Sero-prevalence of Anti-SARS-CoV-2 Antibody Among the Asymptomatic and Confirmed COVID-19 Positive Population of Chattogram Metropolitan Area

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Session: 2019-2020

A thesis submitted in the partial fulfillment of the requirements for the degree of MPH (Public Health)

One Health Institute

Chattogram Veterinary and Animal Sciences University

Chattogram-4225

June: 2022

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Md. Tarek Ul Quader

June 2022

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This is to certify that we have examined the above MPH (Public Health) thesis and have found that is complete and satisfactory in all respects, and that all required by the thesis examination committee have been made.

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Acknowledgements

All praises to almighty Allah who gave me the opportunity to be enrolled in the **One Health Institute** for achieving Master in Public Health. I would like to express my veneration to honorable supervisor **Prof. Dr. Sharmin Chowdhury,** Professor, Department of Pathology and Parasitology, Faculty of Veterinary Medicine, Chattogram Veterinary and Animal Sciences University (CVASU) for her coherent and articulated instructions. It wouldnot be possible to complete such a laborious task without her scholastic guidelines. It was an exquisite experience for me to work under her supervision. I feel much pleasure to convey my gratitude to Prof. Dr. M. A. Hassan Chowdhury, Professor, Department of Medicine, Chittagong Medical College Hospital for his valuable suggestions. Special thanks to him for his encouragement and cordial co-operation throughout thework. I would like to acknowledge the support and encouragement received during MPH program from other teachers, technical and non-technical staffs of the One Health Institute, CVASU.

I am also grateful to my family members for their allowance and heartiest support.

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List of Abbreviations

RT-PCR	Real Time Polymerase Chain Reaction
HCW	Health Care Worker
ARDS	Acute Respiratory Distress Syndrome
CRP	C-Reactive Protein
LDH	Lactate Dehydrogenase
IL-6	Inter Leukin-6
RBD	Receptor Binding Domain
EMA	European Medicines Agency
FDA	Food and Drug Administration
CRS	Cytokine Release Syndrome
ACE-2	Angiotensin Converting Enzyme-2
CMA	Chattogram Metropolitan Area

Abstract

Seroprevalence studies of coronavirus disease 2019 (COVID-19) assess the degree of undetected transmission in the community. Different groups, such as healthcare workers (HCWs), garment workers, and others, are deemed vulnerable due to their workplace hazards and immense responsibility. The present study was conducted to estimate the seroprevalence of anti-SARS-CoV-2 antibody (IgG) and its association with different explanatory variables. Further, the antibody was quantified to assess the increasing or decreasing trend over different intervention periods and according to other factors. This cross-sectional study observed health workers - doctor, nurse, hospital staff, etc. in and outpatients (non-COVID-19) and garments workers of Chattogram metropolitan area (CMA, N=748) from randomly selected six government and private hospitals and two garment factories. Study subjects were included upon written consent, fulfilling specific inclusion criteria. Venous blood was collected following standard aseptic methods. Qualitative and quantitative ELISA was used to identify and quantify antibodies (IgG) in serum samples. Descriptive, univariable, and multivariable statistical analysis was performed. Overall seroprevalence was estimated as 66.99% (95% CI: 63.40%-70.40%). Seroprevalence among HCWs, in and outpatients, and garments workers were 68.99 % (95% CI: 63.8%-73.7%), 81.37 % (95% CI: 74.7%-86.7%), and 50.56 % (95% CI: 43.5%-57.5%), respectively. Sero-prevalence was 44.47 % (95% CI: 38.6%-50.4%) in the non-vaccinated population while it was significantly (p<0.001) higher in the population receiving the first dose (61.66 %, 95% CI: 54.8%-68.0%) and both (first and second) doses of vaccine (100%, 95% CI: 98.4%-100%). The mean titer of the antibody was estimated as 255.46 DU/ml and 159.08 DU/ml in the population with both doses and one dose of vaccine, respectively, compared to 53.71 DU/ml of the unvaccinated population. A decreasing trend in the titer of antibodies with increasing time after vaccination was observed. Sero-prevalence and mean antibody titer varied according to different factors in this study. The second dose of vaccine significantly increased the sero-prevalence and titer, which decreased to a certain level over time. Although antibody was produced following natural infection, the mean titer was relatively low compared to antibody after vaccination. This study emphasizes the role of the vaccine in antibody production. Based on the findings, interventions like continuing extensive mass vaccination of the leftover unvaccinated population and

bringing the mass population with a second dose under a third dose campaign might be planned.

Chapter 1: Introduction

1.1 Background

Coronavirus disease of 2019 (COVID-19) is caused by the novel corona virus that has emerged in China in 2019. The recent COVID-19 pandemic is threatening the world population, with 99.3 million reported cases in 219 countries and territories worldwide and 2 international conveyances; the recent COVID-19 pandemic is intimidating the world population. Among the infected ones, 71.3 million people have recovered while 2.1 million infected individuals have already passed away. Hence, till date, the recovery and death rate are respectively reported 97% and 3%. Up to the same date, more than 531 thousand Bangladeshi populations have been infected, and 8,003 people died of this pandemic. Meanwhile, the source further claimed that 475,899 patients have received the medical certificate as they recovered from COVID-19 (Worldometer, 2021). Such data insights that coronavirus has widely spread in the country.

The virus can spread from an infected person‘s mouth or nose in respiratory droplet particles when they cough, sneeze, speak, sing or breathe heavily. These respiratory droplet particles are different sizes, ranging from larger respiratory droplets to smaller aerosols. Other people can catch COVID-19 when the virus gets into their mouth, nose or eyes, which is more likely to happen when people are in direct or closecontact (less than 1 meter apart) with an infected person (WHO, 2020).

The virus has the ability to live in the air for 2-3 hours. Furthermore, if an infected person contacts some surface or item, it survives there for 8 hours. This disease has incubation duration of 2-14 days. Novel coronavirus infects the upper and lower respiratory tract. Cold, cough, sore throat, moderate muscle ache, lack of appetite, fever and diarrhea are some mild signs of this disease. The critical symptoms of this disease are: pneumonia, shortness of breathing and organ failure that may lead to death. People with comorbidity (having diabetes, blood pressure, heart disease and kidney disease) or acute respiratory disease like asthma, are more vulnerable to its infection (Guan *et al.*, 2020, Islam *et al.*, 2020).

Chattogram, the port city of Bangladesh, is classified as a high-risk zone for SARS-CoV-2 contact transmission and is one of the most crowded economic and trading centers (Rana *et al.*, 2020). On April 3, 2020, Chattogram city witnessed its first Coronavirus Disease 2019 (COVID-19) positive case, followed by the first death on 9 April. The disease can manifest itself in various ways, from asymptomatic and minor upper respiratory symptoms to severe pneumonia and acute respiratory distress syndrome (Goenka *et al.*, 2020). While nucleic acid amplification, such as polymerase chain reaction (PCR), is the gold standard for diagnosing acute SARS-CoV-2 infection and is widely recommended, the antibody-based approach improves diagnosis accuracy by capturing asymptomatic testing and recovered infections (Vernet *et al.*, 2021).

During an infectious disease outbreak, seroprevalence investigations are crucial in revealing undetected infection in the population and preventing post-pandemic reappearance (Bryant *et al.*, 2020). Determining the actual burden of infection is also vital for epidemic forecasting and response planning. Seroprevalence studies are potent in identifying the number of undiagnosed missing cases with mild or no symptoms or who cannot undergo testing that may contribute significantly to the transmission (Shakiba *et al.*, 2021). Further, seroprevalence studies estimate the susceptible population in a community. A current investigation discovered that up to 23% of the patients diagnosed with COVID-19 from December 2020 to February 2021 in Bangladesh were asymptomatic (Hossain *et al.*, 2021). Thus, antibody testing could be crucial to determine the actual SARS-CoV-2 exposure rates since PCR only identifies the viral nucleic acid in individuals with existing symptoms (Thomas *et al.*, 2021).

According to numerous research, seropositivity fluctuates considerably depending on parameters such as location and time (Shakiba *et al.*, 2021). Antibody titers reach their peak one month after the onset of symptoms, and their levels are directly proportional to the severity of the illness (Wang *et al.*, 2021). Titers continue to fall after that, with IgM and IgA titers falling fast and IgG titers falling more slowly. However, a greater understanding of antibody responses to SARS-CoV-2 after natural infection might aid in the development of more successful vaccination strategies in the future. Bangladesh started administering COVID-19 vaccinations on January 27, 2021, and mass immunization commenced on February 7, 2021. As of December 21,

2021, 50.27% of the target population had received the first dose, and 34.60% received the second dose. Bangladesh has already started administering third doses to senior persons aged 60 and up, people with comorbidities, and frontline workers. According to a web-based anonymous cross-sectional survey conducted among the general Bangladeshi population between January 30 and February 6, 2020, 61.16% of respondents were inclined to accept/take the COVID-19 vaccine (Mahmud *et al.*, 2021). However, vaccination coverage and seroprevalence among the general public must be investigated nationwide to know the herd immunity.

In the COVID-19 pandemic, HCWs are facing immense challenges worldwide. Occupational exposures among HCWs have been documented in numerous nations as worrying (Mahmud *et al.*, 2021). Likewise, COVID-19 has had a significant impact on the healthcare system of Bangladesh. According to the latest data from the Bangladesh Medical Association, between March 8, 2020, and November 11, 2021, 9455 HCWs, including physicians, nurses, and other staff, were infected with COVID-19, as well as 188 doctors died as a result. Front liners directly involved in diagnosing, treating, and caring for COVID-19 patients are at risk of physical and psychological distress (Chen *et al.*, 2020). Similarly, workers in the garment industry confront different problems in the workplace all around the world. According to the Bangladesh Garment Manufacturers and Exporters Association (BGMEA), 4500 garment companies employ over 4.5 million people or nearly 2.5 percent of the country's entire population (Khan and Ullah, 2017). The bulk of the industries operate with limited space, making it challenging to enforce physical distancing norms (Hosen *et al.*, 2020). SARS-CoV-2 transmission might be exacerbated by crowded workplaces, transportation, and lack of physical distancing (Dyal, 2020). Hence, it is necessary to put in place measures including risk management in the workplace, vulnerable employee care, the development of an occupational surveillance system, and vaccination policy administration to address the COVID-19 issues. Thus, knowing the true seroprevalence both in the risk groups and community might assist in planning interventions efficiently(Islam *et al.*, 2021).

In this study, we reported population-based SARS-CoV-2 seropositivity among HCWs, indoor and outdoor patients of various government and private hospitals, and garment workers of CMA, as determined by enzyme-linked immunosorbent assay (ELISA). Moreover, we measured the antibody titer, and both outcomes

(seropositivity and antibody titer) were tested to know the association of different factors.

1.2 Rationale

Through November 9, 2021, Bangladesh had reported >1.57 million COVID-19 cases and 27,904 deaths, with incidence and mortality rates substantially lower than in many other countries. It is difficult to know whether differences in rates of illness and death reflect undercounts due to limited surveillance and healthcare-seeking or actual differences in incidence due to interventions or different biological responses to infection without performing population-based seroprevalence estimates.In early March 2021, cases across Bangladesh began to rise.The majority of currently available data are restricted to laboratory-confirmed cases for symptomatic patients, and the SARS-CoV-2 infection can manifest as an asymptomatic or mild disease. Therefore, the true extent of the burden of COVID-19 may be underestimated. Improved serological detection of specific antibodies against SARS-CoV-2 could help

estimate the true numbers of infections. Publicly available sequencing data indicate that the SARS-CoV-2 Delta variant was first detected in the Chattogram region of Bangladesh in mid-May 2021, and 99% (98/99)of the viral genomes submitted during July 1–October 1, 2021 have been of the Delta variant, similar to national trends (Bhuiyan *et al.*, 2021).

Serological surveys help to determine the extent of infectionby a viral agent in a population and identify associatedrisk factors. There were very few studies that have explored the seroprevalence of anti SARS-COV-2 antibody in Bangladesh. Therefore, the present study aimed to determine the seroprevalence of anti SARS-COV-2 antibody among the asymptomatic and confirmed COVID 19 population. The findings of which may enrich the knowledge of physician to monitor exposure and side-by-side determine and differentiate between infection- and vaccine-induced humoral immunity. Such data might be crucial to inform public health decisionmakersto improve vaccine distribution and allocation, and appraise booster dose requirementsduring the COVID-19 pandemic.

