Bukti Korespondensi

Judul	Seroprevalence of SARS-CoV-2 and risk factors in Bantul Regency in March-April 2021, Yogyakarta, Indonesia
Jurnal	PLOS Global Public Health
Penulis	Riris Andono Ahmad, MD, MPH, PhD * Citra Indriani, MD, MPH Risalia Reni Arisanti, MD, MPH Ratih Oktri Nanda, MPH Yodi Mahendradhata, MD, MSc, PhD Tri Wibawa, MD, Sp.MK(K), PhD

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No	Tanggal	Perihal/Keterangan
1	9-May-2022	Submit manuskrip ke Plos Global Public Health
2	9-May-2022	Draft manuskrip submitted
3	30-Aug-2022	Korespondensi review 1
4	13-Oct-2022	Perbaikan manuskrip sebagai respon review 1
5	17-Jan-2023	Korespondensi review 2
6	29-Jan-2023	Perbaikan manuskrip sebagai respon review 2
7	7-Mar-2023	Korespondensi review 3
8	12-Apr-2023	Perbaikan manuskrip sebagai respon review 3
9	25-May-2023	Manuskrip accepted
10	26-May-2023	Korespondensi review 4 setelah accepted
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12	26-Jun-2023	Article published

Riwayat Korespondensi Manuskrip

Seroprevalence of SARS-CoV-2 and risk factors in Bantul Regency in March-April 2021, Yogyakarta, Indonesia

Correspondence Date	Letter	Recipient	Revision
Jun 05 2023 08:13AM	Formal Accept Letter (GPH)	Riris Andono Ahmad, MD, MPH, PhD	3
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Jun 02 2023 01:38PM	Prod - FAR Completed	Jay-ar Formentera Medes	
Jun 02 2023 01:40AM	FTC - FAR Editorial Task Reminder (pre-due)	Riris Andono Ahmad, MD, MPH, PhD	
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May 28 2023 01:31AM	FTC - FAR Editorial Task Reminder (pre-due)	Riris Andono Ahmad, MD, MPH, PhD	
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May 25 2023 04:23PM	Editor Decision - Provisional Accept (GPH)	Riris Andono Ahmad, MD, MPH, PhD	3
Apr 12 2023 12:30PM	Author Submits Revision Confirmation	Riris Andono Ahmad, MD, MPH, PhD	3
Apr 12 2023 12:25PM	PDF Built and Requires Approval	Riris Andono Ahmad, MD, MPH, PhD	3
Apr 06 2023 01:42AM	Author Revision Reminder - Pre-due	Riris Andono Ahmad, MD, MPH, PhD	2
Apr 05 2023 01:43AM	Author Revision Reminder - Due Tomorrow	Riris Andono Ahmad, MD, MPH, PhD	2
Mar 30 2023 01:39AM	Author Revision Reminder - Pre-due	Riris Andono Ahmad, MD, MPH, PhD	2
Mar 23 2023 01:34AM	Author Revision Reminder - Pre-due	Riris Andono Ahmad, MD, MPH, PhD	2
Mar 07 2023 08:50PM	Editor Decision - Minor Revision	Riris Andono Ahmad, MD, MPH, PhD	2
Jan 29 2023 10:27AM	Author Submits Revision Confirmation	Riris Andono Ahmad, MD, MPH, PhD	2
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Dec 29 2022 10:54PM	PDF Built and Requires Approval	Riris Andono Ahmad, MD, MPH, PhD	1
Dec 27 2022 07:30PM	Author Notice - Technical Check Failure	Riris Andono Ahmad, MD, MPH, PhD	1
Dec 22 2022 12:24AM	PDF Built and Requires Approval	Riris Andono Ahmad, MD, MPH, PhD	1
Dec 04 2022 11:10PM	Author Notice - Technical Check Failure	Riris Andono Ahmad, MD, MPH, PhD	1
Nov 23 2022 10:51AM	PDF Built and Requires Approval	Riris Andono Ahmad, MD, MPH, PhD	1
Oct 20 2022 10:48PM	Author Notice - Technical Check Failure	Riris Andono Ahmad, MD, MPH, PhD	1
Oct 13 2022 07:09AM	Author Submits Revision Confirmation	Riris Andono Ahmad, MD, MPH, PhD	1
Oct 13 2022 07:05AM	PDF Built and Requires Approval	Riris Andono Ahmad, MD, MPH, PhD	1
Oct 13 2022 06:43AM	PDF Built and Requires Approval	Riris Andono Ahmad, MD, MPH, PhD	1
Oct 13 2022 01:17AM	Author Revision Reminder - Due Tomorrow	Riris Andono Ahmad, MD, MPH, PhD	0
Oct 07 2022 01:16AM	Author Revision Reminder - Pre-due	Riris Andono Ahmad, MD, MPH, PhD	0
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Aug 30 2022 05:00PM	Editor Decision - Major Revision	Riris Andono Ahmad, MD, MPH, PhD	0
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May 14 2022 01:13AM	Automated Reminder - Submission SBTA	Riris Andono Ahmad, MD, MPH, PhD	0
May 10 2022 03:27PM	Author Notice - Technical Check Failure	Riris Andono Ahmad, MD, MPH, PhD	0
May 10 2022 03:26PM	Tech Check - Copyright Query	Riris Andono Ahmad, MD, MPH, PhD	0
May 09 2022 11:51AM	Author Submits New Manuscript Confirmation	Riris Andono Ahmad, MD, MPH, PhD	0
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May 09 2022 11:49AM	PDF Built and Requires Approval	Riris Andono Ahmad, MD, MPH, PhD	0

