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Seroprevalence of SARS-COV-2 Exposure among "High-Risk" Populations (Healthcare Workers, People Who Attend Markets, and School Children) in Zanzibar

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Abstract

In Zanzibar, from the start of the pandemic in March 2020 to the time of sampling in December 2020, SARS-CoV-2 seroprevalence data was limited. We conducted a seroprevalence study to evaluate the magnitude of SARS-CoV-2 exposure among healthcare workers, school children, and people who attended general markets in Zanzibar. The objectives of the study were to analyse the total antibodies from selected higher-risk population groups in order to determine magnitude in SARS CoV-2 exposure. Blood samples were collected from eligible and consented participants (adults and children), and their serum was analyzed for total antibodies against SARS-CoV-2 using ELISA. A questionnaire was used to collect participants' demographic and clinical data. The overall SARS-CoV-2 seroprevalence across all age groups was 33%, and a higher seroprevalence was observed in the 40 - 49 years' age group relative to other ages as well as in those who attended markets. A runny nose (18.8% of participants) was the most frequently reported SARS-CoV-2 infection-related symptom. Multivariable analysis showed significantly higher odds of infection in people living in urban districts. The findings provide insight into SARS-CoV-2 infection among school children, health workers,

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and people who attended markets in Zanzibar in the early stages of the pandemic. Exposure in these groups might have been influenced by infection and prevention strategies taken by the government, as well as shopping behavior, school overcrowding, and population density in urban settings. The study had methodological limitations, including cross-sectional design. Further, well-designed, longitudinal studies are recommended to understand exposure and transmission at a population level.

Keywords

SARS-CoV-2, Seroprevalence, Zanzibar, ELISA, COVID-19

1. Introduction

COVID-19, caused by the SARS-CoV-2 virus, was declared a pandemic on 11th March 2020 [1]. Like many other African countries, the first confirmed case of COVID-19 in Zanzibar was reported shortly after the pandemic declaration (19th March 2020 [2]). From the first reported case to the 7th May 2020, Zanzibar recorded a total of 134 confirmed cases before the Minister for Health declined to release further situation reports in early May 2020 [3]. Due to the suspension of COVID-19 situation reporting in Zanzibar and Tanzania as a whole, the actual burden of COVID-19 remained unknown until mid-2021 when the United Republic of Tanzania (URT) resumed reporting, including previously detected COVID-19 cases, resulting in 33,836 confirmed cases and a death toll of 803 by April 1, 2022 [4] [5].

The detection and spread of an emerging respiratory pathogen is accompanied by uncertainty regarding the key epidemiological and serologic characteristics of the novel pathogen particularly its transmissibility and its virulence [6]. For any outbreak or epidemic of emerging or re-emerging disease, surveillance is of paramount importance to inform decision-making on timely interventions and mitigation actions [7]. Initially, surveillance of SARS-CoV-2 globally focused primarily on patients with symptoms of severe disease, and, as such, the full spectrum of the disease, including the extent and prevalence of mild or asymptomatic infections that do not require medical attention was not clear [6] [8]. Good public health planning requires information on the full range of exposure and disease symptoms to assess the potential burden of the pandemic on the healthcare system and the collateral effects of any mitigation measures [9].

During the COVID-19 pandemic, Zanzibar, like other African countries, imposed community-based infection, prevention and control (IPC) measures to lower the trajectory of disease transmission. However, as part of URT, the measures taken were not stringent compared to other countries [3]. Zanzibar did not impose any form of official lockdown. Businesses remained open, and citizens were not restricted from attending religious gatherings or shopping in markets [3] [10]. Zanzibar, as part of URT, stopped releasing COVID-19 epidemiological data on April 29, 2020 and resumed sharing for the first time on June 28, 2021 [11]. During the period when data was not shared, the figures for disease testing, patient recoveries, active cases, and fatalities were based on epidemiological estimates and mathematical model predictions [12].

Seroprevalence studies estimate the prevalence of anti-SARS-CoV-2 antibodies in a group or population [13]. These studies are crucial to understand the overall extent of exposure, by demographic group, and geographic area, as well as to identify COVID-19 infection or disease in undiagnosed cases. Immunoassays using anti-SARS-CoV-2 antibodies offer a prediction of immune protection [14] [15], and seroprevalence studies can also be indicative of population levels of protection [13]. They are therefore important to inform scenario modeling, public health planning, and national policies in response to the pandemic [13]. Prior to this study, no COVID-19 seroepidemiological data had been recorded in Zanzibar. To obtain baseline data to plan for appropriate interventions, the government of Zanzibar requested a study investigating the of extent SARS-CoV-2 exposure among healthcare workers, people who attend markets, and school children. Healthcare facilities, markets, and schools are occupational settings described to have higher SARS-CoV-2 transmission [16] [17] [18] [19]. Therefore, a serosurveillance study was done to determine the SARS-CoV-2 seroprevalence from selected higher-risk settings (markets, hospitals, and schools) to determine differences in exposure.

2. Methods

2.1. Study Area

The study was carried out in the Zanzibar archipelago (Unguja and Pemba Islands). Zanzibar is part of the United Republic of Tanzania (URT) after the unification of Tanganyika and the islands of Zanzibar [20].

The Zanzibar Ministry of Health oversees all health-related matters within the islands [21]. In 2022, Zanzibar had a population of 1,889,773 people with 543,441 and 1,346,332 inhabitants from Pemba and Unguja islands, respectively [22]. Data and sample collection in our study were completed in eight districts (4 urban and 4 rural) of the Zanzibar archipelago (**Figure 1**).