1.3 Research Question

Is there any distinction in the seroprevalence of Covid-antibody among asymptomatic and Covid positive patients?

1.4 Objectives

1.4.1 General Objective:

To evaluate the seroprevalence of anti-SARS-COV-2 antibody among the asymptomatic and confirmed COVID 19 population

1.4.2 Specific Objectives:

1. To measure seroprevalence of anti-SARS-COV-2 antibody among the confirmed COVID 19 population.
2. To measure seroprevalence of anti-SARS-COV-2 antibody among the asymptomatic COVID 19 population.
3. To compare the seroprevalence of anti-SARS-COV-2 antibody between confirmed COVID 19 and asymptomatic COVID 19 population.

Chapter 2: Literature Review

2.1 COVID-19 pandemic and its causative agent

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), has led to a global pandemic. Though SARS-CoV-2 only emerged at the end of 2019, the associated disease COVID-19 has spread rapidly to more than 180 countries by the early 2020. Over two years have passed since the World Health Organization announced on January 30, 2020 that COVID-19 is a public health emergency of international concern, yet many questions persist about the spread and impact of the virus driving this crisis. As of May 15, 2021, there were over 160 million confirmed cases of SARS-CoV-2 infection and 3.3 million deaths worldwide. However, these case counts might be inevitably an underestimation of the true cumulative incidence of infection because of limited diagnostic test availability, barriers to testing accessibility, and asymptomatic infections (Buitrago-Garcia *et al.*, 2020, Cheng *et al.*, 2020).

Thousands of SARS-CoV-2 variants are grouped into either clades or lineages. The WHO, in collaboration with partners, expert networks, national authorities, institutions and researchers, have established nomenclature systems for naming and tracking SARS-CoV-2 genetic lineages by GISAID, Nextstrain and Pango. At the present time, the expert group convened by WHO has recommended the labeling of variants using letters of the Greek Alphabet, for example, Alpha, Beta, Delta, and Gamma, giving the justification that they "will be easier and more practical to discussed by non-scientific audiences (Happi *et al.*, 2021).

Several notable variants of SARS-CoV-2 emerged throughout 2020. As of December 2021, there are five dominant variants of SARS-CoV-2 spreading among global populations: the Alpha variant (B.1.1.7, formerly called the UK variant), first found in London and Kent, the Beta variant (B.1.351, formerly called the South Africa variant), the Gamma variant (P.1, formerly called the Brazil variant), the Delta variant (B.1.617.2, formerly called the India variant), and the Omicron variant (B.1.1.529), which had spread to 57 countries as of 7 December (World Health Organization, 2021).

2.2 Symptoms of COVID-19

SARS-CoV-2 is a novel severe acute respiratory syndrome coronavirus. It was first isolated from three people of Shenzhen with pneumonia connected to the cluster of acute respiratory illness cases in Wuhan. All structural features of the novel SARS-CoV-2 virus particle occur in related coronaviruses in nature. It is thought to have an animal (zoonotic) origin. Genetic analysis has revealed that the coronavirus genetically clusters with the genus Betacoronavirus, in subgenus Sarbecovirus (lineage B) together with two bat-derived strains. It is 96% identical at the whole genome level to other bat coronavirus samples (BatCov RaTG13). The structural proteins of SARS-CoV-2 include membrane glycoprotein (M), envelope protein (E), nucleocapsid protein (N), and the spike protein (S). The M protein of SARS-CoV-2 is about 98% similar to the M protein of bat SARS-CoV, maintains around 98% homology with pangolin SARS-CoV, and has 90% homology with the M protein of SARS-CoV; whereas, the similarity is only around 38% with the M protein of MERS-CoV.

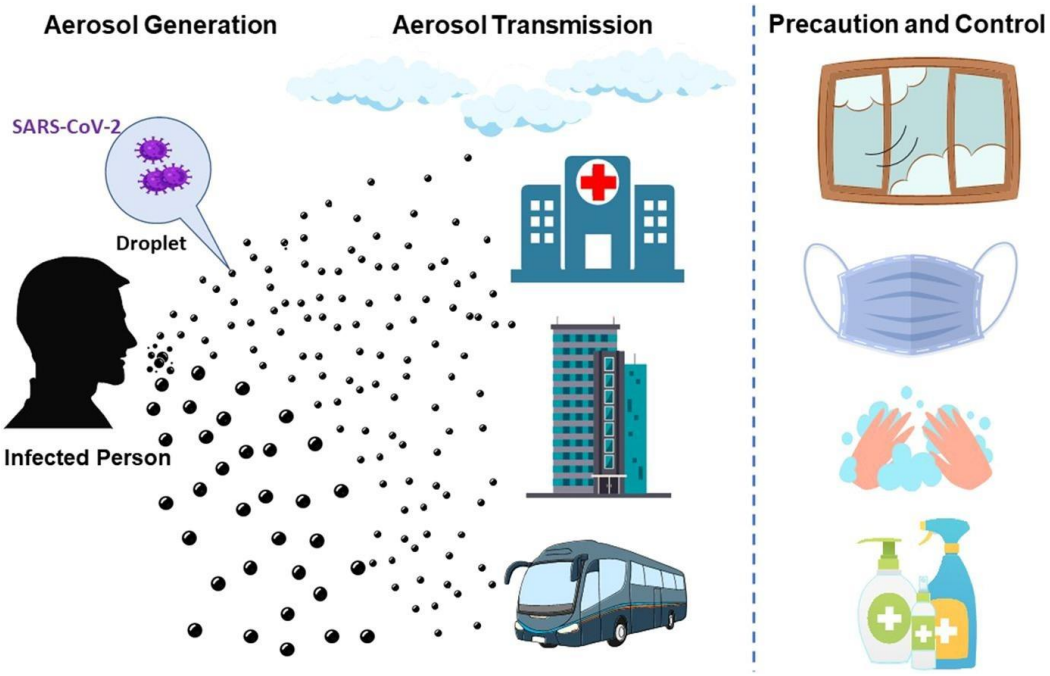


Figure 2.1: Mode of spread of COVID-19 (Karia *et al.*, 2020)

2.3 Pathogenesis and host response

The SARS-CoV-2 virus can infect a wide range of cells and systems of the body. COVID-19 is most known for affecting the upper respiratory tract (sinuses, nose, and throat) and the lower respiratory tract (windpipe and lungs). The lungs are the organs most affected by COVID-19 because the virus accesses host cells via the receptor for the enzyme angiotensin-converting enzyme 2 (ACE2), which is most abundant on the surface of type II alveolar cells of the lungs. The virus uses a special surface glycoprotein called a "spike" to connect to the ACE2 receptor and enter the host cell (Harrison *et al.*, 2020).

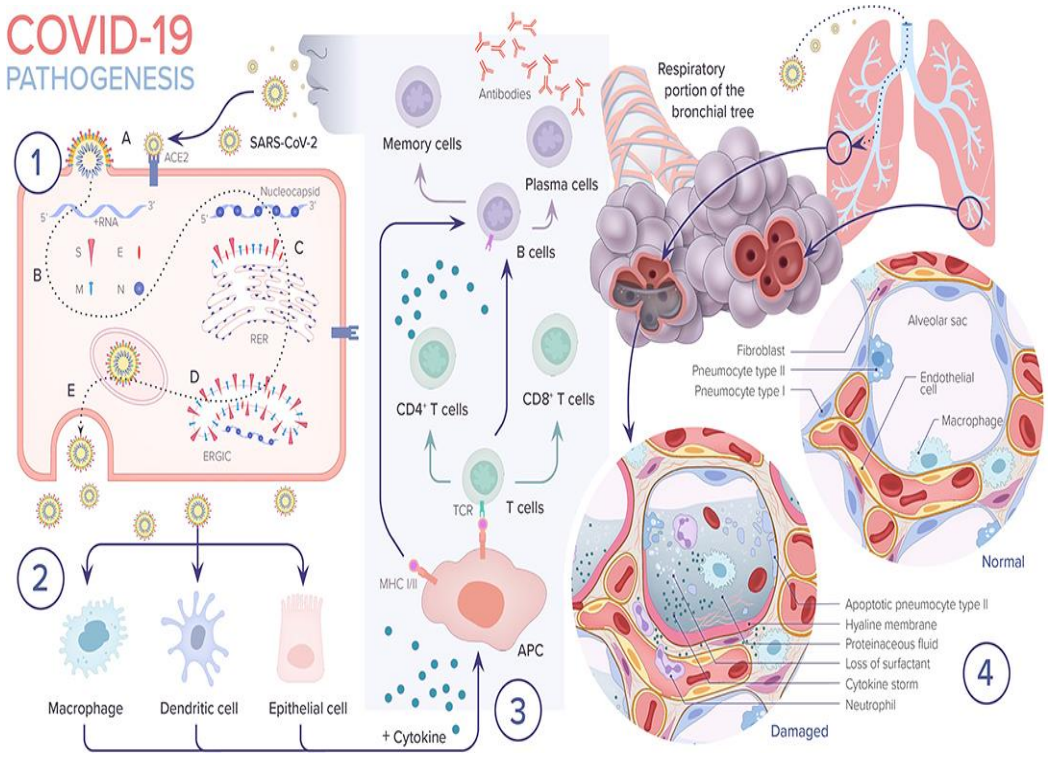


Figure 2.2: Pathogenesis of COVID-19 (Chams *et al.*, 2020)

Although SARS-CoV-2 has a tropism for ACE2-expressing epithelial cells of the respiratory tract, people with severe COVID-19 have symptoms of systemic hyperinflammation. Clinical laboratory findings of elevated Interleukin-2, IL-7, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma-induced protein 10 (IP-10), monocyte chemoattractant protein 1 (MCP1), macrophage inflammatory protein 1-alpha (MIP-1-alpha), and tumour necrosis factor (TNF-α) indicative of cytokine release syndrome (CRS) suggest an underlying immunopathology (Huang *et al.*, 2020).

Additionally, people with COVID-19 and acute respiratory distress syndrome (ARDS) have classical serum biomarkers of CRS, including elevated C-reactive protein (CRP), lactate dehydrogenase (LDH), D-dimer, and ferritin. Systemic inflammation results in vasodilation, allowing inflammatory lymphocytic and monocytic infiltration of the lung and the heart. In particular, pathogenic GM-CSF-secreting T cells were shown to correlate with the recruitment of inflammatory IL-6-secreting monocytes and severe lung pathology in people with COVID-19 (Zhang *et al.*, 2020).

Multiple viral and host factors affect the pathogenesis of the virus. The S-protein, otherwise known as the spike protein, is the viral component that attaches to the host receptor via the ACE2 receptors. It includes two subunits: S1 and S2. S1 determines the virus-host range and cellular tropism via the receptor-binding domain. S2 mediates the membrane fusion of the virus to its potential cell host via the H1 and HR2, which are heptad repeat regions. Studies have shown that S1 domain induced IgG and IgA antibody levels at a much higher capacity. It is the focus spike proteins expression that are involved in many effective COVID-19 vaccines (Dai and Gao, 2021).

The M protein is the viral protein responsible for the transmembrane transport of nutrients. It is the cause of the bud release and the formation of the viral envelope. The N and E protein are accessory proteins that interfere with the host's immune response. Human angiotensin converting enzyme 2 (hACE2) is the host factor that SARS-COV2 virus targets causing COVID-19 (Boopathi *et al.*, 2021).

The effect of the virus on ACE2 cell surfaces leads to leukocytic infiltration, increased blood vessel permeability, alveolar wall permeability, as well as decreased secretion of lung surfactants. These effects cause the majority of the respiratory symptoms. However, the aggravation of local inflammation causes a cytokine storm eventually leading to a systemic inflammatory response syndrome. Among healthy adults not exposed to SARS-CoV-2, about 35% have CD4+ T cells that recognize the SARS-CoV-2 S protein (particularly the S2 subunit) and about 50% react to other proteins of the virus, suggesting cross-reactivity from previous common colds caused by other coronaviruses (Dai and Gao, 2021).

The severity of the inflammation can be attributed to the severity of what is known as the cytokine storm. Levels of interleukin 1B, interferon-gamma, interferon-inducible protein 10, and monocyte chemoattractant protein 1 were all associated with COVID-19 disease severity. Treatment has been proposed to combat the cytokine storm as it remains to be one of the leading causes of morbidity and mortality in COVID-19 disease (Quirch *et al.*, 2020).