09 Mei 2022	Bukti Submit Manuskrip dan Draft Manuskrip

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Date: May 09 2022 11:51AM
To: "Riris Andono Ahmad" risandono.ahmad@gmail.com
From: "PLOS Global Public Health" globalpubhealth@plos.org
Subject: Submission Confirmation for %ARTICLE_TITLE%

PGPH-D-22-00793
Seroprevalence of SARS-CoV-2 and risk factors in Bantul Regency, Yogyakarta, Indonesia
PLOS Global Public Health

Dear Dr. Ahmad,

Thank you for submitting your manuscript entitled 'Seroprevalence of SARS-CoV-2 and risk factors in Bantul Regency, Yogyakarta, Indonesia' to PLOS Global Public Health. Your assigned manuscript number is PGPH-D-22-00793.

We will now begin processing your manuscript and may contact you if we require any further information. You will receive an update once your manuscript passes our in-house technical check; you can also check the status of your manuscript by logging into your account at https://www.editorialmanager.com/pgph/.

If you have any inquiries or other comments regarding this manuscript please contact globalpubhealth@plos.org.

Thank you for your support of PLOS Global Public Health.

Kind regards,
PLOS Global Public Health

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30 Agustus 2022	Korespondensi review 1

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Date: Aug 30 2022 05:00PM
To: "Riris Andono Ahmad" risandono.ahmad@gmail.com
cc: hannah.clapham@nus.edu.sg
From: "PLOS Global Public Health" globalpubhealth@plos.org
Subject: PLOS Global Public Health Decision: Revision required [PGPH-D-22-00793]

PGPH-D-22-00793
Seroprevalence of SARS-CoV-2 and risk factors in Bantul Regency, Yogyakarta, Indonesia
PLOS Global Public Health

Dear Dr. Ahmad,

Thank you for submitting your manuscript to PLOS Global Public Health. After careful consideration, we feel that it has merit but does not fully meet PLOS Global Public Health's publication criteria as it currently stands. Therefore, we invite you to submit a revised version of the manuscript that addresses the points raised during the review process.

Please submit your revised manuscript by Oct 14 2022 11:59PM. If you will need more time than this to complete your revisions, please reply to this message or contact the journal office at globalpubhealth@plos.org. When you're ready to submit your revision, log on to https://www.editorialmanager.com/pgph/ and select the 'Submissions Needing Revision' folder to locate your manuscript file.

Please include the following items when submitting your revised manuscript:

- A rebuttal letter that responds to each point raised by the editor and reviewer(s). You should upload this letter as a separate file labeled 'Response to Reviewers'.
- A marked-up copy of your manuscript that highlights changes made to the original version. You should upload this as a separate file labeled 'Revised Manuscript with Track Changes'.
- An unmarked version of your revised paper without tracked changes. You should upload this as a separate file labeled 'Manuscript'.

Guidelines for resubmitting your figure files are available below the reviewer comments at the end of this letter.

We look forward to receiving your revised manuscript.