2.2. Study Design and Population

A one-time, cross-sectional investigation was performed to evaluate the extent of exposure to the SARS-COV-2 virus in people in higher-risk settings. An evaluation of the literature suggested that people frequenting healthcare facilities, markets and schools had a higher risk of being exposed to SARS-CoV-2 [16] [17] [18] [19]. It was therefore decided by the Zanzibar Ministry of Health that these were priority settings to investigate in this study.

2.3. Timing of the Study

Demographic and clinical data were collected in December 2020. During this

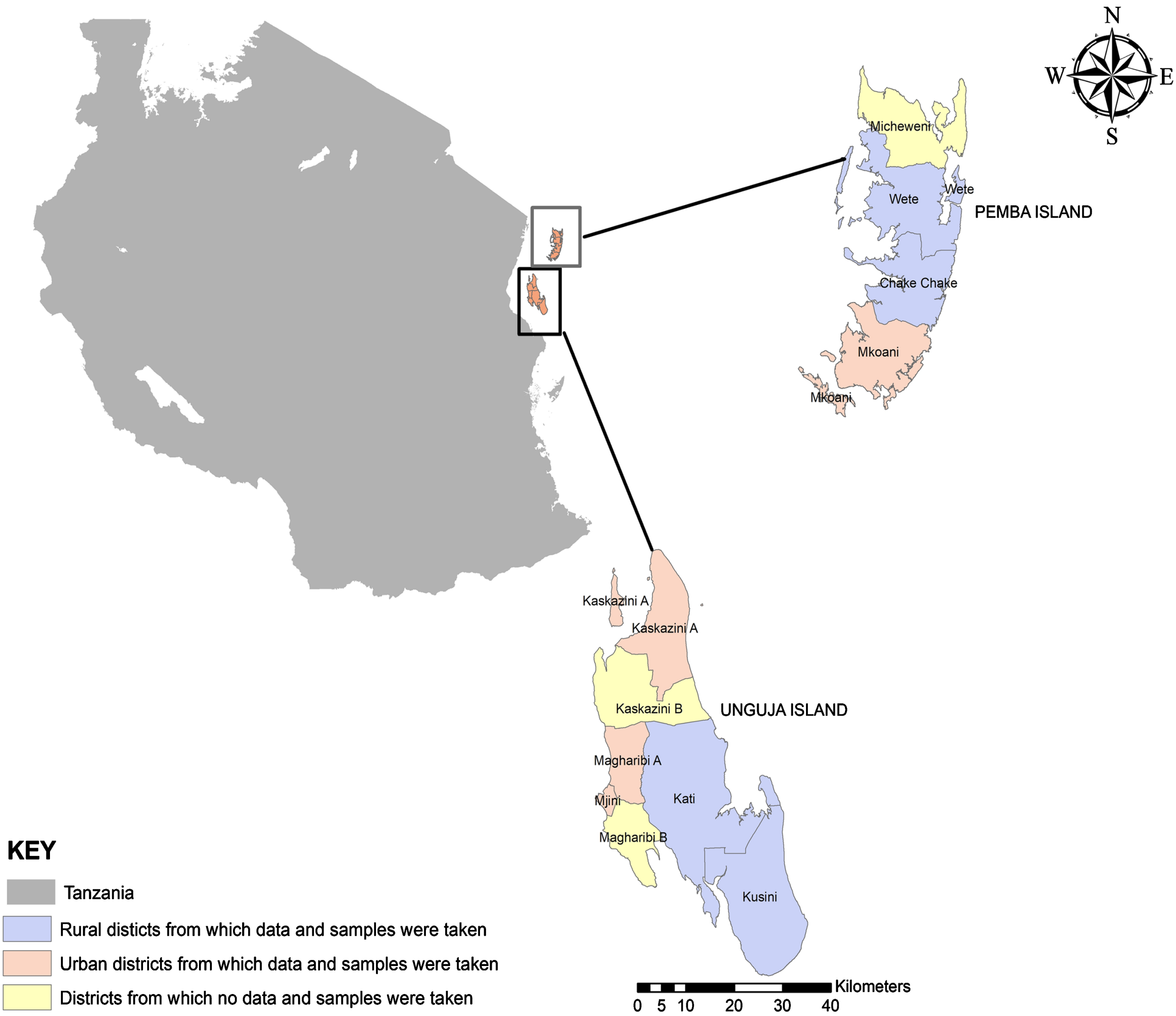

Figure 1. Map of the United Republic of Tanzania, indicating the position of the two islands of the Zanzibar archipelago and the districts (urban and rural) from which data and samples collection were done.

period, the COVID-19 situation report shared with WHO by the URT authority, indicated a total of 509 confirmed cases and 21 deaths [23]. From the date of the first case in Zanzibar to the date of sampling by this study, no research had been conducted to document the trend of SARS-CoV-2 exposure in the URT. However, evidence of community transmission had been documented in Zanzibar and an official statement of ongoing active transmission was proclaimed by the President [24] [25]. During this period, a report by WHO indicated a surge of COVID-19 cases and deaths in Africa due to new, more contagious variants of the SARS-CoV-2 virus spreading across many countries [26] [27]. The report further showed that infections rose by 50% on the continent between 29 December 2020 and 25 January 2021 when compared with the previous four weeks, indicating that our sampling was done during the peak of the second wave of community transmission [26] [27].

