

## A sero-epidemiological study after two waves of the COVID-19 epidemic

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### Abstract

**Background:** The COVID-19 situation in Maldives have evolved since the epidemic began in March 2020 with unprecedented increase in cases since mid-July 2019 with over 8000 cases at the end of August 2020.

**Objective:** The aim of the sero-epidemiological investigation is to obtain a sense of the population exposure to the SARS-CoV-2 by measuring the seroprevalence of antibodies to COVID-19 in the general population.

**Methods:** A population-based, age-stratified prospective method was employed to find out the key epidemiological and serologic characteristics of COVID-19 virus in this study.

**Results:** The results showed that seroprevalence in the population was 13%. The factors that were associated with antibody results included age (OR: 4.0, CI: 1.7-9.0), nationality (OR: 12.9, CI: 8.3-19.7), being diagnosed for COVID-19 (OR: 24.7, CI: 15.9-38.4) and having symptoms of COVID-19 (OR: 2.0, CI: 1.5-2.8). There was a gradual decrease in the antibody levels from 19 days to 250 days. The mean duration of the presence of antibodies in this study was found to be 124 days.

**Conclusion:** While the seroprevalence provides a measure that can be used to predict community transmission risk of the disease, the extent of functional immunity provided by antibody titres is still not clear. It is acknowledged that other mechanisms of protection such as T cell mediated immunity will play an important role in providing individual protection.

**Key words:** seroprevalence, COVID-19, antibody levels, Maldives, symptoms, age

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### Introduction

COVID-19 has affected all the countries around the world except a few. The crude mortality rate varies markedly by country depending on the populations affected, the point a country is at in the course of its outbreak, and the availability and application of testing. The crude clinical case fatality is currently over 3%, increasing with age and rising to approximately 15% or higher in patients over 80 years of age.<sup>1</sup> Morbidity associated with COVID-19 is reported to be very high. Underlying health conditions that affect the cardiovascular, respiratory and immune systems confer an increased risk of severe illness and death.<sup>2</sup> Countries are at different stages of national and subnational outbreaks. Where there has been early action and implementation of comprehensive public health measures – such as rapid case identification, rapid testing and isolation of cases, comprehensive contact tracing and quarantine of contacts – countries and subnational regions

have suppressed the spread of COVID-19 below the threshold at which health systems become unable to prevent excess mortality.<sup>1</sup>

The COVID-19 situation in the Maldives have evolved since the epidemic began in March 2020 with unprecedented increase in cases since mid-July 2019 with over 8000 cases at the end of August 2020.<sup>3</sup> Infection was concentrated in Male' area, though imported cases were being reported from other islands among travelers who were under quarantine.<sup>3</sup> Seroprevalence has not been undertaken in the country, though some antibody testing for COVID-19 has been done by private hospitals. Information available on public domain from a project conduct by ADK hospital in Male' in July 2020 shows about 4% seroprevalence from COVID-19 exposure.<sup>4</sup> With the exponential increase of cases, it is expected that the cases to be much higher in August than was observed in July. The vaccination program also has not been started when the study was conducted.

Due to the high proportion of asymptomatic or mild infections, data restricted to laboratory-confirmed cases do not capture the true extent of the spread or burden of the virus, severity or its infection-fatality ratio. A review study conducted by Portugal showed that under-ascertainment is very common for COVID-19 science.<sup>5</sup> Therefore, a number of countries have resorted to serological detection of specific antibodies against SARS-CoV-2 that can better estimate the true number of infections. A Spanish study, which included more than 60000 participants, showed a nationwide seroprevalence of 5.0%, with urban areas around Madrid showing more than 10%.<sup>6</sup> Similar findings were observed in a Swiss study, with seroprevalence data from Geneva reaching 10.8%.<sup>7</sup> While seroprevalence provides information to better understand the extent of the population affected, it must be noted that such seroprevalence studies provide information only about previous exposure, rather than immunity, as no neutralising antibodies are measured.<sup>8</sup>