Kind regards,

Hannah E. Clapham
Academic Editor
PLOS Global Public Health

Journal Requirements:

1. We have amended your Competing Interest statement to comply with journal style. We kindly ask that you double check the statement and let us know if anything is incorrect.

2. Please amend your detailed Financial Disclosure statement. This is published with the article. It must therefore be completed in full sentences and contain the exact wording you wish to be published.

a. State the initials, alongside each funding source, of each author to receive each grant.
b. State what role the funders took in the study. If the funders had no role in your study, please state: "The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript."
c. If any authors received a salary from any of your funders, please state which authors and which funders.

3. Please ensure that Funding Information and Financial Disclosure Statement are matched.

4. We ask that a manuscript source file is provided at Revision. Please upload your manuscript file as a .doc, .docx, .rtf or .tex with pdf.

5. We noticed that you regular figures 1,2, tables 1 -3 are the same with supplementary figures and tables. Please clarify if this figures should treated us regular figures/tables and not as a supporting information files. If yes, please remove the duplicates.

6. Please update the figures 1 and 2 captions and include the direct link for base map layer in the manuscript file.

7. Please include S1 Data in the Supporting information legends in the manuscript file.

8. In the online submission form, you indicated that "The data that support the findings of this study are available from the corresponding author upon reasonable request.". All PLOS journals now require all data underlying the findings described in their manuscript to be freely available to other researchers, either 1. In a public repository, 2. Within the manuscript itself, or 3. Uploaded as supplementary information.

This policy applies to all data except where public deposition would breach compliance with the protocol approved by your research ethics board. If your data cannot be made publicly available for ethical or legal reasons (e.g., public availability would compromise patient privacy), please explain your reasons by return email and your exemption request will be escalated to the editor for approval. Your exemption request will be handled independently and will not hold up the peer review process, but will need to be resolved should your manuscript be accepted for publication. One of the Editorial team will then be in touch if there are any issues.

5. Review Comments to the Author

Please use the space provided to explain your answers to the questions above. You may also include additional comments for the author, including concerns about dual publication, research ethics, or publication ethics. (Please upload your review as an attachment if it exceeds 20,000 characters)

Reviewer #1: Androno et al conducted a seroprevalence study in Bantul Regency, Yogyakarta, Indonesia among the general population between March and April 2021. Summary findings were that 31.1% of the population in Bantul Regency had evidence of prior infection with SARS-CoV-2, with significant differences observed by age group but not other characteristics.

How does difference in access to health services cause (non-significant) difference in seroprevalence between urban and semi-urban areas? I feel that this statement is not supported by any data and can be removed from the abstract.

Major comments
Methods
Sample size estimation- please simplify description to make it easier to understand. For example is 6% sampling error meant to be precision (±6 %)?. What is meant by cluster size was 11? Is this the number of individuals or homes in a cluster?
Please justify reason for excluding children <5 years old
Did the investigators conduct local validation of the Fine assay that they used? If so what were the sensitivity and specificity figures? And were the seroprevalence figures adjusted to take into account the sensitivity of the assay?
Results
I suggest that the first paragraph of the results be a simple description of the study population without going into factors that were associated with seropositivity, which can come later. This would also apply to table 1 which at the moment is both a description of the characteristics of study participants and results of testing for factors associated with seropositivity.
Lines 203-212. As currently drafted, these sentences are potentially misleading because none of these differences in seropositivity were statistically significant. Please consider revising appropriately
Table 3
The denominator number (132) could be placed at the top of the column to avoid repetition of the number in each row.
Discussion
Line 262-264: The comment about seropositivity being affected by access to health services needs to be fleshed out some more to make the link (if it exists) more obvious
Line 285: Obesity: a distiniction needs to be made between rsk factors for seropositivity and risk factors for severe disease for those who do get infected. As currently drafted this argument is very confusing.
Minor comments:
Please consider modifying the title of the manuscript to include the dates when the serosurvey was conducted.
Introduction- last sentence- please modify sentence to specify that the study looked at antibodies to SARS-CoV-2 and not SARS-CoV-2 itself.
General comment- language used is in several instances not very clear and grammar can certainly be improved.

Reviewer #2: The authors present the results of a seroprevalence survey of antibodies against SARS-CoV-2 in the Indonesian population. This report is relevant considering that there is a lack of serosurveys from this region.