2.4. Sample Size

Assuming a prevalence of 50% for unknown disease prevalence, and a confidence level of 95% with a margin error of 5%, a convenient sample size of 720 participants was collected. Due to limited financial resources and time, the chosen sample size was sufficient for the study to collect useful data and information necessary for understanding the seroprevalence in selected high-risk settings at that particular time in Zanzibar [28]. The distribution of enrolled participants across the three different occupations and districts is indicated in **Figure 2**. Briefly, the participants recruited included 314 students (from 9 schools), 165 health workers (from 7 health facilities), and 241 market workers (from 6 markets), and were sampled from a total of eight districts in Unguja and Pemba islands. Participants from schools were sampled from seven districts, health workers from six and participants who attended markets were from five districts as shown in **Figure 2**.

2.5. Recruitment of Participants

At least one and a maximum of two schools (primary and/or secondary) were randomly selected in each of the eight districts. In schools, children above ten years were selected based on their availability and assent to participate in the study. The total number of recruited children per school was random and ranged from a minimum of 20 children to a maximum of 43 children.

Healthcare workers were recruited from randomly selected health facilities in six districts. Total number of health facilities ranged from a minimum of one to a maximum of two. The total number of participants recruited per health facility varied from a minimum of 10 to a maximum of 43 participants.

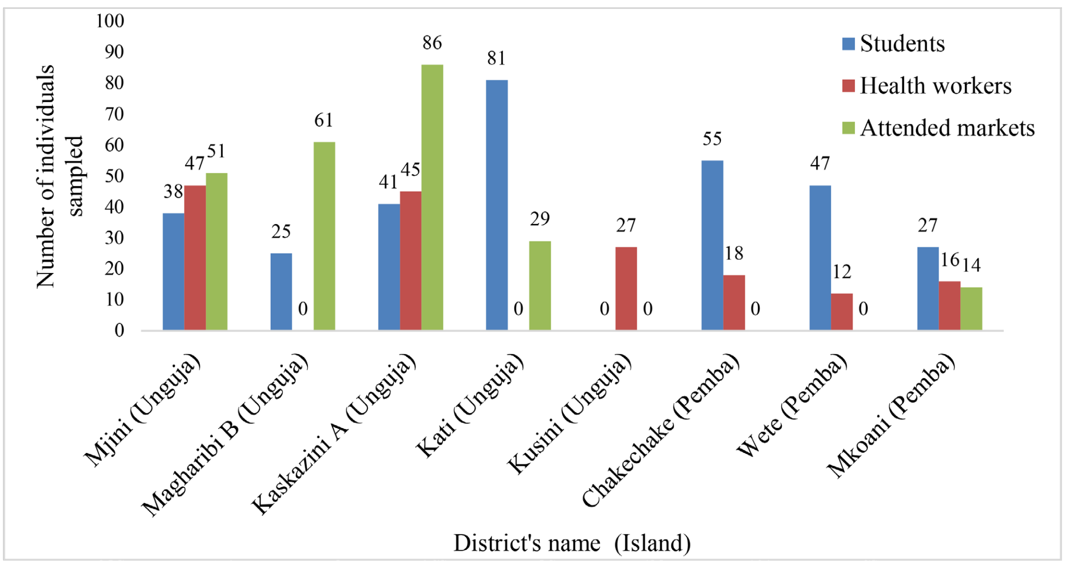

Figure 2. Distribution of sample size across districts and occupations from which participants were recruited during determination of SARS-CoV-2 seroprevalence in Zanzibar, December 2020. Students were sampled in higher numbers than individuals from other risk groups due to the larger student population and willingness to participate in the study.

Participants who attended markets (workers, sellers, and customers) from selected markets were approached and the aims of the study were explained. Market sampling was done in five districts and from each district, only one market was selected for sampling. Eligible participants were recruited, and the total number of participants per market from total of 27 to 97 participants. Selection of sampling sites was based on easy of accessibility and occupancy of larger number of people.

2.6. Demographic and COVID-19-Related Exposure and Symptoms Survey

A structured questionnaire was administered to each enrolled participant to document their demographic, medical history, and recent COVID-19-related exposure and symptoms in the last month. The questionnaire was translated into Kiswahili language, which is a native language in Zanzibar. The accuracy of the questionnaire was tested during training sessions run by the research team, and the survey was back-translated to English to ascertain the translation accuracy. During data collection, a paper-based data capture method was used whereby a trained interviewer administered the questionnaire to participants in the field. The interviewer read the questions to the participants and recorded the responses provided for each question. Data entries were cross-checked by the research team supervisor to ensure completeness before filling in the next question.

2.7. Serology Testing

5 ml of venous whole blood was collected into vacutainer tubes with EDTA anticoagulant from each of the enrolled participants. Samples were transported within 4 hours of collection to the Mnazi Mmoja referral hospital pathology laboratory and the Public Health Laboratory on Unguja and Pemba islands, respectively. Blood sample collection was performed by a trained and experienced phlebotomist and nurses. All samples were labeled using a unique participant identification number, which was linked to participant data in the questionnaires. Samples were centrifuged at 2000 × g for 10 minutes to separate the serum from the red blood cells and transferred into labeled cryogenic tubes. The tubes with serum were immediately stored in −80˚C freezers. Stored serum samples from the PHL laboratory in Pemba were later shipped to the Mnazi Mmoja pathology laboratory for analysis. All the serum samples were stored in duplicate.