In many countries where community transmission has led to outbreaks with near exponential growth, countries have introduced widespread population-level physical distancing measures and movement restrictions in order to slow-down the spread similar to the Maldives. However, these lockdown and restrictive measures can have a profound negative impact on individuals, communities, and societies by bringing social and economic life to a near stop.<sup>9</sup> Hence, there is a need to obtain further evidence on exposure and case severity to plan for interventions that can enable the sustainable suppression of transmission to a low-level whilst enabling the resumption of some aspects of economic and social life. The aim of this investigation is to measure the seroprevalence of antibodies to COVID-19 in the resident population of Male' area in the Maldives.

## Methodology

A population-based, age-stratified method was employed to find out the key epidemiological and serological characteristics of COVID-19 virus. The study was designed as a cross-sectional investigation using probability sampling and conducted between 15 October to 16 November 2020.

The geographic scope of the investigation was limited to greater Male' area where there was community spread since 15 April 2020.<sup>3</sup> The study population were the people living in Male' at the time of survey. (Based on 95% confidence interval and 5% error, a representative sample for four age groups were calculated with a total sample size of 1540 people. To accommodate for a 30% non-response rate the final sample size for the survey was 2002 people. The final sample size was further stratified into age groups (1-17, 18-34, 34-59 and 60+) in order to determine and compare age-specific sero-prevalence. One individual from a household was selected randomly for the study using a household registry. This was verified at the time of enrolment to the study at the site, using the data system used for the COVID-19 outbreak. Individuals were enrolled until the required sample was reached for each age group. Each selected individual was sent a text message and invited to collect their sample from the closest collection point. For elderly people who were bed ridden or found it difficult to visit the collection points, a health worker visited their household to do the survey and collect the sample.

Data was collected from sample collection sites set up in 4 locations. Informed consent was taken for each participant recruited and was asked to complete a questionnaire which covers demographic, clinical and exposure information. For minors, informed consent was sought from parents. The blood samples were collected by health care professionals with a license to collect blood. Three milliliters of whole blood samples were collected in plain tubes and serum were separated. All infection control measures required were complied with while conducting the study. The study was approved by the National Health Research Council.

## Serological testing

The serological tests were conducted at Hulhumale Hospital Laboratory, using the qualitative VITROS anti-SARS-CoV-2 IgG antibody in vitro diagnostic test on the automated VITROS 5600 Immunodiagnostic System. An Immunometric technique was used where spike protein S1 is targeted for confirmation of this test. Verification of the assay performance characteristics of the test kit performed by the CDC testing laboratory reported sensitivity = 90.0%, specificity 100.0% (10). A test's sensitivity can be estimated by determining whether or not it is able to detect antibodies in blood samples from patients who have been confirmed to have COVID-19 with a nucleic acid amplification test (NAAT). Prior to testing of the study samples, verification of the test kits, the VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total, was done using stored frozen serum samples which was previously tested with NAAT. Chemiluminescent immunoassay test intended for the qualitative measurement of total antibody (including IgG, IgA and IgM) to SARS-CoV-2 in human serum and plasma showed 100% compliance. For the result interpretation, a cut off values of 1.0 was used for anti-SARS-CoV-2 IgG antibody titre; reactive for antibody = 1.0 and above, non-reactive is below 1.0.

## Results

A total of 1940 people responded to the survey which gives a response rate of 96.9%. There were 57.8% males and 42.2% females in the study. Out of the samples tested, 13.4% were reactive for COVID-19 antibodies and 7% of the participants were tested positive for COVID-19 before the study. Majority of the participants (82.2%) were Maldivians and 17.8% were foreigners, consistent with the population proportion. The percentage of respondents in the three age categories, 0-17, 18-59 and 60+ were 21.6%, 74.3% and 4.1% respectively. **Table 1** shows the demographic information of the respondents who were reactive to antibodies and who were not reactive. There is a significant relationship between age group and respondents being reactive to COVID-19 antibodies. Similarly, there is a significant relationship between nationality and respondents being reactive to COVID-19 antibodies. Other factors that are related to being reactive to COVID-19 antibodies include, sex, occupation, having had symptoms for COVID-19 and being diagnosed with COVID-19. It must also be noted that in this study, 42.1% of the people who said they were tested positive for COVID-19 did not have antibodies (antibodies were below 1.0) when the serologic tests were done.