Comments:
Introduction: it is necessary to include information about the behavior of the epidemic in the country, for example, how many peaks have taken place, in which months of 2020 and 2021, report the R0. A graph with this information would be very highly appreciated. Methods: data collection: present time/ duration of collection. Add a section of Serological Analyses, add information about the used test and their sensitivity and specificity. Please add a supplementary table with the number of households, segments and participants included and the response rate for each city. The Statistical analysis should be presented in detail and clearly and in accordance with the hierarchy of objectives. This section needs to be reformulated because it is not clear. Results: Include abbreviations for all tables. results of table 2 not introduced in the objective of the study. The results should be displayed according to the objectives (1: SARS-CoV-2 prevalence; 2: seropositive risk factors, 3: COVID-19 vaccine acceptance). According to third 3 objectives: display the results in a table or figure.

13 Oktober 2022	Perbaikan manuskrip sebagai respon review 1

PLOS Global Public Health

Seroprevalence of SARS-CoV-2 and risk factors in Bantul Regency in March-April 2021, Yogyakarta, Indonesia.

--Manuscript Draft--

Manuscript Number:	PGPH-D-22-00793R3
Article Type:	Research Article
Full Title:	Seroprevalence of SARS-CoV-2 and risk factors in Bantul Regency in March-April 2021, Yogyakarta, Indonesia.
Short Title:	Seroprevalence of SARS-CoV-2 in Bantul, Indonesia.
Corresponding Author:	Riris Andono Ahmad, MD, MPH, PhD Universitas Gadjah Mada Fakultas Kedokteran: Universitas Gadjah Mada Fakultas Kedokteran Kesehatan Masyarakat dan Keperawatan Yogjakarta, DIY INDONESIA
Order of Authors:	Riris Andono Ahmad, MD, MPH, PhD
	Citra Indriani, MD, MPH
	Risalia Reni Arisanti, MD, MPH
	Ratih Oktri Nanda, MPH
	Yodi Mahendradhata, MD, MSc, PhD
	Tri Wibawa, MD, Sp.MK(K), PhD
Keywords:	Indonesia; SARS-CoV-2; seroprevalence; urban; Semi-urban
Abstract:	COVID-19 case counts in Indonesia inevitably underestimate the true cumulative incidence of infection due to limited diagnostic test availability, barriers to testing accessibility and asymptomatic infections. Therefore, community-based serological data is essential for understanding the true prevalence of infections. This study aims to estimate the seroprevalence of SARS-CoV-2 infection and factors related to the seropositivity in Bantul Regency, Yogyakarta, Indonesia. A cross-sectional study involving 425 individuals in 40 clusters was conducted between March and April 2021. Participants were interviewed using an e-questionnaire developed in the Kobo toolbox to collect information on socio-demographic, COVID-19 suggestive symptoms, history of COVID-19 diagnosis and COVID-19 vaccination status. A venous blood sample was collected from each participant and tested for immunoglobulin G (Ig-G) SARS-CoV-2 antibody titers using the enzyme-linked immunosorbent assay (ELISA). Seroprevalence was 31.1% in the Bantul Regency: 34.2% in semi-urban and 29.9% in urban villages. Participants in the 55-64 age group demonstrated the highest seroprevalence (43.7%; p=0.00), with a higher risk compared to the other age group (aOR= 3.79; 95% CI, 1.46-9.85, p<0.05). Seroprevalence in the unvaccinated participants was 29.9%. Family clusters accounted for 10.6% of the total seropositive cases. No significant difference was observed between seropositivity status, preventive actions, and mobility. Higher seroprevalence in semi-urban rather than urban areas indicates a gap in health services access. Surveillance improvement through testing, tracing, and treatment, particularly in areas with lower access to health services, and more robust implementation of health protocols are necessary.
Opposed Reviewers:	
Additional Information:	
Question	**Response**

Financial Disclosure Enter a financial disclosure statement that	This research was funded by the Ministry of Research and Technology/National Research and Innovation Agency and Indonesia (BRIN) and the Indonesia Endowment Fund for Education (LPDP) of the Ministry of Finance of the Republic of Indonesia. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
describes the sources of funding for the work included in this submission and the role the funder(s) played. This includes grants and any commercial funding of the work or authors. This statement will be typeset if the manuscript is accepted for publication. *Please review the submission guidelines and the instructions link below for detailed requirements and guidance.*	
Competing Interests On behalf of all authors, disclose any competing interests that could be perceived to bias this work. This statement will be typeset if the manuscript is accepted for publication. *Please review the instructions link below and PLOS Global Public Health's competing interests policy to determine what information must be disclosed at submission.*	The authors have declared that no competing interests exist.