Serological assays to detect SARS-CoV-2 total antibodies were done at Mnazi Mmoja referral hospital pathology laboratory. Briefly, total antibodies against SARS-CoV-2 from stored serum were detected using the WHO-recommended WANTAI SARS-CoV-2 Ab ELISA kit (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd., Beijing China) [6] [29] following the manufacturer's instructions [30]. Serum samples were considered negative (having no antibodies against SARS-CoV-2) when the ratio of absorbance (A) to cut-off (C.O.) (A/C.O.) was less than one and positive (having antibodies against SARS-CoV-2) when greater or equal to one. Samples that were borderline at the time of testing

(A/C.O. = 0.9 - 1.1) were retested in duplicate and considered borderline when the results retained the same ratio range [30].

2.8. Data Management and Statistical Analysis

Data management was done at the Zanzibar Health Research Institute (ZAHRI). Completed questionnaires were submitted to the data manager and checked for errors i.e., legibility, range of values, inconsistency, and missing identification numbers. All submitted questionnaires were entered into the database at ZAHRI using Microsoft Excel software, and data entry was completed by trained data entry staff. Data checks and cleaning of the database were done by the data manager, and only authorized personnel had access to the collected data.

Data were transferred to the statistical analysis package Stata version 14.0 (STATA Corp, Texas-USA) for cross-checking, further data cleaning, and analysis. Descriptive statistics were conducted and frequency and percentage for categorical variables and mean, standard deviation, range, minimum, and maximum for continuous variables were presented. Pearson Chi-square tests were used to compare group differences for categorical variables. A binary logistic regression model was used to obtain the Crude Odds Ratio (COR) and Adjusted Odd Ratio (AOR) with a 95% Confidence interval of the factors associated with COVID-19 infection Variables with a p-value of <0.05 in the bivariate analysis using Chi Square were included in the logistic regression analysis. All variables were checked for multi-collinearity before being included in the multivariate model, all tests were two-sided and the level of significance was set at 5%.

3. Results

A total of 720 participants were enrolled in the study comprising 545 and 175 participants from Unguja and Pemba islands, respectively. Out of the 720 enrolled participants, three participants gave consent but their blood samples were not collected due to unsuccessful venipuncture (n = 2) or failure to collect a good quality blood sample after three attempts of venipuncture (n = 1). 717 participants completed the questionnaire and had their blood collected for analysis. However, during laboratory analysis, 10 samples were excluded from testing due to insufficient volume of serum harvested (n = 7) or hemolysis (n = 3). 707 enrolled participants had a complete dataset that was eligible for analysis.

3.1. Participants' Demographic characteristics

The average age of participants was 29.0 (SD = ±15.7) years. Among the participants, 57.6% (n = 407) were male and 42.4% (n = 300) were female. Age ranges were stratified into 10-year groups; the 10 - 19 age group comprised the majority of the enrolled participants (38.8%, n = 274)), while the above 60 years' group presented the lowest enrollment (6.9%, n = 49 participants). Students presented with the highest enrollments (43.0%, n = 310), while the lowest enrollments were from health workers (23.3%, n = 65). 51.2% (n = 362) and 48.8% (n = 345)) participants were enrolled from rural and urban settings, respectively. Unguja Island presented with more enrollments than Pemba with 75% (n = 530) and 25% (n = 177), respectively (Table 1).

3.2. SARS-CoV-2 Seroprevalence

The overall seroprevalence amongst all groups was 33%. Table 1 shows the distribution of SARS-CoV-2 seropositivity based on different demographic and socioeconomic characteristics of the enrolled participants. SARS-CoV-2 seroprevalence was higher in males (35.4%) compared to females (30.3%), however, the difference was not significant (p = 0.06. When age groups were compared, there was a significant difference between the groups (p = 0.001), with people aged between 40 and 49 exhibiting the highest seroprevalence (40.6%). When occupations were compared, people who reported to engaged in business (trade) had a seroprevalence of 50%, which was significant compared to other occupations (p = 0.002). When "high-risk occupation" groups were compared, people recruited from markets showed the highest seroprevalence (42.9%), and the difference was significant compared to students and health workers (p = 0.001). The islands were also compared for rates of seroprevalence and no statistical difference was found (p = 0.17).

Table 1. Seroprevalence of SARS-CoV-2 antibodies based on participants' demographic and occupational characteristics in Zanzibar, December 2020. Seroprevalence was highest in males, people aged 40 - 49, students, urban areas and the district of Mkoani.

Demographic & occupational characteristics	Total N (%)	SARS-CoV-2 serological status			p-value
		Positive n (%)	Negative n (%)	Borderline zone n (%)	
Sex					
Male	407 (57.6)	144 (35.4%)	251 (61.7%)	12 (2.9%)	
Female	300 (42.4)	91 (30.3%)	206 (68.7%)	3 (1.0%)	0.057
Total	707 (100)	235 (33.2%)	457 (64.6%)	15 (2.1%)	
Age group					
10-19	274 (38.8)	74 (27.0%)	198 (72.3%)	2 (0.7%)	
20-29	154 (22.0)	58 (37.7%)	92 (59.7%)	4 (2.6%)	
30-39	101 (14.3)	37 (36.6%)	62 (61.4%)	2 (2.0%)	
40-49	69 (9.8)	28 (40.6%)	41 (59.4%)	0 (0.0%)	
50-59	60 (8.5)	23 (38.3%)	36 (60.0%)	1 (1.7%)	
60 or above	49 (6.9)	15 (30.6%)	28 (57.1%)	6 (12.2%)	0.001
Total	707 (100)	235 (33.2%)	457 (64.6%)	15 (2.1%)	
Occupation					
Agriculture	35 (5.0)	9 (25.7%)	24 (68.6%)	2 (5.7%)	
Health Care worker	141 (19.9)	40 (28.4%)	96 (68.1%)	5 (3.6%)	

