkbox"/> NS/NR <input type="checkbox"/> | Há quanto tempo: |
| 12.8 Nariz Congestionado /Corrimento Nasal  | Sim <input type="checkbox"/> Não <input type="checkbox"/> NS/NR <input type="checkbox"/> | Há quanto tempo: |
| 12.9 Falta de Ar (Dificuldade Respiratória) | Sim <input type="checkbox"/> Não <input type="checkbox"/> NS/NR <input type="checkbox"/> | Há quanto tempo: |

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CASCAIS  
Tudo começa nas pessoas

# COVID-19

## INQUÉRITO SEROLÓGICO

Código de Identificação do laboratório:

Código de Identificação do Teste:

- 12.10 Pieira Sim  Não  NS/NR  Há quanto tempo:
- 12.11 Dor Torácica Sim  Não  NS/NR  Há quanto tempo:
- 12.12 Outras Queixas Respiratórias Sim  Não  NS/NR  Há quanto tempo:
- 12.13 Conjuntivite Sim  Não  NS/NR  Há quanto tempo:
- 12.14 Dor de Cabeça** Sim  Não  NS/NR  Há quanto tempo:
- 12.15 Alterações do Estado de Consciência Sim  Não  NS/NR  Há quanto tempo:
- 12.16 Convulsões Sim  Não  NS/NR  Há quanto tempo:
- 12.17 Outras Queixas Neurológicas Sim  Não  NS/NR  Há quanto tempo:
- 12.18 Náusea ou Vômitos** Sim  Não  NS/NR  Há quanto tempo:
- 12.19 Diarreia** Sim  Não  NS/NR  Há quanto tempo:
- 12.20 Dor Abdominal Sim  Não  NS/NR  Há quanto tempo:
- 12.21 Perda de Cheiro Sim  Não  NS/NR  Há quanto tempo:
- 12.22 Perda de Paladar Sim  Não  NS/NR  Há quanto tempo:
- 12.23 Perda de Apetite Sim  Não  NS/NR  Há quanto tempo:
- 12.24 Hemorragia Nasal Sim  Não  NS/NR  Há quanto tempo:
- 12.25 Outras Queixas Sim  Não  NS/NR  Há quanto tempo:

13. Procurou apoio médico devido a algum destes sintomas? Sim  Não  NS/NR

14. Faltou ao trabalho/escola devido a algum destes sintomas? Sim  Não  NS/NR

15. Ficou hospitalizado/a devido a algum destes sintomas? Sim  Não  NS/NR

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# CASCAIS

Tudo começa nas pessoas



# COVID-19

## INQUÉRITO SEROLÓGICO

A PREENCHER PELOS SERVIÇOS

Código de Identificação do Laboratório:

Código de Identificação do Teste:

Preenchimento do Inquérito: No Ponto de Colheita

Externamente

### CARACTERIZAÇÃO SOCIOECONÓMICA

1. Sexo  Masculino  Feminino  Outro

2. Ano de Nascimento

3. País de Nascimento \_\_\_\_\_

4. Código Postal

5. Qual o seu nível de escolaridade (ano mais elevado de escolaridade que completou ou ao qual obteve equivalência)?

Menos que a 4ª Classe

1º Ciclo Completo (4º ano)

2º Ciclo Completo (6º ano)

3º Ciclo Completo (9º ano)

Ensino Secundário (12º ano - cursos gerais, tecnológicos ou profissionais)

Ensino Pós-Secundário Não Superior (Curso Especialização Tecnológica)

Frequência Universitária ou Bacharelato/ Curso Médio

Ensino Superior (Licenciatura, Mestrado, Doutoramento)

Não Sabe/ Não Responde

6. Em que situação se encontra, quanto à ocupação ou atividade profissional?

Empregado/a

Desempregado/a

(inclui estágios pagos, licenças de maternidade/paternidade, baixas médicas por doença, lay-off ou bolsas de investigação)

Estudante /Em Formação  Reformado/a  Prestador/a de Trabalho Doméstico

Prestador/a de Serviço Comunitário OU Cuidador Informal  Incapacitado/a permanentemente

Outra situação de inatividade  Não Sabe/ Não Responde

# COVID-19

## INQUÉRITO SEROLÓGICO

A PREENCHER PELOS SERVIÇOS

Código de Identificação do laboratório:

Código de Identificação do Teste:

Preenchimento do Inquérito: No Ponto da Colheita

Externamente

6.1. Caso tenha respondido que se encontra empregado/a, qual a sua atividade profissional principal?

7. Quantas pessoas constituem o seu agregado familiar?

### ANTECEDENTES | DOENÇAS CRÓNICAS

8. Está diagnosticado para alguma das seguintes doenças crónicas?

- |  |                              |                              |                                   |
|--|------------------------------|------------------------------|-----------------------------------|
| Diabetes                                   | <input type="checkbox"/> Sim | <input type="checkbox"/> Não | <input type="checkbox"/> Não sabe |
| Doença Pulmonar Crónica (exclui asma)      | <input type="checkbox"/> Sim | <input type="checkbox"/> Não | <input type="checkbox"/> Não sabe |
| Asma                                       | <input type="checkbox"/> Sim | <input type="checkbox"/> Não | <input type="checkbox"/> Não sabe |
| Doença Cardiovascular (inclui hipertensão) | <input type="checkbox"/> Sim | <input type="checkbox"/> Não | <input type="checkbox"/> Não sabe |
| Obesidade                                  | <input type="checkbox"/> Sim | <input type="checkbox"/> Não | <input type="checkbox"/> Não sabe |
| Doença Autoimune                           | <input type="checkbox"/> Sim | <input type="checkbox"/> Não | <input type="checkbox"/> Não sabe |
| Doença Renal Crónica/Insuficiência Renal   | <input type="checkbox"/> Sim | <input type="checkbox"/> Não | <input type="checkbox"/> Não sabe |
- Outras Doenças. Quais?

### ANTECEDENTES - COVID 19

9. Esteve em contacto com algum caso suspeito ou confirmado de COVID-19?

Sim  Não  Não sabe/Não responde

9.1 Se respondeu "Sim", indique a última data de contacto: //

10. Nos meses de Janeiro e Fevereiro 2020, viajou para fora de Portugal?

Sim  Não  Não sabe/Não Responde

# COVID-19

## INQUÉRITO SEROLÓGICO

A PREENCHER PELOS SERVIÇOS

Código de Identificação do laboratório:

Código de Identificação do Teste:

Preenchimento do Inquérito: No Ponto de Colheita

Externamente

10.1. Se sim, para que países viajou?

11. Já realizou algum teste para deteção de COVID-19?  Sim  Não  Não sabe

11.1 Caso tenha respondido "Sim", qual foi o resultado?

Positivo. Em caso afirmativo, indique a data do primeiro teste positivo:

/ /

Negativo

Inconclusivo

Não sabe/ Não Responde

12. Desde o início de março, já teve algum dos seguintes sintomas, mesmo que passageiros?

Em caso afirmativo, identificar se os primeiros sintomas ocorreram:

**A)** Última Semana **B)** Há 2 Semanas **C)** Há 3 Semanas **D)** Há 1 Mês **E)** Há mais de 1 mês

- |  |  |                                       |
|--|--|---------------------------------------|
| 12.1 Febre ( $\geq 37,5^{\circ}\text{C}$ )     | Sim <input type="checkbox"/> Não <input type="checkbox"/> NS/NR <input type="checkbox"/> | Há quanto tempo: <input type="text"/> |
| 12.2 Arrepios                                  | Sim <input type="checkbox"/> Não <input type="checkbox"/> NS/NR <input type="checkbox"/> | Há quanto tempo: <input type="text"/> |
| 12.3 Fadiga (Cansaço)                          | Sim <input type="checkbox"/> Não <input type="checkbox"/> NS/NR <input type="checkbox"/> | Há quanto tempo: <input type="text"/> |
| 12.4 Dores Musculares                          | Sim <input type="checkbox"/> Não <input type="checkbox"/> NS/NR <input type="checkbox"/> | Há quanto tempo: <input type="text"/> |
| 12.5 Dores Articulares                         | Sim <input type="checkbox"/> Não <input type="checkbox"/> NS/NR <input type="checkbox"/> | Há quanto tempo: <input type="text"/> |
| 12.6 Dor de Garganta                           | Sim <input type="checkbox"/> Não <input type="checkbox"/> NS/NR <input type="checkbox"/> | Há quanto tempo: <input type="text"/> |
| 12.7 Tosse                                     | Sim <input type="checkbox"/> Não <input type="checkbox"/> NS/NR <input type="checkbox"/> | Há quanto tempo: <input type="text"/> |
| 12.8 Nariz Congestionado<br>/Corrimento Nasal  | Sim <input type="checkbox"/> Não <input type="checkbox"/> NS/NR <input type="checkbox"/> | Há quanto tempo: <input type="text"/> |
| 12.9 Falta de Ar<br>(Dificuldade Respiratória) | Sim <input type="checkbox"/> Não <input type="checkbox"/> NS/NR <input type="checkbox"/> | Há quanto tempo: <input type="text"/> |