Data Availability Before publication, Authors are required to make fully available and without restriction all data underlying their findings. Please see our PLOS Data Policy page for detailed information on this policy. A **Data Availability Statement**, detailing where the data can be accessed, is required at first submission. Insert your Data Availability Statement in the box below. Please see the data reporting section of	The data supporting the findings of this study are available within the supplementary materials.
our submission guidelines for instructions on what you need to include in your Data Availability Statement. This statement will be typeset if the manuscript is accepted for publication. *PLOS allows rare exemptions to address legal and ethical concerns. If you have legal or ethical restrictions, please use the box below to detail these in full sentences for the Journal team to consider.*	

Dr. Catherine Kyobutungi

Editor-in-Chief

PLOS Global Public Health April 24, 2022
Dear Editor,

We wish to submit an original research article entitled **"Seroprevalence of SARS-CoV-2 and Risk Factors in Bantul Regency, Yogyakarta, Indonesia**" for consideration by PLOS Global Public Health.

The reported cases of COVID-19 in Indonesia inevitably underestimate the true cumulative incidence of infection due to limited barriers in testing and asymptomatic infections. The absence of a reasonable estimate of the number of infections poses a challenge in estimating this need. A seroprevalence survey involving more diverse groups of people among urban and semi-urban communities is necessary to grasp the overall picture of SARS-CoV-2 infection.

Therefore, this study aims to estimate the seroprevalence of COVID-19 and the risk factors in Bantul Regency, Yogyakarta.

This study indicates a discrepancy with COVID-19 confirmed cumulative incidence data reported in the same period, suggesting that silent transmission might have occurred within the community. Furthermore, a higher seroprevalence was observed in semi-urban areas than urban areas, indicating a gap in access to health services, especially in testing, tracing, and treating COVID-19 cases. This finding will be beneficial to assist policymakers in surveillance improvement, particularly in areas with lower access to health services.

We believe the findings presented in this study will be of interest to PLOS Global Public Health readers. Thus, we sincerely hope you would consider this paper for publication.

We confirm that this work is original and has not been published elsewhere, nor it is currently under consideration for publication elsewhere. We have no conflicts of interest to disclose.

Sincerely,
Riris Andono Ahmad, MD, MPH, PhD

Director of Center for Tropical Medicine
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Seroprevalence of SARS-CoV-2 and risk factors in Bantul Regency in March-April 2021, Yogyakarta, Indonesia

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Abstract

COVID-19 case counts in Indonesia inevitably underestimate the true cumulative incidence of infection due to limited diagnostic test availability, barriers to testing accessibility and asymptomatic infections. Therefore, community-based serological data is essential for understanding the true prevalence of infections. This study aims to estimate the seroprevalence of SARS-CoV-2 infection and factors related to the seropositivity in Bantul Regency, Yogyakarta, Indonesia. A cross-sectional study involving 425 individuals in 40 clusters was conducted between March and April 2021. Participants were interviewed using an e-questionnaire developed in the Kobo toolbox to collect information on socio-demographic, COVID-19 suggestive symptoms, history of COVID-19 diagnosis and COVID-19 vaccination status. A venous blood sample was collected from each participant and tested for immunoglobulin G (Ig-G) SARS-CoV-2 antibody titers using the enzyme-linked immunosorbent assay (ELISA). Seroprevalence was 31.1% in the Bantul Regency: 34.2% in semi-urban and 29.9% in urban villages. Participants in the 55-64 age group demonstrated the highest seroprevalence (43.7%; p=0.00), with a higher risk compared to the other age group (aOR= 3.79; 95% CI, 1.46-9.85, p<0.05). Seroprevalence in the unvaccinated participants was 29.9%. Family clusters accounted for 10.6% of the total seropositive cases. No significant difference was observed between seropositivity status, preventive actions, and mobility. Higher seroprevalence in semi-urban rather than urban areas indicates a gap in health services access. Surveillance improvement through testing, tracing, and treatment, particularly in areas with lower access to health services, and more robust implementation of health protocols are necessary.