Continued

Students	310 (43.9)	90 (29.0%)	218 (70.3%)	2 (0.6%)	
Business (Buying/selling	124 (17.5)	62 (50.0%)	59 (47.6%)	3 (2.4%)	
Fishermen	23 (3.3)	10 (38.1%)	12 (52.2%)	1 (4.4%)	
Skilled manual work	42 (5.9)	16 (38.1%)	25 (59.5%)	1 (2.4%)	
Other	32 (4.5)	8 (25.0%)	23 (71.9%)	1 (3.1%)	
Total	707 (100)	235 (33.2%)	457 (64.6%)	15 (2.1%)	0.002
Setting					
Rural	362 (51.2)	115 (31.8%)	237 (65.5%)	10 (2.8%)	
Urban	345 (48.8)	120 (34.8%)	220 (63.8%)	5 (1.4%)	0.368
Total	707 (100)	235 (33.2%)	457 (64.6%)	15 (2.1%)	
Districts					
Chakechake	70 (9.9)	15 (21.4%)	54 (77.1%)	1 (1.4%)	
Kaskazini A	173 (24.5)	48 (27.7%)	118 (68.2%)	7 (4.1%)	
Kati	107 (15.1)	35 (32.7%)	71 (66.4%)	1 (0.9%)	
Kusini	27 (3.8)	5 (18.5%)	20 (74.1%)	2 (7.4%)	
Magharibi B	78 (11.0)	28 (35.9%)	46 (59.0%)	4 (5.1%)	
Mjini	145 (20.5)	64 (44.1%)	81 (55.9%)	0 (0.0%)	
Mkoani	55 (7.8)	27 (49.1%)	28 (50.9%)	0 (0.0%)	
Wete	52 (7.3)	13 (33.2%)	39 (75.0%)	0 (0.0%)	0.001
Total	707 (100)	235 (33.2%)	457 (64.6%)	15 (2.1%)	
Occupation risk groups					
Students	304 (43.0)	88 (28.9%)	214 (70.4%)	2 (0.7%)	
Health care worker	165 (23.3)	45 (27.3%)	115 (69.7%)	5 (3.0%)	
Market attendees	238 (33.7)	102 (42.9%)	128 (53.8%)	8 (3.4%)	0.001
Total	707 (100)	235 (33.2%)	457 (64.6%)	15 (2.1%)	
Island					
Unguja	530 (75.0)	180 (34.0%)	336 (63.4%)	14 (2.6%)	
Pemba	177 (25.0)	55 (31.1%)	121 (68.4%)	1 (0.5%)	0.171
Total	707	235 (33.2%)	457 (64.6%)	15 (2.1%)	

3.3. Reported SARS-CoV-2 Related Symptom History

About 6.8% (n = 48) reported to have contacted a person with confirmed COVID-19 disease. A runny nose (18.8%) was the most frequently reported SARS-CoV-2 related symptom followed by 16.4% of participants experiencing a headache and/or a cough (13.9%). Table 2 summarizes the history of SARS-CoV-2 infection related symptoms in the past month as reported by enrolled participants. Out of 707 participants, 15.5% (n = 110) reported health outcome related

Table 2. Prevalence of SARS-CoV-2 infection-related symptoms and health outcome as reported by participants. The reported symptoms and health outcome were experienced by participants in the month prior to the survey, and runny nose was the most frequently reported symptom.

SARS-CoV-2 infection related symptoms history	Yes (%)	No (%)
Sore Throat	45 (6.4)	662 (93.6)
Runny Nose (Rhinorrhea)	133 (18.8)	574 (81.2)
Cough	98 (13.9)	609 (86.1)
Shortness of breath (Dyspnea)	19 (2.7)	688 (97.3)
Other respiratory symptoms	7 (1.0)	700 (99.0)
Chills	21 (3.0)	686 (97.0)
Vomiting	10 (1.4)	697 (98.6)
Nausea	26 (3.7)	681 (96.3)
Diarrhea	43 (6.1)	664 (93.9)
Headache	116 (16.4)	591 (83.6)
Rash	27 (3.8)	680 (96.2)
Conjunctivitis	9 (1.3)	698 (98.7)
Muscle aches	35 (4.9)	672 (95.1)
Joint aches (Myalgia)	42 (5.9)	665 (94.1)
Loss of appetite	28 (4.0)	679 (96.0)
Loss of smell (Anosmia)	10 (1.4)	697 (98.6)
Loss of Test (Ageusia)	13 (1.8)	694 (98.2)
Nose bleed	9 (1.3)	698 (98.7)
Fatigue	19 (2.7)	688 (97.3)
Seizures	8 (1.1)	699 (98.9)
Altered Consciousness	6 (0.9)	701 (99.1)
Other Neurological signs	10 (1.4)	697 (98.6)
Other symptoms	7 (1.0)	700 (99.0)
Participants' reported health outcome		
Sought medical attention	49 (6.9)	658 (93.1)
Missed school/work	26 (3.7)	681 (96.3)
Required hospitalization	35 (4.9)	672 (95.1)

to, 6.9% (n = 49) required medical attention, 3.7% (n = 26) failed to attend school/work while sick and 4.9% (n = 35) reported being hospitalized (Table 2).