A cytokine storm is due to an acute hyperinflammatory response that is responsible for clinical illness in an array of diseases but in COVID-19, it is related to worse prognosis and increased fatality. The storm causes acute respiratory distress

syndrome, blood clotting events such as strokes, myocardial infarction, encephalitis, acute kidney injury, and vasculitis. The production of IL-1, IL-2, IL-6, TNF-alpha, and interferon-gamma, all crucial components of normal immune responses, inadvertently become the causes of a cytokine storm. The cells of the central nervous system, the microglia, neurons, and astrocytes, are also involved in the release of pro-inflammatory cytokines affecting the nervous system, and effects of cytokine storms toward the CNS are not uncommon (Bhaskar *et al.*, 2020).

In a study of antibody response to SARS-CoV-2 were initially characterized in a cohort of individuals convalescing from COVID-19 at approximately 40 days (1.3 months) after infection. The participants with RT–PCR-confirmed SARS-CoV-2 infection and/or seroconversion returned for analysis at approximately 191 days (6.2 months; range of 165 to 223 days) after the onset of symptoms. In this cohort, symptoms lasted for a median of 12 days (range of 0 to 44 days) during the acute phase, and 10 (11%) of the participants were hospitalized. Consistent with other reports (44%) of the participants reported persistent long-term symptoms attributable to COVID-19. The duration and severity of symptoms during acute disease was significantly greater among participants with persistent post-acute symptoms at the second study visit (Robbiani *et al.*, 2020).

Bertoglio et al. (2021) reported that COVID-19 is a severe acute respiratory disease caused by SARS-CoV-2, a new recently emerged sarbecovirus. This virus uses the human ACE2 enzyme as receptor for cell entry, recognizing it with the receptor binding domain (RBD) of the S1 subunit of the viral spike protein. The researchers present the use of phage display to select anti-SARS-CoV-2 spike antibodies from the human naïve antibody gene libraries HAL9/10 and subsequent identification of 309 unique fully human antibodies against S1. 17 antibodies are binding to the RBD, showing inhibition of spike binding to cells expressing ACE2 as scFv-Fc and neutralize active SARS-CoV-2 virus infection of VeroE6 cells. The antibody STE73-2E9 is showing neutralization of active SARS-CoV-2 as IgG and is binding to the ACE2-RBD interface. Thus, universal libraries from healthy human donors offer the advantage that antibodies can be generated quickly and independent from the availability of material from recovering patients in a pandemic situation (Bertoglio *et al.*, 2021)bodies against the spike protein of coronaviruses are potential candidates for therapeutic development. Antibodies against the S1 subunit, especially against RBD,

can potently neutralize SARS-CoV and MERS. Monoclonal human antibodies against SARS-CoV are described to cross-react with SARS-CoV-2; some of them are also able to neutralize SARS-CoV-2. Other reports show how monoclonal antibodies against SARS-CoV-2 can be selected by rescreening memory B cells from a SARS patient, selected from COVID-19 patients by single B cell PCR or using phage display. The antibody palivizumab is European Medicines Agency (EMA)/Food and Drug Administration (FDA) approved for treatment of a severe respiratory infection of infants caused by the respiratory syncytial virus (RSV) and can be used as a guideline to develop therapeutic antibodies against SARS-CoV-2. Antibody phage display is a powerful tool to generate human antibodies against infectious diseases (van Mechelen *et al.*, 2016).

2.4 Symptoms and diagnosis of COVID-19

COVID-19 showed both symptomatic and asymptomatic infections. As a consequence, the global prevalence of SARS-CoV-2 infection remains unknown. Confirmed cases for symptomatic patients, and the SARS-CoV-2 infection can manifest as an asymptomatic or mild disease. Currently, the diagnosis of COVID-19 is confirmed by the detection of SARS-CoV-2 via real-time reverse transcription polymerase chain reaction (qRT-PCR) assays that target open reading frame-1 antibodies, envelope proteins, nucleocapsid proteins, RNA-dependent RNA polymerase genes, and the N1, N2, and N3 target genes, among suspected cases with an exposure history and signs/symptoms of SARS-CoV-2 infection (Lai *et al.*, 2021). However, the clinical manifestations of COVID-19 include both respiratory and extra-respiratory signs and symptoms and can range from an asymptomatic mild disease to severe disease or acute respiratory tract infections. Therefore, misdiagnosis of COVID-19 can occur in patients without a characteristic presentation, even for asymptomatic and mild infections, and in places where qRT-PCR is unavailable. These issues could limit our understanding of the extent of SARS-CoV-2 infection and further affect the implementation of infection control and prevention policies. The use of a serological test to detect antiSARS-CoV-2 antibodies could be a better way to estimate the burden of SARS-CoV-2 infection than the PCR method, and help improve understanding of the associated epidemiology (Lai *et al.*, 2020).

On 3 February 2020, in Japan, 712 (19.2%) of 3711 passengers and crew had tested positive to COVID-19. At the time of testing, 331 (46.5%) of those with positive results were asymptomatic. Although the later infected persons reported no symptoms, some actually had subclinical changes in their lungs. When computed tomography scans for 76 of these persons were examined, 54% showed lung opacities (Oran and Topol, 2020).

Another study revealed the proportion of asymptomatic persons as 17.9%. the proportion of asymptomatic individuals appears to be 16.1% (35/218) before 13 February, 25.6% (73/285) on 15 February, 31.2% (111/355) on 16 February, 39.9% (181/454) on 17 February, 45.4% (246/542) on 18 February, 50.6% (314/621) on 19 February and 50.5% (320/634) on 20 February (Mizumoto *et al.*, 2020).

On 28 March, an initial case of COVID-19 was diagnosed with a positive test result at a homeless shelter in downtown of Los Angeles, California. After a cluster of symptomatic persons was identified early in the week of 20 April, the shelter was closed to new occupants and testing was started for current occupants. As of 22 April, 43 (24.2%) of 178 completed tests were positive for SARS-CoV-2 and 27 (63.8%) of the persons who tested positive were asymptomatic (Chou, 2020).

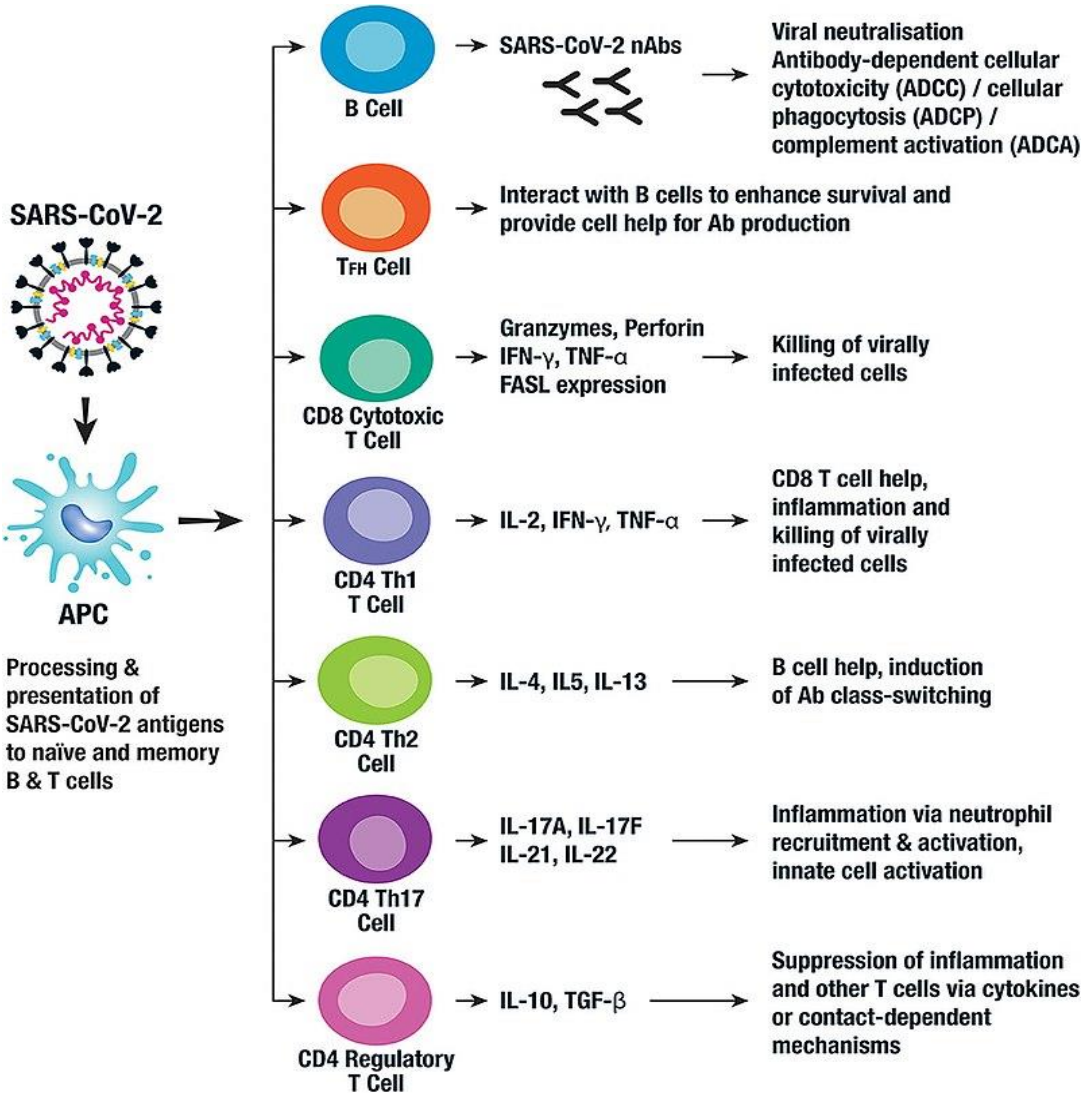


Figure 2.3: Key components of the adaptive immune response to SARS-CoV-2 (Flanagan *et al.*, 2020)

Widespread outbreaks of COVID-19 in the correctional facilities of several states of USA have led to large-scale screening programs. According to research by Reuters journalists, as of 25 April 2020, SARS-CoV-2 test results that include data on symptom status were available for 4693 inmates in the state prison systems of Arkansas, North Carolina, Ohio, and Virginia. Among these inmates, 3277 (69.8%) tested positive, of whom 3146 (96%) had no symptoms at the time of testing (So and Smith, 2020).

2.5 Seroprevalence of SARS-CoV-2

Detection of anti-SARS-CoV-2 antibodies among people at risk of infection is crucial for understanding both the past transmission of COVID-19 and vulnerability of the population to continuing transmission and, when done serially, the intensity of ongoing transmission over an interval in a community. SARS-CoV-2 infections are symptomatic, presymptomatic, or asymptomatic; asymptomatic individuals have similar viral loads to those who are symptomatic6 and have a considerable role in transmission of the disease. Without symptoms, most asymptomatic infections are not detected, except in seroprevalence studies. Nevertheless, seroprevalence estimates vary widely depending on country and risk groups. For example, some states such as San Francisco in the USA reported seroprevalence as low as 0·26%, Wuhan, China had 3·2–3·8%, Switzerland had 11%, New York City, USA had 19%, and more recently the highest was reported in Manaus, Brazil, with 55·1–61·4% (Álvarez-Antonio *et al.,* 2021). In Peru, preliminary results from two seroprevalence studies have been reported: one study done in July, 2020, reported a seroprevalence of 29·7% in the region of Lambayeque; and another done in December, 2020, found a seroprevalence of 39·3% in the regions of Lima and Callao using population-based sampling techniques (Álvarez-Antonio *et al.*, 2021).As of February 2022, approximately 75% of children and adolescents had serologic evidence of previous infection with SARS-CoV-2, with approximately one third becoming newly seropositive since December 2021.The greatest increases in seroprevalence during September 2021–February 2022, occurred in the age groups with the lowest vaccination coverage; the proportion of the U.S. population fully vaccinated by April 2022 increased with age (5–11, 28%; 12–17, 59%; 18–49, 69%; 50–64, 80%; and ≥65 years, 90%). Lower seroprevalence among adults aged ≥65 years, who are at greater risk for severe illness from COVID-19, might also be related to the increased use of additional precautions with increasing age (Clarke *et al.*, 2022).

Serological assays identify SARS-CoV-2 antibodies indicates previous infection in unvaccinated persons. Population-based serological testing provides better estimates of the cumulative incidence of infection by complementing diagnostic testing of acute infection and helping to inform the public health response to COVID-19. Furthermore, as the world moves through the vaccine and variant era, synthesizing seroepidemiology findings is increasingly important to track the spread of infection,

identify disproportionately affected groups, and measure progress towards herd immunity (Cheng *et al.*, 2020).