# COVID-19

## INQUÉRITO SEROLÓGICO

A PREENCHER PELOS SERVICOS

Código de Identificação do laboratório:

Código de Identificação do Teste:

Preenchimento do Inquérito: No Ponto de Colheita

Externamente

- 12.10 Pieira Sim  Não  NS/NR  Há quanto tempo:
- 12.11 Dor Torácica Sim  Não  NS/NR  Há quanto tempo:
- 12.12 Outras Queixas Respiratórias Sim  Não  NS/NR  Há quanto tempo:
- 12.13 Conjuntivite Sim  Não  NS/NR  Há quanto tempo:
- 12.14 Dor de Cabeça Sim  Não  NS/NR  Há quanto tempo:
- 12.15 Alterações do Estado de Consciência Sim  Não  NS/NR  Há quanto tempo:
- 12.16 Convulsões Sim  Não  NS/NR  Há quanto tempo:
- 12.17 Outras Queixas Neurológicas Sim  Não  NS/NR  Há quanto tempo:
- 12.18 Náusea ou Vômitos Sim  Não  NS/NR  Há quanto tempo:
- 12.19 Diarreia Sim  Não  NS/NR  Há quanto tempo:
- 12.20 Dor Abdominal Sim  Não  NS/NR  Há quanto tempo:
- 12.21 Perda de Cheiro Sim  Não  NS/NR  Há quanto tempo:
- 12.22 Perda de Paladar Sim  Não  NS/NR  Há quanto tempo:
- 12.23 Perda de Apetite Sim  Não  NS/NR  Há quanto tempo:
- 12.24 Hemorragia Nasal Sim  Não  NS/NR  Há quanto tempo:
- 12.25 Outras Queixas Sim  Não  NS/NR  Há quanto tempo:

13. Procurou apoio médico devido a algum destes sintomas? Sim  Não  NS/NR

14. Faltou ao trabalho/escola devido a algum destes sintomas? Sim  Não  NS/NR

15. Ficou hospitalizado/a devido a algum destes sintomas? Sim  Não  NS/NR

16. Se ficou hospitalizado/a, precisou de cuidados intensivos? Sim  Não  NS/NR

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# CASCAIS

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## ANNEX III: Informed Consent Germano Sousa



**GERMANO DE SOUSA**  
CENTRO DE MEDICINA LABORATORIAL



Utente:

NIF:

### Nota informativa ao utente

**O utente, acima identificado, confirma ter sido informado:**

1. Que a empresa, designou um encarregado de proteção de dados, e que pode ser contactado, preferencialmente por email: dpo.privacidade@germanodesousa.com.
2. Que o tratamento dos seus dados se destina à execução das suas análises clínicas.
3. Que os dados recolhidos são conservados de acordo com os prazos legais.
4. Que o processamento e conservação dos seus dados, em caso de necessidade, podem ser executados por outra entidade, continuando garantida a política de privacidade.
5. Que os resultados das análises clínicas serão comunicados ao próprio, ao representante legal ou ao seu médico.
6. Dos direitos de acesso, de retificação, de eliminação, de limitação da utilização, da portabilidade e da oposição ao tratamento dos seus dados pessoais.
7. Que os dados pessoais serão, se necessário, comunicados para efeitos de faturação, à sua seguradora ou subsistema de saúde.
8. Do direito de apresentação de reclamação na Comissão Nacional de Proteção de Dados (CNPD).
9. Que o presente documento continua válido para futuras análises clínicas, salvo indicação expressa em contrário.

**O utente, acima identificado, aceita que os seus dados pessoais sejam utilizados:**

10. De forma anonimizada para fins estatísticos e/ou de estudos científicos,
11. Para receber informação da empresa, clínica, médica e sobre novos testes de análises clínicas.

NÃO	SIM

Confirmo ter compreendido este documento, e que me foram prestadas todas as informações que solicitei.

Terça-feira, 26 Maio 2020

Assinatura: \_\_\_\_\_

Mod.201.03

Centro Medicina Laboratorial Germano de Sousa, S.A. | Rua Cupertino de Miranda, nº9, Lt 8, P.Tecnologico, 1600-513 Lisboa