3.4. Association between Reported Symptoms and SARS-CoV-2 Serological Results

Chi-square tests were used to establish the association between participants' reported symptoms and serological findings. 692 participants were included in the analysis (participants with borderline serologic results were excluded). We found that only chills were significantly associated with positive (p = 0.02) SARS-CoV-2 serology results. Other symptoms were not significantly associated with positive serological findings. Also when we established the association between SARS-CoV-2 health outcomes and serological findings, no significant associations were found (**Table 3**).

3.5. Multivariable Analysis of SARS-CoV-2 Exposure Risk Factors

Multivariable logistic regression analysis was done to evaluate the risk of SARS-CoV-2 exposure. Multiple factors considered to be important risk factors for infection were analyzed against positive serological findings. Individuals with borderline serological findings were not included in the analysis. In crude analysis, the age group between 20-29 years had 1.69 (95% CI 1.10 - 2.57, p = 0.016) times higher odds of having a positive SARS-CoV-2 serological result than 10 - 19-year-olds. However, when adjusted for other factors, no significant difference existed among age groups. Mkoani and Mjini districts, from Pemba and Unguja island, respectively, showed significantly higher odds of infection when compared to the reference district (**Table 4**). Other risk factors were not significantly associated with a positive SARS-CoV-2 serological result in both crude and adjusted analysis.

4. Discussion

The spread of infections in a population depends heavily on social interactions and population density, therefore, assessing the proportion of potentially protected individuals in populations with different levels of exposure is crucial [31] [32]. While the cumulative incidence of infection has been described for different population demographics such as age groups and ethnicity [33] [34], antibody detection, measurement, and characterization in individuals from occupations with high-risk of SARS-CoV-2 exposure due to frequent or high-risk social human interactions (e.g., supermarket and health worker) is critical for notifying target populations for implementing public health measures [35]. Demographic data allows public health professionals to study health disparities in a population [36]. From the 707 enrolled participants, more men were sampled from people who attended markets than women. More men enrolled in markets could reflect the cultural and religious influence of the inhabitants of Zanzibar. Based on guidelines within the Islam religion (about 98% of Zanzibar inhabitants are Muslims), men are obliged to go shopping and most of market sellers are male [37] [38]. In health facilities and schools, more females were enrolled in the study than men. While the number of employees and school children might be higher in a fever of males, high female enrollment could be due to female nature of general altruistic considerations after informed consent accounting for universal approach during informed consenting [39].

Table 3. Seroprevalence of SARS-CoV-2 antibodies in relation to reported symptoms and health outcomes in the month prior to the survey as reported by enrolled participants (n = 692). Participants with borderline serology results were not included in the analysis. Chills as a symptom were significantly associated with SARS-CoV-2 seropositivity.

SARS-CoV-2 symptom history	Total	SARS-CoV-2 serological status (n = 692)		*p*-Value
		Positive (%)	Negative (%)	
Contact with SARS-CoV-2 infected person	48 (6.8)	14 (29.2)	34 (70.8)	0.467
Sore Throat	45 (6.4)	17 (37.9)	28 (62.2)	0.576
Runny Nose (Rhinorrhea)	131 (18.5)	39 (29.8)	92 (70.2)	0.261
Cough	95 (13.4)	28 (29.5)	47 (70.5)	0.320
Shortness of breath (Dyspnea)	19 (2.7)	5 (26.3)	14 (73.7)	0.476
Other respiratory symptoms	7 (1.0)	1 (14.3)	6 (85.7)	0.433
Chills	21 (3.0)	12 (57.1)	9 (42.9)	0.023*
Vomiting	10 (1.4)	3 (30.0)	7 (70.0)	1.000
Nausea	26 (3.7)	10 (38.5)	16 (61.5)	0.621
Diarrhea	42 (5.9)	12 (30.9)	29 (69.1)	0.671
Headache	113 (16.0)	44 (38.9)	69 (61.1)	0.222
Rash	27 (3.8)	8 (29.6)	19 (70.4)	0.620
Conjunctivitis	9 (1.3)	3 (33.3)	6 (66.7)	1.000
Muscle aches	35 (5.0)	16 (45.7)	19 (54.3)	0.132
Joint aches (Myalgia)	42 (5.9)	16 (38.1)	26 (61.9)	0.559
Loss of appetite	28 (4.0)	10 (35.7)	18 (64.3)	0.841
Loss of smell (Anosmia)	10 (1.4)	4 (40.0)	6 (60.0)	0.685
Loss of Test (Ageusia)	13 (1.8)	6 (46.1)	7 (53.8)	0.349
Nose bleed	9 (1.3)	3 (33.3)	6 (66.7)	1.000
Fatigue	18 (2.5)	10 (55.6)	8 (44.4)	0.050
Seizures	8 (1.1)	2 (25.0)	6 (70.0)	0.723
Altered Consciousness	6 (0.8)	3 (50.0)	3 (50.0)	0.414
Other Neurological signs	10 (1.4)	3 (30.0)	7 (70.0)	1.000
Other symptoms	6 (0.8)	3 (50.0)	3 (50.0)	0.414
Participants' reported health outcome				
Seeking medical attention	47 (6.6)	16 (34.0)	31 (66.0)	0.614
Miss school/work	25 (3.5)	8 (32.0)	17 (68.0)	0.833
Required hospitalization	34 (4.8)	11 (32.4)	23 (67.6)	0.839

Table 4. Multivariable logistic regression analysis of risk factors associated with SARS-CoV-2 exposure and serology results from enrolled participants in Zanzibar.