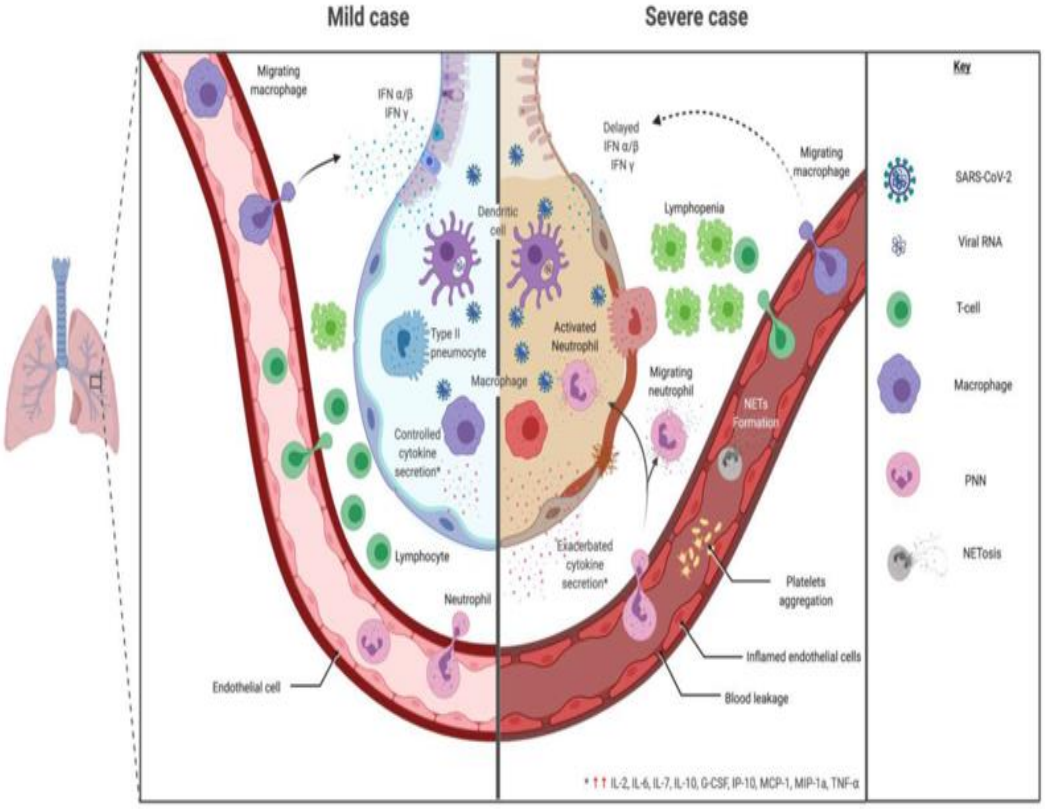


Figure 2.4: Mild versus severe immune response during virus infection (Lebeau *et al.*, 2020)

SARS-CoV-2 seroprevalence estimates are reported not only in published articles and preprints, but also in government and health institute reports, and media. Consequently, few studies have comprehensively synthesized seroprevalence findings that include all of these sources. Describing and evaluating the characteristics of seroprevalence studies conducted over the first year of the pandemic may provide valuable guidance for serosurvey investigators moving forward (Chen *et al.*, 2021).

Serological surveys help to determine the extent of infection by a viral agent in a population and identify associated risk factors. Since January 2020, serological surveys of SARS-CoV-2 have been performed in the general population on all continents. The median seroprevalence adjusted for imperfect test accuracy was estimated to be 3.2% (interquartile range 1–6.4%) in 184 studies conducted in the

general population. Associations between a positive serological test (i.e. seropositivity) and demographic characteristics, such as a younger age, non-White ethnicity, occupational exposure, lower socio-economic status and higher population density, have been reported (Bobrovitz *et al.*, 2021).

According to Carrat et al. (2021) seropositivity to anti-SARS-CoV-2 antibodies in the French adult population was ≤10% after the first wave. Modifiable and non-modifiable risk factors were identified (Carrat *et al.*, 2021).

Chen et al. (2021) in their study reported that close contacts (18·0%, 95% CI 15·7–20·3) and high- risk health-care workers (17·1%, 9·9–24·4) had higher seroprevalence than did low-risk health-care workers (4·2%, 1·5–6·9) and the general population (8·0%, 6·8–9·2) (Chen *et al.*, 2021). Seroprevalence varied greatly across WHO regions, with the lowest seroprevalence of general populations in the Western Pacific region (1·7%, 95% CI 0·0–5·0). The pooled infection-to-case ratio was similar between the region of the Americas (6·9, 95% CI 2·7–17·3) and the European region (8·4, 6·5–10·7), but higher in India (56·5, 28·5–112·0), the only country in the South-East Asia region with data (Chen *et al.*, 2021).

According to Naranbhai et al. (2020), SARS-CoV-2 antibody testing allows quantitative determination of disease prevalence, which is especially important in high-risk communities. They performed anonymized convenience sampling of 200 currently asymptomatic residents of Chelsea, the epicenter of COVID-19 illness in Massachusetts, by BioMedomics SARS-CoV-2 combined IgM-IgG point-of-care lateral flow immunoassay. The seroprevalence was 31.5% (17.5% IgM+IgG+, 9.0% IgM+IgG−, and 5.0% IgM−IgG+). Of the 200 participants, 50.5% reported no symptoms in the preceding 4 weeks, of which 24.8% (25/101) were seropositive, and 60% of these were IgM+IgG−. These data are the highest seroprevalence rates observed to date and highlight the significant burden of asymptomatic infection (Naranbhai *et al.*, 2020).

Figueiredo Campos et al. (2020) quantified IgM, IgG, and IgA antibodies recognizing the SARS-CoV-2 receptor-binding domain (RBD) or the Spike (S) protein over a period of 6 months following COVID-19 onset. They reported the detailed setup to monitor the humoral immune response from over 300 COVID-19 hospital patients and healthcare workers, 2500 university staff, and 198 post-COVID-19 volunteers.

Anti-SARS-CoV-2 antibody responses follow a classic pattern with a rapid increase within the first three weeks after symptoms. Although titres reduce subsequently, the ability to detect anti-SARS-CoV-2 IgG antibodies remained robust with confirmed neutralization activity for up to 6 months in a large proportion of previously virus-positive screened subjects. Their work provides detailed information for the assays used, facilitating further and longitudinal analysis of protective immunity to SARS-CoV-2. Importantly, it highlighted a continued level of circulating neutralizing antibodies in most people with confirmed SARS-CoV-2 (Figueiredo-Campos *et al.*, 2020).

In a study it was found that, the point prevalence of SARS-CoV-2 viral carriage was 2.4%. The overall seroprevalence of SARS-CoV-2 antibodies was 24.4%. Participants who reported prior symptomatic illness had higher seroprevalence (37.5% vs 17.1%, $\chi 2 = 21.1034$, $p<0.0001$) and quantitatively greater antibody responses than those who had remained asymptomatic. Seroprevalence was greatest among those working in housekeeping (34.5%), acute medicine (33.3%) and general internal medicine (30.3%), with lower rates observed in participants working in intensive care (14.8%). BAME (Black, Asian and minority ethnic) ethnicity was associated with a significantly increased risk of seropositivity. Working on the intensive care unit was associated with a significantly lower risk of seropositivity compared with working in other areas of the hospital. They identified differences in the occupational risk of exposure to SARSCoV-2 between hospital departments and confirm asymptomatic seroconversion occurs in healthcare workers (Shields *et al.*, 2020).

Another study reported a seroprevalence of 70% (95% CI 67–73) at baseline and 66% (95% CI 62–70) at 1 month of follow-up, with a test-retest positivity of 65% (95% CI 61–68), and an incidence of new exposures of 2% (95% CI 1–3). They observed significant differences in the seroprevalence between age groups, with participants aged 18–29 years having lower seroprevalence than those aged younger than 12 years. After the first epidemic peak, Iquitos had one of the highest rates of seroprevalence of anti-SARS-CoV-2 antibodies worldwide. Nevertheless, the city experienced a second wave starting in January, 2021, probably due to the emergence of the SARS-CoV-2 P1 variant, which has shown higher transmissibility and reinfection rates (Álvarez-Antonio *et al.*, 2021).

Currently, more than 300 tests are available for SARS-CoV-2 antibody detection. These tests are produced in several formats and they detect different types of antibodies including IgG, IgM or IgA subtypes or total immunoglobulin. In addition, the target proteins used to detect antibodies vary between the tests. Commercial tests are usually designed to detect antibodies against SARS-CoV-2 nucleocapsid (N), spike1 (S1), spike2 (S2), or receptor-binding domain of the spike (S-RBD) protein, or their combinations, though not all commercial providers specify the viral proteins used. Given the large variability in antibody tests, discrepancies between test results are expected. Concordantly, at the moment no agreement exists upon which viral protein should be used as a gold standard in serodiagnosis of COVID-19 patients. Naaber et al. (2020) highlighted the importance to consider clinical symptoms, time of testing, and using more than one viral antigen in SARS-CoV-2 antibody testing. The majority of clinical studies and validations of commercial tests have been performed in patient groups with severe disease and thus reported sensitivity data may not be the same for COVID-19 patients with mild symptoms (Naaber *et al.*, 2020).

According to Nah et al. (2021), asymptomatic active infection might be an important contributor to the COVID-19 outbreak. Serological tests can assess the extent of exposure and herd immunity to COVID-19 in general populations. The overall seroprevalence of anti-SARS-CoV-2 was 0.39% for men and 0.48% for women. The rate of anti-SARS-CoV-2 positivity varied significantly between different regions of Korea ($p=0.003$), but not with age group, sex, or the statuses of obesity, diabetes, hypertension or smoking (Nah *et al.*, 2021).

A study conducted by Wiens et al. (2021) quantified IgG antibody responses to SARS-CoV-2 spike protein receptor-binding domain and estimated seroprevalence using a Bayesian regression model accounting for test performance. They recruited 2,214 participants from August 10 to September 11, 2020, and 22.3% had anti-47 SARS-CoV-2 IgG titers above levels in pre-pandemic samples. After accounting for waning antibody levels, age, and sex, 38.5% (32.1 - 46.8) of the population had been infected with SARS-CoV-2. For each RT-PCR confirmed COVID-19 case, 104 (87-126) infections were unreported. Background antibody reactivity was higher in pre-pandemic samples from Juba compared to Boston, where the serological test was validated. The estimated proportion of the population infected ranged from 30.1% to

60.6% depending on assumptions about test performance and prevalence of clinically severe infections (Wiens *et al.*, 2021).

Timely diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is a prerequisite for treatment and prevention. The serology characteristics and complement diagnosis value of the antibody test to RNA test need to be demonstrated. The seroconversion rates for Ab, IgM and IgG were 98.8%, 93.8% and 93.8%, respectively. The first detectible serology marker was Ab, followed by IgM and IgG, with a median seroconversion time of 15-, 18- and 20-days post exposure (d.p.e.) or 9-, 10- and 12-days post onset (d.p.o.), respectively. The antibody levels increased rapidly beginning at 6 d.p.o. and were accompanied by a decline in viral load. For patients in the early stage of illness (0–7 d.p.o), Ab showed the highest sensitivity (64.1%) compared with IgM and IgG (33.3% for both; $p<0.001$). The sensitivities of Ab, IgM and IgG increased to 100%, 96.7% and 93.3%, respectively, 2 weeks later. When the same antibody type was detected, no significant difference was observed between enzyme-linked immunosorbent assays and other forms of immunoassays. A typical acute antibody response was induced during SARS-CoV-2 infection. Serology testing provides an important complement to RNA testing in the later stages of illness for pathogenic-specific diagnosis and helpful information to evaluate the adapted immunity status of patients (Lou *et al.*, 2020).

The population-based serosurveys are essential for estimating COVID-19 burden and monitoring the progression of this pandemic. Javed et al. (2022) reported that the overall seroprevalence of SARS-CoV-2 antibodies was 16.0% (2810/17,764). The total antibody seropositivity was higher in males than females (OR 1.22, 95% CI 1.110–1.340). The symptomatic subjects had 2.18 times higher odds of IgG seropositivity while 1.2 times for IgM seropositivity than the asymptomatic subjects. The multivariable logistic regression model showed that the odds of SARS-CoV-2 total antibody seroprevalence were affected by the number of dependents (OR = 1.077; 95% CI 1.054–1.099), apparent symptomology (OR = 1.288; 95% CI 1.011–1.643), close unprotected contact with a confirmed or probable case of COVID-19 (OR 2.470; 95% CI 2.164–2.819), traveling history (last 14 days) (OR = 1.537; 95% CI 1.234–1.914) and proximity with someone who traveled (OR = 1.534; 95% CI 1.241–1.896) (Javed *et al.*, 2022).