A multivariate logistic regression model was used to further analyse the factors that are associated with being exposed to COVID-19 in the past. As shown in **Table 2**, after adjusting for confounding factors. (Occupation and being a contact of COVID-19 positive case), the factors that are associated with antibody results include age, nationality, being diagnosed for COVID-19 and having symptoms of COVID-19. The results show that being a foreign national had a 13 times higher risk of being exposed to COVID-19 in the past than a Maldivian (OR: 12.9, CI: 8.3-19.7). A person being diagnosed with COVID-19 earlier were 25 times more likely to have reactive antibodies than people who did not know they were diagnosed for COVID-19 (OR: 24.7, CI: 15.9-38.4). Respondents who said that they had COVID-19-like symptoms earlier were two times more likely to have reactive antibodies for COVID-19 compared with people who did not have any symptoms (OR: 2.0, CI: 1.5-2.8). Compared with children under the age of 18 years, people of age group 18-59 years and people who are above 60 years were more likely to be exposed to COVID-19 before (OR: 4.0, CI: 1.7-9.0) and (OR: 1.4, CI: 1.4-5.6) respectively.

As described earlier, more than 40% of the respondents who were diagnosed for COVID-19 earlier, did not have antibodies (below 1.0) during the time of the survey. **Figure 1** is a survival analysis of antibody levels over a period of 250 days. As shown in **figure 1**, there is a gradual decrease in the antibody levels from 19 days to 200 days. The mean duration of the presence of antibodies in this study was found to be 124 days (CI: 109.8-138.2).

**Table 1. Demographic characteristics of people who are reactive to and non-reactive to COVID-19 antibodies**

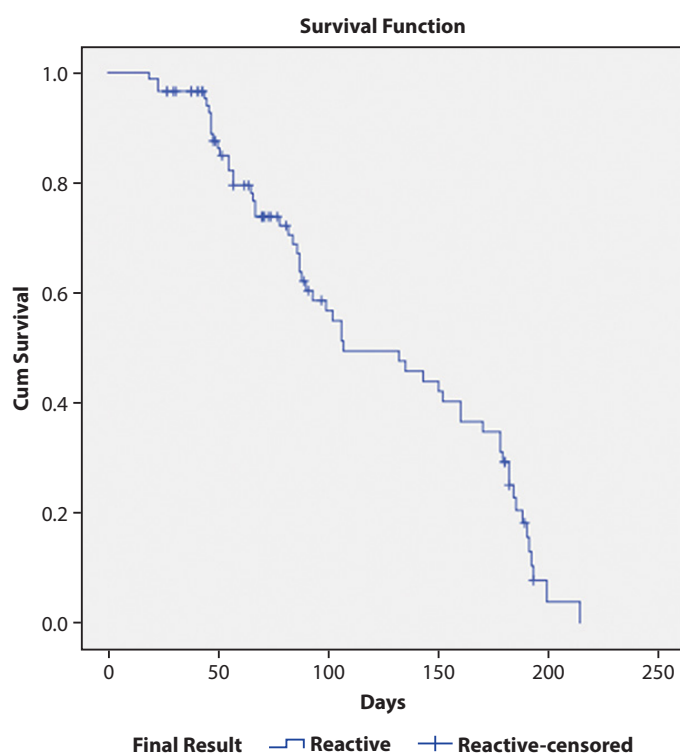
Demographic Information	Number/ Percentage of respondents who were reactive	Number/ Percentage of respondents who were non-reactive
<b>Age***</b>		
0-17 years	20 (4.9%)	390 (95.1%)
18-34 years	131 (13.9%)	814 (86.1%)
35-59 years	81 (18.0%)	370 (82.0%)
60+ years	20 (26%)	57 (74.0%)
<b>Nationality***</b>		
Maldivian	133 (8.5%)	1423 (91.5%)
Foreign	119 (33.6%)	208 (63.6%)
<b>Sex***</b>		
Male	68 (16.2%)	677 (83.8%)
Female	63 (9.5%)	655 (90.5%)
<b>Occupation***</b>		
Enforcement	6 (14.3%)	36 (85.7%)
Executive	1 (3.0%)	32 (97.0%)
Food business- restaurant, cafe, bakeries etc.	2 (18.2%)	9 (81.8%)
Health care worker	10 (9.9%)	91 (90.1%)
Home-based trader	1 (12.5%)	7 (87.5%)
Office support - admin/ clerical	10 (8.1%)	114 (91.9%)
Professional	18 (9.8%)	165 (90.2%)
Skilled worker - vocational	7 (11.9%)	52 (88.1%)
Student	22 (4.4%)	481 (95.6%)
Unskilled worker/labourer	108 (39.1%)	168 (60.9%)
Wholesale, retail	2 (4.5%)	42 (95.5%)
Other	65 (13.0%)	434 (87.0%)
<b>Diagnosed for COVID-19 before***</b>		
Yes	77 (57.9%)	56 (42.1%)
No	175 (10.0%)	1575 (90.0%)
<b>Has symptoms of COVID-19 before***</b>		
Yes	157 (18.4%)	694 (81.6%)
No	95 (9.2%)	937 (90.8%)