Keywords: Indonesia; SARS-CoV-2; Seroprevalence; Urban; Semi-urban

Introduction

Coronavirus disease 2019 (COVID-19) is a respiratory illness caused by the newly discovered severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), leading to a global pandemic, including Indonesia. Since the first confirmed SARS-CoV-2 on March 2 2020 [1], Indonesia has experienced exponential growth of COVID-19 cases in 34 provinces. As of May 2 2021, the government of Indonesia reported 1.677.274 confirmed COVID-19 cases and 45.796 deaths [2] and became one of the countries with high cumulative and incidence cases of COVID-19. The number of COVID-19 cases in Indonesia has been

increasing rapidly since January, leading to the highest peak thus far, with provinces in Java making up 64% of the national cases. Two notable waves were observed from March 2020 to December 2021 **(Fig 1).** Based on the daily trend of COVID-19 cases, the first wave of COVID-19 cases was observed in January, with the highest number of reported cases reaching 14518 cases on January 16. After that, the cases fluctuated and reached the highest during the third wave in September 2022, with 56.757 recorded cases.

Fig 1. COVID-19 cases daily trend in Indonesia March 2020 – December 2021 (data source: https://ourworldindata.org/coronavirus/country/indonesia, accessed on October 4 2022)

However, these case counts inevitably underestimate the true cumulative incidence of infection because of limited diagnostic test availability, barriers to testing accessibility, and asymptomatic infections, not to mention underreporting [2]. As a consequence, the national prevalence of SARS-CoV-2 remains unknown.

The lack of clarity about the number of SARS-CoV-2 infections across Indonesia limits the Indonesian government's ability to plan appropriately, prepare and respond to this epidemic. Monitoring the incidence of newly diagnosed cases of severe COVID-19 and the case fatality rate is critical to address the demands on the healthcare system.

One of the epidemiological investigations used to determine the level of disease spread is to conduct a seroprevalence survey. According to the Centers for Disease Control and Prevention (CDC), this survey uses serological tests to detect antibodies in the blood, indicating an infection [3]. This test uses an enzyme-linked immunosorbent (ELISA) where the antigen used is purified SARS-CoV-2 S protein (without live virus). Population-based serological testing provides better estimates of the cumulative incidence of infection by complementing

diagnostic testing of acute illness and helping to inform the public health response to COVID-19. A seroprevalence study could also be a powerful tool to detect subclinical infections and improve policy-making in the country [4, 5]. 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 [2].

Seroprevalence varies geographically; the denser urban areas have higher seropositivity rates than rural areas [6]. A study in East Java, Indonesia, in the second semester of 2020 showed a higher prevalence in Surabaya (13.1%), which is an urban area, than in Jombang (9,9%), a rural area [7]. The epidemiological trend also implicates SARS-CoV-2 spread among rural communities only later in the epidemic wave [8, 9], which would require sound anticipatory interventions. A seroprevalence survey involving more diverse groups of people among urban and rural communities is necessary to grasp the overall picture of SARS-CoV-2 infection. Bantul Regency has become one area that contributes to many cases and leads to the high transmission of COVID-19 in the Yogyakarta Provinces. There was a lack of seroprevalence study during the time of the study. Therefore, this study aims to estimate the SARS-CoV-2 seroprevalence, seropositive risk factors, and COVID-19 vaccine acceptance in Bantul Regency, Yogyakarta.

Materials and methods

Study setting

Bantul Regency is located in the southern region of Yogyakarta Province, covering 506.85 km^2 **(Fig 2).** The regency consists of 17 sub-districts and 75 villages, 30% semi-urban.

Most adults are engaged in the non-formal sector, such as farming, trade and the service industry. [10]. The daily mobility of residents between districts to and from Bantul is high, which may increase the risk of disease transmission. [11].

Fig 2. Map of Bantul Regency, Special Region of Yogyakarta, Indonesia

Study design and sampling

A cross-sectional study was conducted from March to April 2021. The study population consisted of individuals who lived in the Bantul regency for at least six months, with 1,018,402 inhabitants. [10]. EpiInfo was used to calculate the sample size.

Sampling was determined using a multistage cluster random sample adopted from the WHO/EPI rapid survey. [12]. The calculated sample size was 414, which accounted for the estimated prevalence of 20%, 6% sampling error, a significance level of 0.05 with a design effect of 2 and a non-response rate of 15%. There were 40 clusters, with 11 participants per cluster. The cluster was based on the smallest administrative area called RT (*rukun tetangga* or household group). The RT consists of approximately 50-70 households.