Risk factor variables	n (%)	Crude Odds Ratio (COR)			Adjusted Odds Ratio (AOR)		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value
Age group							
10 - 19	274 (38.8)	1	-	-	1	-	-
20 - 29	154 (22.0)	1.69	1.10 - 2.57	0.016	1.77	0.84 - 3.71	0.131
30 - 39	101 (14.3)	1.60	0.98 - 2.60	0.060	1.45	0.57 - 3.70	0.428
40 - 49	69 (9.8)	1.82	1.05 - 3.17	0.032	1.76	0.66 - 4.69	0.255
50 - 59	60 (8.5)	1.71	0.95 - 3.07	0.074	2.03	0.71 - 5.79	0.181
60 or above	49 (6.9)	1.43	0.72 - 2.83	0.301	1.34	0.45 - 3.93	0.589
Occupation							
Agriculture	35 (5.0)	1	-	-	1	-	-
Health Care worker	141 (19.9)	1.11	0.47 - 2.60	0.808	1.38	0.35 - 5.48	0.642
Students	310 (43.9)	1.10	0.49 - 2.46	0.815	1.05	0.14 - 7.73	0.957
Business	124 (17.5)	2.80	1.20 - 6.52	0.017	2.38	0.96 - 5.92	0.059
Fishermen	23 (3.3)	2.22	0.71 - 6.92	0.168	1.72	0.49 - 5.93	0.392
Skilled manual work	42 (5.9)	1.71	0.63 - 4.59	0.290	1.44	0.51 - 4.13	0.489
Other	32 (4.5)	0.93	0.30 - 2.81	0.305	0.92	0.25 - 3.30	0.900
Chill in the past 1 month							
No	686 (97.0)	1	-	-	1	-	-
Yes	21 (3.0)	2.68	1.11 - 6.45	0.028	0.41	0.16 - 1.05	0.064
Occupation risk groups							
Students	304 (43.0)	1	-	-	1	-	-
Health Care Worker	165 (23.3)	0.95	0.62 - 1.45		0.48	0.05 - 3.94	0.495
Market	238 (33.7)	1.93	1.35 - 2.78		0.73	0.11 - 4.89	0.749
District							
Chakechake	70 (9.9)	1	-	-	1	-	-
Kaskazini A	173 (24.3)	1.46	075 - 2.84	0.26	1.13	0.54 - 2.34	0.737
Kati	107 (15.1)	1.77	0.88 - 3.57	0.109	1.58	0.75 - 3.33	0.221
Kusini	27 (3.8)	0.90	0.28 - 2.79	0.856	0.94	0.28 - 3.15	0.924
Magharibi B	78 (11.0)	2.19	1.04 - 4.59	0.038	1.36	0.60 - 3.08	0.456
Mjini	145 (20.6)	2.84	1.47 - 5.49	0.002	2.30	1.13 - 4.66	0.021
Mkoani	55 (7.8	3.47	1.59 - 7.56	0.002	2.82	1.26 - 6.34	0.012
Wete	52 (7.4)	1.20	0.51 - 2.80	0.674	1.19	0.50 - 2.81	0.690

As recommended by WHO, measuring the extent of SARS-CoV-2 seropositivity could help to understand the rate of disease transmission over time [40]. Our overall seroprevalence (33%) came from a population with a unique COVID-19 management approach compared to other neighboring countries. There were no stringent interventions and control measures taken by the Government of Tanzania compared to other neighboring countries, a factor that is likely to have impacted SARS-CoV-2 prevalence and transmission. A systematic review and meta-analysis study reported a pooled seroprevalence of anti-SARS-CoV-2 antibodies in Africa to be 22%, characterized by high heterogeneity, with central Africa having the highest seroprevalence (41%) compared to southern Africa (34%), west Africa (25%), north Africa (13%) and east Africa, respectively (12%) [31]. Generally, the main factor associated with this reported variation in seroprevalence is likely to be the time point at which the study sample was taken, study design, and testing methods across regions. However, other factors such as chance of selection, cultural practices, political decision-making, policies, mitigation efforts, health infrastructure and prevention/control measures and/or the effectiveness of the implementation of such measures as well as occupations might influence the seroprevalence and heterogeneity across Africa [31]. The high seroprevalence detected in our study relative to other research from East Africa might have been influenced by one or more of these factors. For example, the Tanzanian COVID-19 management approach did not put the country's economy on hold as the markets were open, people were allowed to go to work and no lockdown was declared, undermining the efficiency of containment efforts [3]. Instead, people were encouraged by government officials to use herbal medicine to cure the disease [3] [41].