In a study 3 000 individuals were included conforming asymptomatic and pauci-symptomatic groups (n=1 500 each). From the total sample, only 8.83% (n=265) presented reactivity for IgG anti-SARS-CoV-2. A significant association was observed between positive anti-SARS-CoV-2 IgG and a history of contact with a confirmed case; the transmission rate within households was approximately 30%. In the pauci-symptomatic group, among the seropositive ones, anosmia and fever presented an OR of 16.8 (95% CI 9.5-29.8) and 2.7 (95% CI 1.6-4.6), respectively (p <0.001). In asymptomatic patients, IgG levels were lower compared to pauci-symptomatic patients, tending to decline after 4 months since the symptoms onset. This study observed a low seroprevalence, suggestive of a large population susceptible to the infection. Anosmia and fever were independent significant predictors for seropositivity. Asymptomatic patients showed lower levels of antibodies during the 5-month follow-up. IgG antibodies tended to decrease over the end of this period regardless of symptoms (Rodeles *et al.*, 2021).

2.6 The Bangladesh context of the disease

The first case of coronavirus disease 2019 (COVID-19) was identified on 31 December 2019 in Wuhan, China (Guo et al.; 2020, World Health Organization, 2020). The Government of Bangladesh reported the first case of COVID-19 in Bangladesh on 8 March 2020 (GARDAWORLD, 2020). As of 29 June 2021, 896,770 confirmed cases have been identified in Bangladesh, including 14,276 deaths (Management Information System Directorate General of Health Services, 2021). Bangladesh is estimated to be at high risk for COVID-19 due to its population density, poor sanitary practices, and limited infrastructure and infection control measures. A second wave of COVID- 19 occurred in Bangladesh between April and May 2021, with 90% of cases due to the beta variant of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). By August 2021, all cases of COVID-19 in Bangladesh were due to the delta variant, and the death rate was higher than that in the first wave in 2020 (Rahman et al., 2021). In Bangladesh, the Institute of Epidemiology, Disease Control and Research (IEDCR) directed a national level investigation to evaluate the prevalence of COVID-19, in collaboration with the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), with support from the US Agency for International Development (USAID) and the Bill and Melinda Gates Foundation. Much epidemiological information about this newly

emerging disease remains unknown, including estimates of the proportion of COVID-19 cases in the community, particularly for lower-income regions and countries such as Bangladesh, making it difficult for government policy makers to design optimal containment and mitigation strategies. Prior research has indicated that there may be a considerable number of asymptomatic cases of COVID-19. Other areas requiring further exploration include the incidence rate, prevalence rate, secondary infection rate, incubation period, serial interval and reproductive number of COVID-19 in various settings. Although there have been attempts to gather some of these data in previous studies worldwide, most estimates have been based on small-scale data or on information collected from relatively narrow geographic regions (Anderson et al., 2020). It is also important to determine and characterize the immune responses to SARS-CoV-2 infection to understand how well the response protects people against future SARS-CoV-2 infection and how long this protection lasts. In this context, serological investigation has the potential to provide information about the true number of SARS-CoV-2 infections, allowing for robust estimates of the infection fatality rates, and to guide public health decision-making (Bhuiyan *et al.,* 2022).

2.7 Seroprevalence in Bangladesh

In Bangladesh 30% of the population were found to be seropositive for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) immunoglobulin G antibodies. The highest seroprevalence rate (64%) was found in slum areas in Bangladesh. Thirty-eight percent and 29% of participants from urban and rural areas were SARS-CoV-2 seropositive. The highest seroprevalence rate for coronavirus disease 2019 was observed in August 2020. A study was conducted amongst household members in 32 districts of Bangladesh to build knowledge about disease epidemiology and seroepidemiology of coronavirus disease 2019 (COVID-19). The national seroprevalence rates of immunoglobulin G (IgG) and IgM were estimated to be 30.4% and 39.7%, respectively. In Dhaka, the seroprevalence of IgG was 35.4% in non-slum areas and 63.5% in slum areas. In areas outside of Dhaka, the seroprevalence of IgG was 37.5% in urban areas and 28.7% in rural areas. Between April and October 2020, the highest seroprevalence rate (57% for IgG and 64% for IgM) was observed in August. IgM antibody was more prevalent in younger participants, while older participants had more frequent IgG seropositivity. Follow-up specimens from patients with COVID-19 and their household members suggested that both IgG and IgM

seropositivity increased significantly at day 14 and day 28 compared with day 1 after enrolment. Conclusions: SARS-CoV-2 had spread extensively in Bangladesh by October 2020. This highlights the importance of monitoring seroprevalence data, particularly with the emergence of new SARS-CoV-2 variants over time (Bhuiyan *et al.*, 2022).

Since February 2020, there have been reports of persons who were infected with SARS-CoV-2 but did not develop symptoms of COVID-19. In some cases the viral load of such asymptomatic persons has been equal to that of symptomatic persons, suggesting similar potential for viral transmission (Bi *et al.*, 2020).

In a study author revealed that approximately 40% to 45% of those infected with SARS-CoV-2 will remain asymptomatic suggests that the virus might have greater potential than previously estimated to spread silently and deeply through human populations. Asymptomatic persons can transmit SARS-CoV-2 to others for an extended period, perhaps longer than 14 days. The absence of COVID-19 symptoms in persons infected with SARS-CoV-2 might not necessarily imply an absence of harm. More research is needed to determine the significance of subclinical lung changes visible on computed tomography scans. The focus of testing programs for SARS-CoV-2 should be substantially broadened to include persons who do not have symptoms of COVID-19. The difficulty of distinguishing asymptomatic persons from those who are merely presymptomatic is a stumbling block. To be clear, the asymptomatic individual is infected with SARS-CoV-2 but will never develop symptoms of COVID-19. In contrast, the presymptomatic individual is similarly infected but eventually will develop symptoms. The simple solution to this conundrum is longitudinal testing that is, repeated observations of the individual over time (Oran and Topol, 2020).

In a study it has been suspected that infected persons who remain asymptomatic play a significant role in the ongoing pandemic, but their relative number and effect have been uncertain. The authors sought to review and synthesize the available evidence on asymptomatic SARS-CoV-2 infection. Asymptomatic persons seem to account for approximately 40% to 45% of SARS-CoV-2 infections, and they can transmit the virus to others for an extended period, perhaps longer than 14 days. Asymptomatic infection may be associated with subclinical lung abnormalities, as detected by

computed tomography. Because of the high risk for silent spread by asymptomatic persons, it is imperative that testing programs include those without symptoms. To supplement conventional diagnostic testing, which is constrained by capacity, cost, and its one-off nature, innovative tactics for public health surveillance, such as crowdsourcing digital wearable data and monitoring sewage sludge, might be helpful. In the early months of the coronavirus disease 2019 (COVID-19) pandemic, an iconic image has been the "proned" patient in intensive care, gasping for breath, in imminent need of artificial ventilation. This is the deadly face of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which as of 26 May 2020 had claimed more than 348 000 lives worldwide. But it is not the only face, because SARS-CoV-2 now seems to have a dual nature: tragically lethal in some persons and surprisingly benign in others (Oran and Topol, 2020).

Raqib et al. (2022) reported that among the 3220 participants (2444 adults, ≥18 years; 776 children, 10–17 years), the overall weighted seroprevalence was 67.3% with 71.0% in slum and 62.2% in non-slum. The weighted seroprevalence was 72.9% in Dhaka and 54.2% in Chattogram. Seroprevalence was positively associated with limited years of formal education, lower income, overweight, diabetes and heart disease. Contrarily, negative associations were found between seropositivity and regular wearing of masks and washing hands, and prior BCG vaccination. About 63% of the population had asymptomatic infection; only 33% slum and 49% non-slum population showed symptomatic infection (Raqib *et al.*, 2022).

Shirin et al. (2020) reported that in Bangladesh mildly symptomatic individuals developed IgM and IgA responses by day 14 in 72% and 83% of individuals, respectively, while 95% of individuals developed IgG response, and rose to 100% by day 30. In contrast, individuals infected with SARS-CoV-2 but who remained asymptomatic developed antibody responses significantly less frequently, with only 20% positive for IgA and 22% positive for IgM by day 14, and 45% positive for IgG by day 30 after infection (Shirin *et al.*, 2020).

Only a few studies have investigated the antibody responses in pauci-symptomatic or asymptomatic persons. Several studies have shown stronger antibody response in patients with severe disease as compared with mildly symptomatic ones. Also, higher rate of absence of seroconversion in asymptomatic patients has been described.

However, other studies have failed to find any correlation between clinical course and immune response. Since the majority of COVID-19 cases are asymptomatic, the performance of the tests in this group is important to evaluate the reliability of ntibody tests in seroepidemiological studies and clinical diagnostics.

Chapter 3: Materials and Methods

3.1 Study design

An observational cross-sectional analytical study.

3.2 Place of study

This study was conducted in the six government and private hospitals each, and two garment factories in CMA. All hospitals belonging to the study area were stratified according to their affiliation status; government and private.

3.3 Period of study

This study was carried out from 1st February 2021 to 31stJanuary 2022.

3.4 Study population

HCWs (e.g., doctors, nurses, hospital staff, ward boy, and cleaner), garment workers, and indoor and outdoor patients (non-COVID-19) of six government and private hospitals each, and two garment factories in CMA.

3.5 Sampling technique

Consecutive type of purposive sampling was done according to the availability of the study subjects who fulfilled the inclusion criteria.

3.6 Sample size determination

To conduct this study, the sample size was determined by using this formula. To estimate single proportion : (Haque, 2009)

$$Sample\ size\ n = \frac{Z^2 pq}{d^2}$$

z = z value of standard normal distribution at given level of significance or 95%

Confidence level, Z= 1.96

p= Expected prevalence is 65 % = 0.65

q = 1-p

d = Absolute error or precision (0.5)

All values are taken from (Tabassum *et al.*, 2013)

$$Therefore, \quad \frac{(1.96)^2 \; x \; 0.65 \; (1 - 0.65)}{(0.05)^2}$$

Sample size (n) = 349.58 ≈ 350

Adding design effect of 2, total sample size was 350 X 2 = 700

Considering 10% non-response rate and missing value calculated sample size was

n = 770.

Due to time constrains748 participants were enrolled in this study.

3.7 Selection criteria

Inclusion criteria:

- **Asymptomatic**: Only an asymptomatic group was included to ensure the presence of antibodies. Participants had no COVID-19 related clinical signs, e.g., fever, coughing, runny nose, sore throat, dyspnea, shortness of breath, aches and pain at the time of sample collection
- **In case of having past confirmed COVID-19 status (by Rt PCR**):

    i. Participants who had already passed at least 28 days after a negative Rt-PCR test.

    ii. Participants who did not take a repeated test to ensure negativity had passed at least 42 days after the first COVID-19 test.

Exclusion criteria:

- Persons under 18 were excluded, as were those with an incomplete questionnaire.
- Respondents who did not give informed written consent and not willing to participate.

3.8 Variables

a. Independent variables:

Socio-demographic variables:

- Age
- Gender
- Education
- Occupation
- Vaccination

b. Dependent variables:

Seroprevalence of anti SARS-COV-2 antibody

3.9 Research instruments

A semistructured questionnaire with data collection sheet was prepared for this purpose, which included all the variables of interest.

3.10 Data collection technique

Subjects were selected purposively according to the availability of the respondents. Relevant history and clinical information were obtained by a preformed questionnaire.

3.11 Study procedure

After obtaining Institutional ethical approval from the authorized committee of Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh, this observational cross sectional study was conducted study among HCWs (e.g., doctors, nurses, hospital staff, ward boy, and cleaner), garment workers, and indoor and outdoor patients (non-COVID-19) of six government and private hospitals each, and two garment factories in CMA. All hospitals belonging to the study area were stratified according to their affiliation status; government and private, from 1st March 2021 to 12th December 2021. From each strata, six hospitals were randomly selected. Sample size was calculated considering the following parameter: 0.65 proportion, 5%

margin of error, 95% confidence limit and design effect 2. Each organization's human resources department provided a list of personnel. Following a simple random sampling technique, samples were collected from a total of 748 respondents.