We used systematic random sampling to select households at the selected cluster. In the selected households, we recruited all eligible household members, i.e. anyone who lived under the same building and resided in the study area for at least six months, with a minimum age of 5 years old.

Participants must meet the following inclusion criteria: residing in the study location at least six months before the survey commences, age of 5 years old or older, able to communicate verbally, give written consent to participate in the research or consent from parents/ guardians for respondents under 18 years old. Participants aged 13-17 were interviewed by their parents/guardians. However, for participants under 13 years old, interviews were conducted

with the parents. Meanwhile, the exclusion criteria include people with existing chronic illnesses (including immunocompromised individuals with a history of blood disorders and people with mental disorders.)

Data collection

A door-to-door visit was conducted to collect the primary data. Written informed consent was obtained from each study participant before data collection. Fifteen enumerators, including a phlebotomist, were involved in the data collection. Two supervisors were assigned to ensure the methodology and conduct the spot check. All study teams were involved in four training days regarding methods, data entry, phlebotomy and ethics in research.

Using an aseptic procedure, two millilitres of blood were drawn from a cubital vein with a disposable sterile syringe. The specimen was then kept in an EDTA tube. Before transportation, blood samples were placed in a cool box with an ice pack and transported within the same day to the laboratory. Blood samples were further stored in the refrigerator. Each participant's data included a unique identifier (barcode label) linked to their blood sample and data for tracking and confidentiality. The blood sample was transported, examined and stored in the Laboratory of Microbiology, Faculty of Medicine, Public Health, and Nursing UGM. Plasma was then tested for Ig-G anti-SARS-Cov2 procedures using (Human Anti-2019 n-CoV(N) IgG ELISA Kit V1.5 FineTest [13].

Risk factors information at household and individual levels were obtained using an electronic structured questionnaire developed in the KoBo toolbox [14, 15]. Variables included in the household questionnaire were socio-demographic information (age, gender, relationship with the head of household, and household income). Individual questionnaires were used to gather socio-demographic information (age, gender, highest education, occupation), COVID-19 vaccination status, previous diagnosis of COVID-19 and symptoms related to COVID-19

within the last six months, preventive actions taken, and mobility in the previous two weeks. The data manager validated data daily. A day after the data entry, the data manager sent the supervisor feedback and confirmation regarding completeness and data consistency.

Updated cumulative data on notified cases were obtained through the COVID-19 surveillance system conducted by Bantul District Health Office Bantul from March to April 2021. COVID-19 was defined as a symptomatic or asymptomatic person with a positive PCR result tested for SARS-CoV-2. Population numbers per sub-district were obtained through Bantul Statistical Bureau for calculating incidence per 1000 population [10]. In addition, data on urban-rural classification was obtained from the National Statistical Bureau [16].

Statistical analysis

Proportion and percentage describe the seroprevalence and socio-demographic characteristics of study participants. A map developed in ArcGIS spatially compared seroprevalence and incidence of notified cases by district and urban-rural status [17, 18]. Bivariate analysis was conducted to identify the association between presumed risk factors (age, gender, occupation, comorbidities, prevention taken and mobility over the last two weeks) and anti-SARS CoV-2 seropositivity. After considering collinearity, a multiple logistic regression was used to evaluate risks between seropositive and seronegative groups with adjustment for sex. The P-value would be considered statistically significant at $p < 0.05$. All statistical analysis was performed using STATA 14.0.

Ethics

The Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada approved the study (Ref: KE/1242/12/2020).

Written informed consent was obtained from adult respondents and parents of enrolled children. Confidentiality of information from the respondents was upheld with utmost care throughout data collection, processing and analysis for all data collected. Therefore, their names were included in the notes only for traceability and referral during the data analysis.

Results

Characteristics of study participants

A total of 425 people participated and were tested during the survey. The number of females (59.1%) is higher than that of males (40.9%); 47.2% of the participants were unemployed/students/housewives, and 72.5% lived in urban areas. The majority of the participants had no comorbidities and were not vaccinated.

The prevalence of SARS-CoV-2 seropositivity among the participants in this study was 31.1% (n=132/425). A significant difference was observed in the seroprevalence among age groups (p=0.000), with the highest proportion reported in the 55-64 age group (43.7%; n=31/71). Meanwhile, the under-15 years age group showed no seropositivity.