\*\*\* $p < 0.001$

**Table 2. Multivariate logistic regression of factors associated with being exposed to COVID-19 in the past.**

Variables	P-value	Adjusted OR	95% CI for EXP (B)	
			Lower	Upper
Sex (Female)	1			
Sex (Male)	0.6	1.11	.74	1.68
Nationality (Maldivians)				
Nationality (Foreigners)	< 0.001	13.08	8.53	20.05
Diagnosed (Not tested positive earlier)	1			
Diagnosed (Had tested positive earlier)	< 0.001	25.03	16.10	38.92
Symptoms (No symptoms)	1			
Symptoms (Had symptoms)	< 0.001	2.11	1.53	2.90
Age ≤ 17	1			
Age (18-34)	< 0.001	3.95	1.732	9.03
Age (35-59)	< 0.001	3.33	1.63	6.81
Age ≥ 60	< 0.05	2.06	1.00	4.28

-2 Log likelihood (deviance) 1061.80, df = 8 All variables in the final model were variables for which the change in deviance was significant compared with the corresponding  $\chi^2$  on the relevant degrees of freedom



**Means and Medians for Survival Time**

Final Result	Mean <sup>a</sup>				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
Reactive	124.020	7.246	109.817	138.222	107.000	21.764	64.343	149.657
Overall	124.020	7.246	109.817	138.222	107.000	21.764	64.343	149.657

<sup>a</sup> Estimation is limited to the largest survival time if it is censored.

**Figure 1. Survival Analysis curve for antibody level over a period of 250 days.**

## Discussion

This population-based study was conducted soon after the second wave of community transmission in Male' area in July-September 2020, and a high seroprevalence was expected than what was observed following the first wave. While the earlier study reported about 4% seroprevalence, the current study observed 13%. These findings compare with the findings of studies conducted in other countries where with the first wave seroprevalence was reported between 1-4%.<sup>11,12</sup> Since then, much higher seroprevalence are reported; 13% in London, 22% in Iran and 25% in Niger State.<sup>13,14,15</sup> Systematic review of seroprevalence studies observed that pooled estimates of seroprevalence in the general population was highest from four studies done in the India with 19.6%.<sup>16</sup> All these studies concluded that the spread of the infection was much higher than that was reported in the country. With the findings from current study the range of infections are estimated to be between 38-40,000 cases, five times higher than reported in the country. While there is no significant difference by gender as also observed in the systemic review of the studies, there were important differences in the seroprevalence of COVID-19 among locals and foreign residents and by age.<sup>16</sup>