We interviewed participants to collect information after receiving written consent. Answering a questionnaire and taking blood to test SARS CoV-2 antibodies were part of the study procedure. Our study followed a World Health Organization protocol for population-level COVID-19 antibody testing. The questionnaire included sociodemographic details and factors hypothesized to be associated with seropositivity. Participants were included in the study based on several inclusion criteria.

3.12 Baseline blood collection and processing

Heparinized blood specimens (6mL) were collected and transported to the clinical pathology laboratory (CPL) of Chattogram Veterinary and Animal Sciences University (CVASU) within three hours of collection. The serum was separated to evaluate the IgG antibody and kept at -20 ˚C until serological investigation.

3.13 Serological test examination

Antibody was determined by a commercial qualitative assay using COVID-19 IgG ELISA test (Beijing Kewei Clinical Diagnostic Reagent Inc., China; Ref: 601340) as per the manufacturer's instructions. The assay is an enzyme-linked immunoassay (ELISA) that detects IgG against the SARS-CoV-2. An index (Absorbance/Cutt-off) of <1 was interpreted as negative, 0.9 to 1.1 as borderline (retesting of these specimens in duplicates was done to confirm results), and ≥1 index as positive. Per the manufacturer, the sensitivity and specificity of the assay for IgG are 93.8% and 97.3%, respectively. Positive and negative controls were included in all assay batches. Repeated testing using the same specimen yielded the same interpretation.

The concentration of IgG antibodies was determined by SARS-CoV-2 S1-RBD IgG (DiaSino® Laboratories Co., Ltd. Zhengzhou, China, Ref: DS207704), which is based on enzyme-linked immunoassay for the quantitative detection of IgG antibodies. The assay's sensitivity and specificity for IgG quantification, according to the manufacturer, are 98.41% and 98.02%, respectively. Quantitative results were calculated as a ratio of the extinction of the control or tested specimen over the

extinction of the calibrator. Results were reported in standardized units for the quantitative kits that included six calibrators to quantify the antibody concentration (i.e., DiaSino units/mL). A value of <10 DU/mL was considered negative, and values >10 DU/mL were positive.

3.14 Data management

The linearity of the quantitative variables was evaluated by categorizing them into four categories using quartiles as cut-off values. Logistic regression analysis was conducted on the categorized variables, and parameter estimates were observed for an increasing or decreasing trend. In case of linear increase or decrease in the parameter estimates, linearity in the quantitative variable was assumed and used without modification. In the case of nonlinearity, a quartile was used to categorize it. However, some quantitative variables were categorized considering research interest. For instance, the number of days between the first dose of vaccine and quantification of antibody titer was categorized as 'after one month' and 'after two months' and between the second dose of vaccine and quantification of antibody titer was categorized as 'after two months', 'after four months' and 'after six months'. The number of days between the vaccination and the antibody titer was achieved from the date of vaccination and sample collection. The prevalence estimates were adjusted with the test kit performance (sensitivity and specificity), and the adjusted prevalence was denoted as true prevalence.

3.15 Data analysis

In the study period, a total of 748 qualitative and quantitative test results were included in the analysis. To evaluate the correlation and collinearity in the categorical and quantitative variables, Cramer's V test, Spearman correlation coefficient, Chi-square test, t-test or ANOVA, where appropriate, was used. Variables with a significant association or a Spearman correlation coefficient above 0.4 were regarded as correlated. The effects of different potential explanatory variables on the binary outcome - presence/absence of anti-SARS-CoV-2 antibody was evaluated using univariable and followed by multivariable logistic regression models. To select the final multivariable model, all variables with a significant P-value in the univariable models were included in a model and a manually conducted backward selection strategy was followed by deleting one variable at a time with the highest P-value.

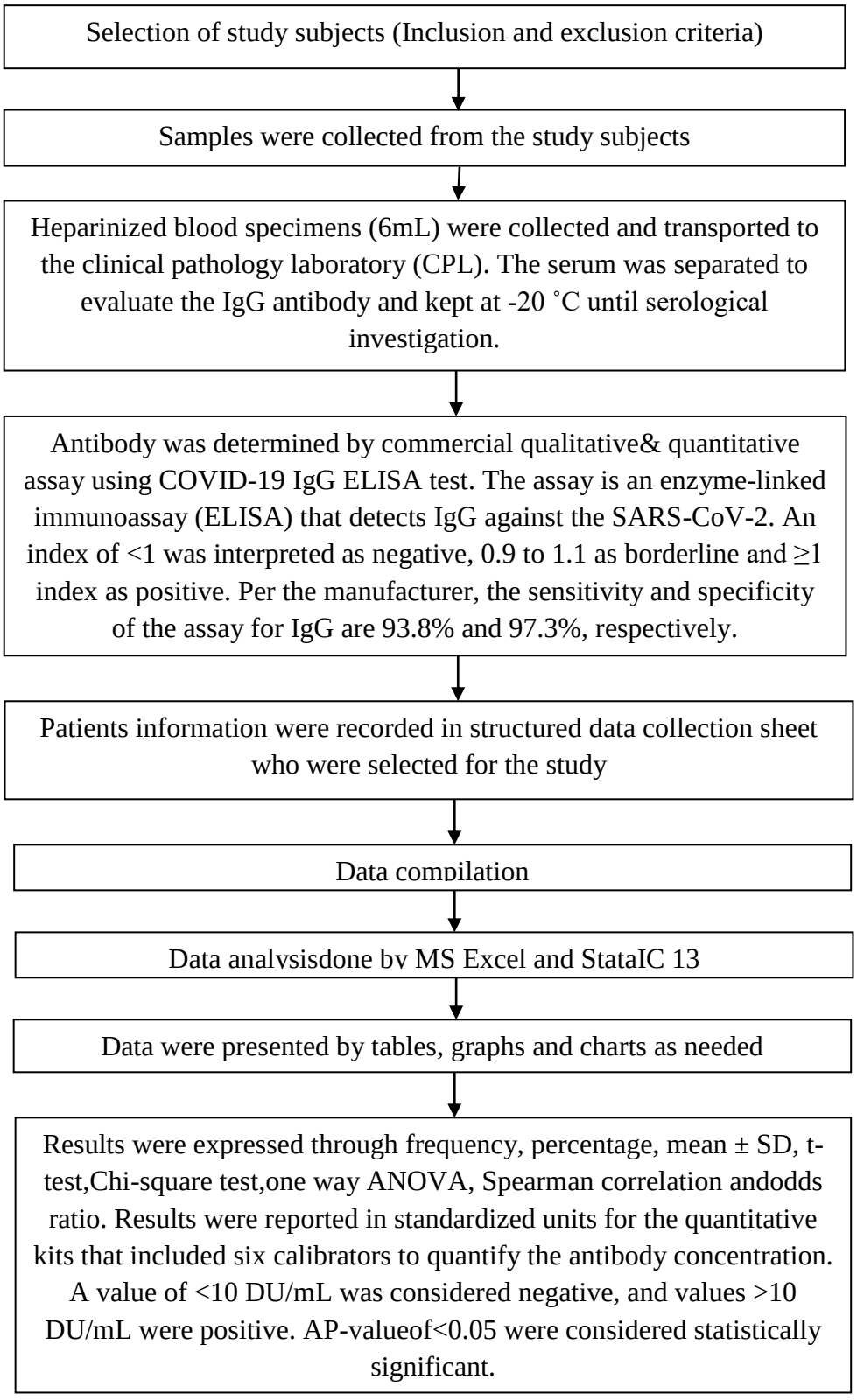
Interactions between all explanatory variables (2 ways) were evaluated in the final model. Effect of variables on the mean titer of the antibody was assessed by t-test and one way ANOVA. P-values <0.05 were considered as significant throughout the analysis. STATA-IC 13 (StataCorp, California, USA) and GraphPad Prism 7.00 for Windows (GraphPad Software, La Jolla, California, USA) were used for statistical analyses and visualization.

3.16 Ethical aspect

- Institutional ethical approval was taken from the authorized committee of Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh.
- Permission for the study was taken from the concerned department from where we collected our study subjects.
- Written consent of all the study subjects was taken free of pressure and without exploiting any weakness of the subjects.
- The entire study subject was thoroughly appraised about the nature, purpose and implications of the study, as well as the entire spectrum of benefits and risks of the study.
- The interest of the study subjects was not compromised to safeguard their rights and health.
- As this study needs only 8 ml of blood of study subjects, the chances of complications were very unlikely. But in case any complications like slight discomfort, mild pain, weakness or vertigo occur, they were treated with assurance, analgesics.
- Subjects were assured about their confidentiality and freedom to withdraw them from the study anytime.

3.17 Data processing plan

Study subjects were selected according to inclusion and exclusion criteria, consents were taken in informed written consent form, and then preceded as follow-



3.18 Procedure for Maintaining Confidentiality

- Signed informed consent was taken from the patient, convincing that the privacy of the patient was maintained.
- There was minimum physical, psychological, social and legal risk during the collection of blood and before physical examinations proper consent was taken.
- For blood collection, proper safety method was followed and during the physical examination and interview, privacy was maintained.
- For safeguarding confidentiality and protecting anonymity, each of the patients was given an unique ID number which was followed in sample collection, transport to lab and reporting, in each step of the procedure.
- A questionnaire (enclosed) was prepared for which a short interview of 15-30 minutes was required.
- No experimental new drug was administered.
- No placebo was used here**.**

Chapter 4: Results

Total 748 patients were selected in CMA, fulfilling the inclusion and exclusion criteria to evaluate the seroprevalence of anti SARS-COV-2 antibody among the asymptomatic and confirmed COVID 19 population. Data were analysed using the appropriate statistical procedures using Microsoft Excel and effect of variables on the mean titer of the antibody was assessed by t-test and one way ANOVA. P-values <0.05 were considered as significant throughout the analysis. STATA- 13. Outcomes were presented in this chapter through tables and graphs.

4.1 Characteristics of study participants

Table 1 states that among 748 respondents CMA service providers 362were HCWs, 205 were garments workers and 179 indoor/outdoor patients. Among them, 27.48% were garment workers, 150 (20.11%) hospital staff, 145 (19.44%) doctors, 148 (19.84%) outdoor patients, 67 (8.98%) nurses, and 31 (4.16%) indoor patients. The majority (n=507; 67.96%) were males. In the total population, 292 (39.14%) did not receive any COVID-19 vaccine, 223 (29.89%) received the first dose of vaccine, and 231 (30.97%) received both doses of the vaccine. The responses regarding contact with confirmed COVID-19 cases were: yes (342; 47.17%), no (307; 42.34%), and unknown (76; 10.48%). One hundred and ninety-seven (32.35%) participants had pre-existing medical conditions**.**

Table 4.1: Baseline characteristics of study participants

Variables	Level	Total population	Known positives (rtPCR positive)	Asymptomatic
Donor type	Doctor	145 (19.44)	40 (35.40)	85 (16.13)
	Nurse	67 (8.98)	19 (16.81)	43 (8.16)
	Hospital staff	150 (20.11)	27 (23.89)	109 (20.68)
	Indoor patient	31 (4.16)	2 (1.77)	26 (4.93)
	Outdoor patient	148 (19.84)	21 (18.58)	109 (20.68)
	Garments worker	205 (27.48)	4 (3.54)	155 (29.41)
Gender	Male	507 (67.96)	73 (65.18)	362 (68.69)
	Female	239 (32.04)	39 (34.82)	165 (31.31)
Age (year)	19 to 29	201 (26.91)	15 (13.27)	149 (28.27)
	30 to 35	184 (24.63)	30 (26.55)	123 (23.34)
	36 to 44	180 (24.10)	34 (30.09)	123 (23.34)
	45 to 84	182 (24.36)	34 (30.09)	132 (25.05)
Vaccination	No	292 (39.14)	11 (9.82)	222 (42.13)
	Only 1st dose	223 (29.89)	38 (33.93)	153 (29.03)
	Both doses	231 (30.97)	63 (56.25)	152 (28.84)
Days passed after 1st dose of vaccine	14 to 30 days	45 (24.06)	8 (25.81)	30 (23.08; 16.1-31.3)
	31 to 60 days	142 (75.94)	23 (74.19)	100 (76.92)
Days passed after 2nd dose vaccine	14 to 60 days	19 (8.26)	6 (9.38)	12 (8.00)
	61 to 120 days	86 (37.39)	20 (31.25)	60 (40.00)
	120 to 180 days	125 (54.35)	37 (59.38)	78 (52.00)
Days between PCR test and antibody test	21 to 60 days	-	17 (15.60)	-
	61 to 120 days	-	16 (14.68)	-
	121 to 180 days months	-	23 (21.10)	-
	>180 days	-	53 (48.62)	-
Contact with confirmed case	Yes	342 (47.17)	79 (71.17)	230 (45.19)
	No	307 (42.34)	17 (15.32)	232 (45.58)
	Don’t know	76 (10.48)	15 (13.51)	47 (9.23)
Family member	1 to 3	186 (26.23)	31 (29.52)	130 (25.79)
	4 to 6	443 (62.48)	64 (60.95)	321 (63.69)
	≥7	80 (11.28)	10 (9.52)	53 (10.52)
Taking immunosuppressive drugs	Yes	15 (2.13)	7 (6.42)	8 (1.63)
	No	688 (97.87)	102 (93.58)	484 (98.37)
Comorbidities	Yes	197 (32.35)	38 (37.25)	291 (68.79)
	No	412 (67.65)	64 (62.75)	132 (31.29)

4.2 Sero-prevalence of SARS-CoV-2 infection

SARS-CoV-2 IgG antibodies were detected in 498 (66.99%) of 748 individuals (Table 2).