While a global seroprevalence in specific populations ranged from 0.6% (neonates) to (59%) in individuals in assisted living and long-term care facilities [42], our findings revealed a higher SARS-CoV-2 seroprevalence of 42.9% (n = 102/238) from people who attended markets, followed by students 28.9% (n = 88/304) and health care workers 27.3% (n = 45/165). The higher prevalence in people who attend markets might have been influenced by the large number of people mixing indoors and having close contact during shopping [43] [44]. Similarly, school settings in Zanzibar are characterized by overcrowded classrooms [45] and therefore might have influenced the SARS-CoV-2 exposure. Overcrowding in schools as a risk factor from Zanzibar is in line with findings from Israel which reported a large COVID-19 outbreak in a high school 10 days after schools' reopening which was associated with crowded classes [46]. Also, transmission of SARS-CoV-2 in schools and clusters of diagnosed COVID-19 cases have been reported in all types of school settings (preschools, primary and secondary schools) elsewhere in the world [47] [48]. However, transmission in schools appears to be affected by how widespread the virus is in the broader community [48]. Most children do not develop symptoms when infected with the virus or will develop a very mild form of COVID-19 [47]. Also, some literature has shown that children can become infected and can spread the virus to other children and adults while they are infectious [49] [50] [51].

Working in healthcare settings is known to be a high risk not only for SARS-CoV-2 transmission but also for other infectious agents [52] [53]. Our findings revealed a lower seroprevalence in healthcare workers (27.3%) compared to other occupations deemed high risk. This may be due to Infection Prevention and Control (IPC) measures and access to Personal Protection Equipment put in place in health facilities during COVID-19, although identifying adherence to these measures is difficult, and thus no conclusions can be made without further studies into IPC adherence. Seroprevalence in health care workers in this study (27.3%) is slightly higher compared to the adjusted prevalence reported in Kenya (20.8%), Malawi (12.3%), and Uganda (21.1%) [54] [55]. The difference in seroprevalence might be due to differences in IPC measures imposed by the government authorities. A more detailed study into the effects of IPC and adherence to it would be needed to understand its impact on SARS-CoV-2 exposure in healthcare settings.

Multivariable analysis revealed an association between seropositivity and living in urban districts. People living in Mjini (Unguja) and Mkoani (Pemba) districts had significantly higher odds of SARS-COV-2 seropositivity with AOR = 2.30 (95% CI = 1.13 - 4.66) and AOR = 2.82 (95% CI = 1.26 - 6.34), respectively. Similar findings have been reported in a study from Mali where the study found an association between seropositivity and the urban community of Sotuba [56]. A cross-sectional cluster sample survey in Zambia, which looked at the prevalence of SARS-CoV-2 in six districts in July 2020, reported a similar finding in which prevalence was higher among people who resided in urban areas compared to rural areas [57]. Urban settlements in Zanzibar are characterized by higher densities and overcrowding of people and a higher number of informal settlements [58] [59], and therefore higher risk of SARS-CoV-2 transmission [60]. Urban settlements and informal settlements are risk factors for SARS-CoV-2 transmission based on the fact that SARS-CoV-2 is an airborne virus transmitted via inhalation [61]. Urban settlements are also characterized by high concentration and density of economic activities, movement of people, and goods [62]. The living conditions in informal settlements have space constraints, violence, and overcrowding making physical distancing and self-quarantine impractical, and the rapid spread of infection is therefore highly likely in these areas [63].

5. Conclusion

This seroprevalence study is the first to collect blood samples and analyze SARS-CoV-2 exposure among healthcare workers, school children, and people who attend markets in Zanzibar during the COVID-19 pandemic. Our findings highlight SARS-CoV-2 exposure in schools, markets, and healthcare facilities from the beginning of the pandemic in March 2020 to the time of sampling in December 2020. As the findings of this study cannot be generalized to the whole population, a larger, systematic, population-based serosurveillance study, encompassing prospective surveillance, is critically needed to enhance understanding of exposure and infections in the general population and monitor the trend of infections in Zanzibar. Serial serological surveys in fixed cohorts enable the study of the dynamics of antibodies against SARS-CoV-2 and the correlation of immune response over time [64]. It is also critical to conduct further studies in longitudinal cohorts in order to understand the dynamics of immune response to SARS-CoV-2 in the Zanzibar population.

6. Study Limitations

The study had some methodological limitations. We employed a one point cross sectional design. Based on the design, our findings might have encountered a temporal relationship between exposure and outcome because we simultaneously assessed the exposure (infection) and outcome (seropositivity). We recommend a future longitudinal study that will generate data to establish a true cause-and-effect relationship. Also, sample size calculation did not account for factors necessary for sample size distribution among risk groups. We used a convenient sample of 720 which were randomly distributed among risk groups and therefore during calculation we didn't account for 1) The effect size (usually the difference between 2 groups); 2) The population standard deviation; 3) The desired power of the experiment to detect the postulated effect. Therefore, our sample size might have suffered from under or over-representation of groups within the sample and unfortunately precluded detailed and robust statistical analysis. Also, we purposefully selected markets, health facilities, and schools as sampling sites as they were deemed 'high-risk' in previous literature. We didn't apply the random selection to select risk clusters out of other higher-risk occupations and sampling units. However, the findings from this study provide a pilot picture of SARS-CoV-2 exposure within the sampled groups in Zanzibar from the beginning of the pandemic to the time of sampling.

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Authors' Contributions

SM, AM, HM and MA designed, managed and supervised the study. EM analysed the data and SM drafted the manuscript. All authors contributed to the interpretation of results and critical review and revision of the manuscript, and have approved the final version.

Ethics Approval and Research Permit

The study received ethical approval with certificate Ref: NO. ZAHREC/03/DEC/2021/27 from the Zanzibar Health Research Ethics Committee and the Research permit was obtained from the second vice president's office. Briefly, participation in the study was voluntary and enrollment was done only for participants who fulfilled the inclusion criteria, voluntarily agreed to participate, and signed the written informed consent.

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Data Availability

The datasets collected and analyzed under this study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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