The results show that being a foreign national had a 13 times higher risk of being exposed to COVID-19 than a Maldivian (OR: 12.9, CI: 8.3-19.7). Other studies have also reported ethnic differences in seroprevalence.<sup>13</sup> This higher risk can be attributed to the existing socio-economic inequalities that exists in the society. The large majority of the foreign migrant workers are labourers residing in overcrowded communal housing. Other studies in France and Singapore have observed that seropositivity for COVID-19 was strongly associated with overcrowding, and living in highly dense residential areas increase risk of exposure.<sup>17,18</sup> This proposition is supported in the Male' area where the first wave of COVID-19 was clustered where majority infected were foreign workers.<sup>19,20</sup> The waning of the antibody response in about 6-7 months observed in this study alongside the prolongation of the pandemic, places the foreign migrants at high risk of re-infection. With the start of the COVID-19 vaccination including migrants, a reduced risk is expected, but this needs to be closely scrutinized to maintain control of the epidemic in the country.

Compared with children under the age of 18 years, people of age group 18-59 years and people who are above 60 years were more likely to be exposed to COVID-19 before (OR: 4.0, CI: 1.7-9.0) and (OR: 1.4, CI: 1.4-5.6) respectively. Lower prevalence among children were observed in other countries in the early pandemic, possibly driven by the lowered risk of exposure with closure of schools and other movement restrictions during that period.<sup>7</sup> In the Maldives, at the time of the study schools were closed for physical classes and education was conducted online. Recent findings from a number of studies indicate that children have similar risk of infections.<sup>6,21</sup> Other studies have reported that children have milder diseases or are asymptomatic and the opening of schools for physically attended classes, is likely to increase the risk of exposure and onward transmission.<sup>22</sup>

The antibody response observed with regard to symptomatic and asymptomatic is consistent with findings reported in other studies. Respondents who said that they had COVID-19-like symptoms earlier were two times more likely to have reactive antibodies for COVID-19 compared with people who did not have any symptoms (OR: 2.0, CI: 1.5-2.8). Another study reported a significant positive correlation of antibody response with disease severity.<sup>23</sup> Other studies have observed that antibody response wanes more quickly in asymptomatic patients compared to those who experience severe symptoms.<sup>24,25</sup> Since a large proportion of the (40-45%) the infections reported in the country are asymptomatic cases, this has significance in predicting immunity of the population and risk of resurgence.<sup>26</sup>

As described earlier, more than 40% of the respondents who were diagnosed for COVID-19 earlier, did not have antibodies during the time of the study. There was a gradual decrease in the antibody levels from 19 days to 250 days. The mean duration of the presence of antibodies in this study was found to be 124 days (CI: 109.8-132.2). This is consistent with studies in other countries that have reported 4-6 months or 5-8 months of sustained antibody response following infection.<sup>27,28</sup> Historic experience of SARS-CoV2 infections indicate that immunity is sustained for longer period only in a small fraction of the infected.<sup>29</sup> A more recent study observed that about 20% of the seasonal human coronaviruses cross reacted with SARS-CoV2, but the antibodies produced from those did not produce protection against SARS-Cov2.<sup>30</sup> Determining survival of humoral immunity is important for epidemiological predictions of the disease in the population and planning the response.

The study has a few limitations; firstly, the participant selection was not stratified by locals and foreigners and secondly, the history of symptoms was collected from participants memory which may introduce some recall bias. Thirdly, the inherent limits of immunoassay test used might have affected the study results.

While the seroprevalence provides a measure that can be used to predict community transmission risk of the disease, the extent of functional immunity provided by antibody titres is still not clear. It is acknowledged that other mechanisms of protection such as T cell mediated immunity will play an important role in providing individual protection. Hence, the study is limited in its utility as a predictor of immunity. Despite this, it provides a yardstick for epidemiological analysis and estimation to inform public health decision.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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