Table 4.2: Prevalence of SARS-CoV-2 IgG antibodies estimation in CMA

Anti-SARS CoV-2 antibody	Total population	Unadjusted seroprevalence, % (95% CI)	Test performance adjusted seroprevalence % (95% CI)	Known positives (rtPCR positive) (%)
Present	498	66.58(63.1-70.0)	66.99 (63.40-70.40)	91 (80.53)
Absent	250	33.42 (30.1-36.9)	32.60 (29.20-36.19)	22 (19.47)

Prevalence of anti-SARS-CoV-2 antibody (IgG) in different donor types along with vaccination percentage is shown in Figure 1.

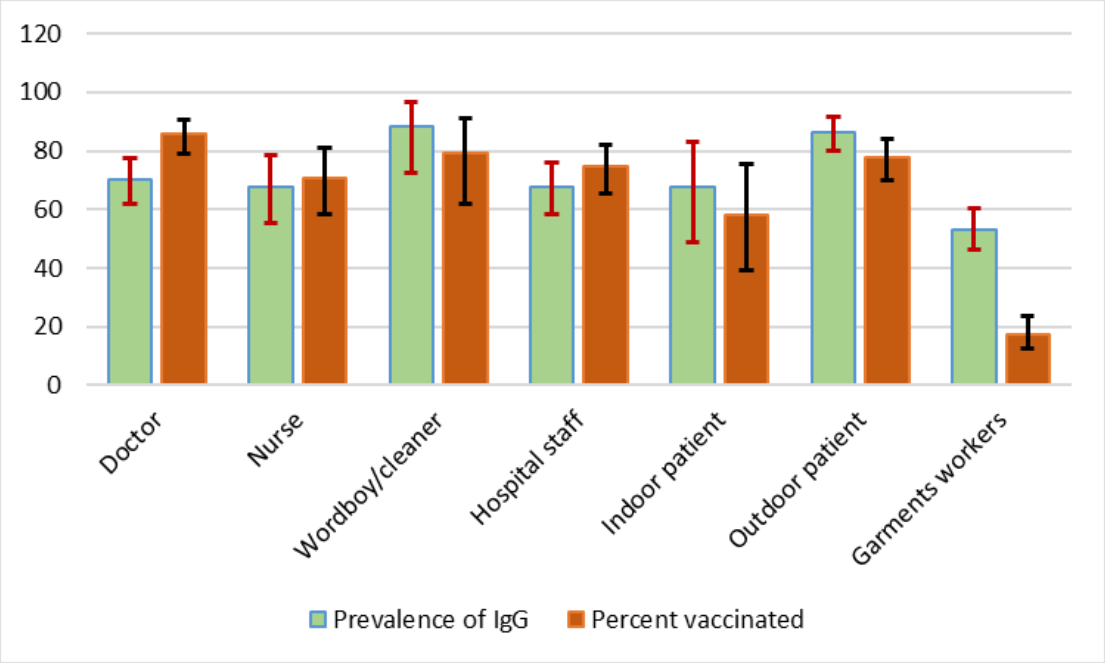


Figure 4.1: Prevalence of anti-SARS-CoV-2 antibody (IgG) in different donor types along with vaccinated percent.

4.3 SARS-CoV-2 antibody titer

Indoor/outdoor patients had the highest mean titer of 197.18 DU/mL, followed by HCWs (163.30 DU/mL) and garment workers (77.05 DU/mL) (p <0.001). The level (mean) of IgG-spike antibodies in both dosage vaccine recipients' was higher (255.46 DU/mL) than in those who received one (159.08 DU/mL) or no doses (53.71 DU/mL) of the vaccine (p <0.001). When the participants had a contact with confirmed cases had a mean titer of 170.89 DU/mL, not known had a titer of 160.05 DU/mL, and in case of noncontact 116.45 DU/mL (p<0.001). The mean titer of different age groups was statistically significant; nevertheless, we removed this variable from further analysis to minimize the bias due to vaccination strategy followed in Bangladesh (priority given to aged); details in Table 3.

Table 4.3: Univariable analysis (t-test, one way ANOVA) to evaluate the mean difference of quantity of anti-SARS-CoV-2 antibody in serum samples

Variable	Level	Mean titer of IgG (DU/ml)	SD	P-value
Doner type	Health worker	163.30	153.54	<0.001
	Indoor/outdoor patient	197.18	147.04	
	Garments worker	77.05	115.63	
Gender	Female	140.09	151.36	0.31
	Male	151.83	148.38	
Age (year)	19 to 29	106.90	132.23	<0.001
	30 to 35	151.16	157.71	
	36 to 44	160.85	143.08	
	45 to 84	176.95	155.92	
Vaccination	No	53.71	91.16	<0.001
	Only 1st dose	159.08	161.05	
	Both doses	255.46	117.04	
Days passed after 1st dose of vaccine	31 to 60 days	131.39	152.08	0.10
	14 to 30 days	175.10	164.09	
Days passed after 2nd dose vaccine	120 to 180 days	147.09	119.29	0.02
	61 to 120 days	255.82	106.00	
	14 to 60 days	324.42	128.42	
Asymptomatic	No	190.01	161.93	<0.001
	Yes	130.03	140.19	
Had COVID-19 confirmed status	No	191.69	142.70	0.005
	Yes	244.87	159.74	
Contact with confirmed case	No	116.45	135.21	<0.001
	Yes	170.89	154.19	
	Don’t know	160.05	158.98	
Taking immunosuppressive drugs	No	143.02	150.09	0.32
	Yes	181.38	152.08	

The changes in mean titer of IgG antibody across different time intervals of intervention (one and both doses of vaccination) is illustrated in Figure 2.

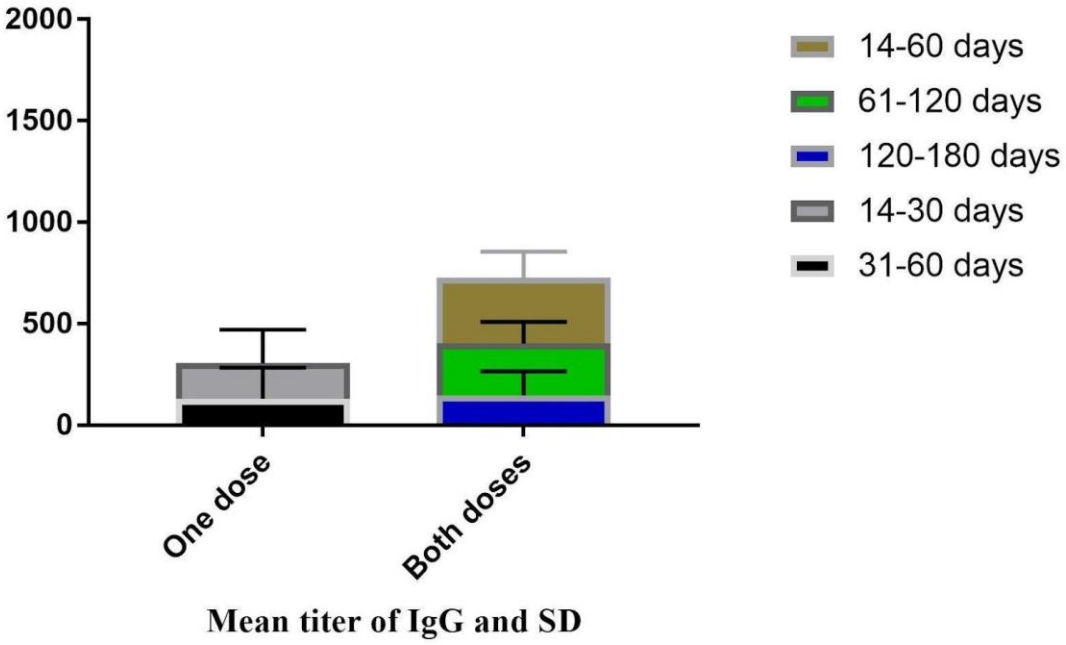


Figure 4.2: Evaluation of time effects of vaccines mean difference of quantitative anti-SARS-CoV-2 antibody (IgG) in serum samples

4.4 Risk factor analysis

Univariable analysis (χ^2 test, logistic regression) to evaluate the association of different variables with the seroprevalence of anti-SARS-CoV-2 antibody

Indoor/outdoor patients amongst the different donor groups had a positivity rate of 81.37% (144 of 179) compared to 68.99% (248 of 362) in the HCWs and 50.56% in the garments workers (104 of 205); the difference was statistically significant ($p<$ 0.001). Both doses of vaccine receivers showed significantly ($p<0.001$) higher seropositivity than one dose or no vaccine receivers. Similarly, contact with confirmed COVID-19 cases showed a higher odds of being seropositive as compared to noncontact ($p=0.01$) [OR= 1.59] (Table 4).

Table 4.4: Univariable analysis (χ^2 test, logistic regression) to evaluate the association of different variables with seroprevalence of anti-SARS-CoV-2 antibody

Variable	Level (n)	Presence of IgG	TP (95% CI of TP)**	OR	P-value
Donor type	Health worker (362)	248	68.99 (63.8-73.7)	Ref.	<0.001
	Indoor/outdoor patient (179)	144	81.37 (74.7-86.7)	1.8	
	Garments worker (205)	104	50.56 (43.5-57.5)	0.47	
Gender	Female (239)	151	63.47 (56.9-69.5)	Ref.	0.15
	Male (507)	347	68.92 (64.6-72.9)	1.26	
Age (year)	19 to 29 (201)	114	56.76 (49.5-63.6)	Ref.	0.002
	30 to 35 (184)	119	65.01 (57.6-71.8)	1.39	
	36 to 44 (180)	132	73.99 (66.8-80.1)	2.09	
	45 to 84 (182)	133	73.73 (66.6-79.8)	2.07	
Vaccination	No (292)	131	44.47 (38.6-50.4)	Ref.	<0.001
	Only 1st dose (223)	137	61.66 (54.8-68.0)	1.95	
	Both doses (231)	229	100 (98.4-100.0)	140.72	
Days passed after 1st dose of vaccine	31 to 60 days (142)	79	55.64 (47.1-63.8)	Ref.	0.29
	14 to 30 days (45)	29	64.78 (49.6-77.5)	1.44	
Days passed after 2nd dose vaccine	120 to 180 days (125)	123	99.9 (95.7-100)	-	-
	61 to 120 days (86)	86	100 (97.2-100)	-	
	14 to 60 days (19)	19	100 (84.2-100)	-	
Asymptomatic	No (220)	160	73.36 (66.9-79.03)	Ref.	0.13
	Yes (528)	355	67.66 (63.4-71.68)	0.76	
Had COVID-19 confirmed status	No (144)	119	83.65 (76.3-89.1)	Ref.	0.66
	Yes (113)	91	81.46 (72.9-87.9)	0.86	
Contact with confirmed case	No (307)	187	61.11 (55.3-66.6)	Ref.	0.01
	Yes (342)	244	71.93 (66.7-76.6)	1.59	
	Don't know (76)	49	64.81 (53.1-75.0)	1.16	
Taking immunosuppressive drugs	No (688)	447	65.32 (61.5-68.9)	Ref.	0.20
	Yes (15)	12	80.91 (54.7-94.3)	2.15	

Multivariable analysis (logistic regression) to determine the potential factors associated with SARS-CoV-2 antibody-positive status in the study area

The multivariable logistic regression model identified two potential factors that might be influencing the seropositivity of SARS-CoV-2 antibodies in the studied population. The chance of being seropositive was 2.22 times higher in indoor/outdoor patients (p= 0.002) and 1.69 times for garments workers than HCWs (p= 0.01). Further, both doses of vaccine receivers had a higher chance of being positive (OR=174.02) than one dose (OR=2.34) or none dose receivers, and the difference was statistically significant (p< 0.001) (Table 5).

Table 4.5: Output from the final multivariable logistic regression model showing the adjusted effect of potential factors on the seroprevalence of anti-SARS-CoV-2 antibody

Variable	Level	OR	95% CI	P-value
Doner type	Health worker	Ref.		
	Indoor/outdoor patient	2.22	1.33-3.68	0.002
	Garments worker	1.69	1.09-2.62	0.01
Vaccination	No	Ref.		
	Only 1st dose	2.34	1.56-3.50	<0.001
	Both doses	174.02	41.46-730.40	<0.001

Chapter 5: Discussion

This cross-sectional population-based studywas carried out to determine the Seroprevalence of anti SARS-COV-2 antibody among the asymptomatic and confirmed COVID 19 population. A total of 748HCWs (e.g., doctors, nurses, hospital staff, ward boy, and cleaner), garment workers, and indoor and outdoor patients (non-COVID-19) of six government and private hospitals each, and two garment factories in CMA were included in this study.

The overall adjusted seroprevalence estimate of SARS-CoV-2 antibodies was 66.99% (95% CI: 63.40%-70.4%) in CMA in this research which is slightly higher than a previous finding (64.1%) using an immunoassay test to detect antibodies in the Sitakunda sub-district (Chattogram district) of Bangladesh from March to June 2021 (Bhuiyan *et al.*, 2021). Another research conducted by icddr'b between October 2020 and February 2021 found a lower (55%) estimate in Chattogram than ours. During the same study period, however, the adjusted seroprevalence in Dhaka, the capital of Bangladesh, was 71% (Ara *et al.*, 2022). Thus, based on several investigations, it can be assumed that seropositivity in Chattogram has been progressively increasing over time. The prevalence might have increased due to either high infection levels or a positive response to the national immunization campaign in its early phases (Ward *et al.*, 2021). According to the findings, 68.99% HCWs and 81.37% indoor/outdoor patients were seropositive. Indoor and outdoor patients were more likelyto be seropositive than health professionals, possibly due to the combined effect of lack of awareness and knowledge about COVID-19 and effect of vaccination. Tripathi *et al.*, 2020 reported that HCWs were more educated of COVID-19 symptoms, incubation time, problems in high-risk patients, and had greater access to therapy than other residents (non HCWs)(Tripathi *et al.*, 2020). In Navi Mumbai in May 2021, serosurveillance of anti-SARS-Cov-2 antibodies among essential workers revealed that police personnel had a 72% seropositivity rate, whereas HCWs had a 48% positivity rate (Maheshwari *et al.*, 2021). Moreover, we observed that, among the garment workers, just fewer than 20% received vaccines and just above 50% were seropositive, which might have majorly been achieved from natural infections. It might indicate their lack of awareness about disease transmission and vaccination.

We found that the IgG antibody was produced in 61.66% of the participants who received the first dose of COVID-19 vaccination. This number increased to 100% among individuals who received a second dose. In a study by Bayram et al., 2021, HCWs' seropositivity rates after the first and second doses of CoronaVac vaccination were found to be 77.8% and 99.6%, respectively(Bayram *et al.*, 2021). Subsequently, when we quantified the antibody titer we observed it higher in those who received the second dose than in those who received just the first. Detection of highly avid anti-S1/-RBD IgG, independent of the causal mechanism, is seen as a very positive indication and indicator of enhanced humoral immunity (Neumann *et al.*, 2021).

Human coronavirus infection may not always result in long-lasting antibody responses, with antibody titers dropping over time(Callow *et al.*, 1990). The waning of antibody responses is an essential element to consider while developing a coronavirus vaccine (Amanat and Krammer, 2020). Our study showed that by the second month following the initial dose, the mean IgG titer in the body had dropped by nearly 25%. However, the antibody's propensity to deteriorate with time was noteworthy. This study revealed that the available mean antibody titers that remained after two months of receiving the second dose had dropped by roughly 21% by the fourth month, and within the sixth month the mean antibody titer was 147.09 DU/mL. So, it can be assumed that the body still retained considerable antibodies against COVID-19 six months after receiving the second dose vaccine, though the threshold level to prevent the virus is not known.

The underreporting of SARS-CoV-2 infection cases makes it difficult to assess the actual infection burden. Limited testing, flaws in the reporting infrastructure, and a substantial proportion of asymptomatic infections contribute to the underreporting (Bhattacharyya *et al.*, 2021). Asymptomatic carriers spread COVID-19, but the clinical characteristics, viral dynamics, and antibody responses of these individuals are unknown (Xiao *et al.*, 2021). According to our findings, 67.66% of the asymptomatic population was seropositive where only 29.03 % of asymptomatic individuals received the first dose of COVID-19 vaccine, and 28.84 % received the second dose too. According to various population-based studies, a considerable majority of seropositive people were asymptomatic or had no known encounter with a COVID-19 patient (Mukhtar *et al.*, 2021). Meanwhile, the observation that asymptomatic people had lower mean IgG levels than symptomatic people backs up

previous findings that asymptomatic carriers have a lesser humoral immune response to COVID-19 infection (Long *et al.*, 2020). The study also revealed that people aged above 35 had a greater seroprevalence. Higher seroprevalence among adults could be associated with increased vaccination exposure. On January 26 of this year, the government began accepting registrations for the COVID-19 vaccine for persons aged 55 and up in the country. In the second phase, the age limit was dropped to 40 years or more, and vaccination of youngsters aged 12-17 has recently begun in the country (Abedin *et al.*, 2021).

The latest and more deadly SARS-CoV-2 viral strains, as well as the possibility of losing immunity with time after vaccination, have prompted health professionals to consider the need for boosters. Research on threshold titers giving protection and time intervals of declining immunity post-immunization for low-middle-income nations like Bangladesh are essential before launching further booster doses. An important application of serological tests is to determine the antibody responses generated upon SARS-CoV-2 infection and vaccination (Krammer and Simon, 2020). The continuation of this study on those who received the second dose more than six months ago will provide an appropriate booster interval, risk population category, and overview of herd immunity. According to a recent study conducted in the greater Chattogram division it is evident that administering the first dose (Oxford-AstraZeneca) vaccine significantly reduces health risk during the COVID-19 infection phase (Rana *et al.*, 2021). So, it is evident that similar research is clamoring for justifications for booster administration. Additionally, more research is required to assess the efficacy of booster doses. Government and healthcare professionals must adopt COVID-19 vaccine booster dose utilization guidelines that consider the risks of fading immunity, new virus strains, and prioritizing vulnerable groups.

Our study has several limitations, such as the fact that we only collected samples from hospitals and the garment industry, but the results would be more representative of the community if we included other groups. We could not compare immunological responses produced by different COVID-19 vaccine brands at the same post-vaccination interval since distinct COVID-19 vaccines were licensed and supplied to CMA at different times. We did not reveal the type and name of COVID-19 vaccines, whereas a sufficient fraction was not covered under the vaccination program, and we were concerned about an infodemic.

Chapter 6: Conclusion

This study revealed that seroprevalence and mean antibody titer varied according to different factors in this study. The second dose of vaccine significantly increased the seroprevalence and titer, which decreased to a certain level over time. Although antibody was produced following natural infection, the mean titer was relatively low compared to antibody after vaccination. This study emphasizes the role of the vaccine in antibody production. Based on the findings, interventions like continuing extensive mass vaccination of the leftover unvaccinated population and bringing the mass population with a second dose under a third dose campaign might be planned.

Chapter 7: Limitations of the study

This study had some limitations as well-

- Because most of the individuals with antibodies were not confirmed cases and reported no COVID-19-related symptoms, we cannot determine when they were infected, which restricts our ability to assess when the antibody was produced.
- The study population was relatively small, so the results of this study may not reflect the exact picture of the whole country.
- The present study was conducted in short period of time.
- The sample was taken purposively. So, there may be a chance of bias that can influence the results.
- Limited resources and facilities.

Therefore, the study findings cannot be generalized to the entire population.

Chapter 8: Recommendations

Based on the finding of this study, following recommendations are made:

- New cases of COVID-19 continuously appear even under the strict interventions adopted in Bangladesh. The present findings suggest that asymptomatic seropositive individuals contribute to virus transmission. The strategy of applying PCR tests to suspected patients and quarantining should be continued. In addition, screening for SARS-CoV-2 using anti-SARS-CoV-2 antibody tests in the asymptomatic populations could be considered in risk region with high incidence rate.
- The policymaker should develop the appropriate provision of sharing up-to date information focusing on all health care workers involved in the laboratory works, especially medical technologists, to expand their perception, knowledge, attitude and practice toward COVID-19 and thus improve their concern level.
- Adequate technical manpower should be deployed in concerned national level laboratories to ensure greater quality of work and limit workload.
- Through proper vaccination, training initiatives with standardized laboratory equipment supply, adequate safety precautions and financial incentives concerns among medical technologists on COVID-19 pandemic in Bangladesh can be improved.

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ANNEXURE

COVID-19 Sero-survey Questionnaire

Chattogram Veterinary and Animal Sciences University

Specimen ID		**Date of specimen collection**	____/____/ 2021

Site*	**Hospital***				**Garments name & Location**	**Slum name & Location**
	Medical Centre	CMC	USTC	Memon		

Name of Interviewee:					
Mobile no.:		Relationship with mobile owner*			Own/Spouse/Child/Other
Age:	__ __ year	Sex*:	M/ F/ Other	No. of family members:	
Profession:		Blood group:			
Present address:	House no./ Road/Village:			Union/ Ward no:	
Upazila/ Thana:		District:			

Did you take vaccine against COVID-19?	Yes	Date.....	Dose:* 1st/ 2nd	
		Hospital registered.....		
		Any side effect*	Yes	No
	No			

If COVID-19 Diagnostic test (Rt PCR) done

Test date:		Test Lab name:			
Test result*:	**(+ve)**	**(-ve)**	If (+ve), negative confirmation test done?*	Yes, Date of test.....	No
Symptoms during test*	Yes *	Fever (.....ºF)/Cough/Shortness of breath/Sore throat/Aches & pain/Anosmia/Ageusia/Dysgeusia/Headache/Rash/Diarrhoea/ Other.....			Onset of symptom (.....) days
	No				
Similar Symptoms any time after Test		Yes, Time & duration.....			No
Maintained isolation?*	Yes	Home Isolation*: Separate bed/ separate room			
		Hospital*: General bed/ ICU/ HDU/Other.....			
	No				
Contact with any confirmed case?*		Yes	No		
Any family member infected?*		Yes, No. of family member infected:			No
Comorbidity*	DM/HTN/Heart disease/Liver disease/Renal disease/Asthma/COPD/Bronchitis/Other.....				
Do you take any immunosuppressive drug?*		Yes*, Steroidal/Other.....			No

COVID-19 Sero-survey Questionnaire

Chattogram Veterinary and Animal Sciences University

If COVID-19 Diagnostic test (Rt PCR) not done

Any COVID like symptoms within last six months?*	Yes*	Fever (.....ºF)/Cough/Shortness of breath/Sore throat/Aches & pain/Anosmia/Ageusia/Dysgeusia/ Headache/Rash/Diarrhoea/ Other.....		
	No			
If yes, Time & onset of symptoms	.....months before, ...days			
If symptoms present, Maintained isolation?*	Yes	Home Isolation*: Separate bed/ separate room		
		Hospitalized		
	No			
Contact with any confirmed case?*	Yes	No		
Any family member had COVID like symptoms within last six months?*	Yes, No. of family member infected:			No
COVID-19 (Rt-PCR) done for Any family member?*	Yes*, (+ve)/ (-ve)	Date of test.....		No
Comorbidity*	DM/HTN/Heart disease/Liver disease/Renal disease/Asthma/ COPD/Bronchitis/Cancer/Other.....			
Do you take any immunosuppressive drug?*	Yes, Steroidal/Other.....		No	

(*) Put tick mark at appropriate option

.....

Interviewer