A significant difference was also observed among occupation groups (p=0.009). The highest seroprevalence was demonstrated by participants working as daily workers/farmers (37.2%), followed by professional/health workers (34.6%), and unemployed/students/housewives (26%). Seroprevalence did not differ between semi-urban and urban areas, even though we observed that semi-urban areas had higher seroprevalence (34.2%; n=40/117) than urban areas (29.9%; n=92/308). Females demonstrated higher seroprevalence; nevertheless, no association was found between genders and seropositivity. Seropositivity also does not differ in occupation category. (Table 1).

201 **Table 1. Distribution of Study Participants by Background Characteristics.**

Characteristic	Total	IgG-positive	IgG-negative	P-value
	n = 425	n=132	n=293	
Sex, n (%)				
Male	174 (40.9)	52 (29.9)	122 (70.1)	
Female	251 (59.1)	80 (31.9)	171 (68.1)	0.663
Age group, years, n (%)				
<=14	16 (3.8)	0 (0.0)	16 (100)	
15-24	52 (12.2)	8 (15.4)	44(84.6)	
25-34	51 (12.0)	8 (15.7)	43(84.3)	
35-44	90 (21.2)	33 (36.7)	57(63.3)	
45-54	92 (21.7)	32 (34.8)	60 (65.2)	
55-64	71 (16.7)	31 (43.7)	40 (56.3)	
65+	53 (12.5)	20 (37.7)	33 (62.3)	< 0.001
Occupation, n (%)				
Unemployed/students/Housewives	200 (47.2)	52 (26.0)	148 (74.0)	
Professional/health worker	130 (30.7)	45 (34.6)	85 (65.3)	0.009
Daily worker/farmer	94 (22.2)	35 (37.2)	59 (62.8)	
Residence set, n (%)				
Urban	308 (72.5)	92 (29.9)	216 (70.1)	
Semi-urban area	117 (27.5)	40 (34.2)	77 (65.8)	0.390
Smoking, n (%)				
Smoker	74 (18.1)	14 (18.9)	60(81.1)	
Non-smoker	335 (81.9)	118 (35.2)	217(64.8)	0.007
History of chronic disease, n (%)				
Yes	96 (22.6)	37 (38.5)	59 (61.5)	
No	329 (77.4)	95 (28.9)	234 (71.1)	0.072
Diabetes mellitus, n (%)				
Yes	15 (3.5)	6 (40.0)	9 (60.0)	
No	394 (92.5)	126 (32.0)	268 (68.0)	0.514
Hypertension, n (%)				

Yes	65 (15.9)	23 (35.4)	42 (64.6)	
No	344 (84.1)	109 (31.7)	235 (68.1)	0.559
Obesity, n (%)				
Yes	8 (1.9)	4 (50)	4 (50)	
No	401 (98.1)	128 (31.9)	273 (68.1)	0.279
Previous COVID-19 diagnosis, n (%)				
Yes	3 (0.7)	1 (33.3)	2 (66.7)	
No	422 (99.3)	131 (31.0)	291 (68.9)	1.000
COVID-19 symptoms n (%)				
Yes	155 (36.4)	44 (28.4)	111 (71.6)	
No	270 (63.4)	88 (32.6)	182 (67.4)	0.4
COVID-19 vaccination, n (%)				
Yes, at least one dosage	13 (3.1)	9 (69.2)	4 (30.8)	
Not yet	412 (96.9)	123 (29.9)	289 (70.2)	0.005
Preventive measures and mobility				
Wearing masks when going out				
Always	380 (89.4)	116 (30.5)	264 (69.5)	0.491
Not always	45 (10.6)	16 (35.6)	29 (64.4)	
Washing hands for at least 20 seconds with running water				
Always	344 (80.9)	106 (30.8)	238 (69.2)	0.822
Not always	81 (19.1)	26 (32.1)	55 (67.9)	
Maintain a physical distancing (1-2m) in the public area				
Always	274 (64.5)	82 (29.9)	192 (70.1)	0.497
Not always	151 (35.5)	50 (33.1)	101 (66.9)	
Mobility				
Attending invitations to traditional or religious activities (e.g. weddings, funerals)				
Always	39 (9.2)	14 (35.9)	25 (64.5)	0.493
Not always	386 (90.8)	118 (30.6)	268 (69.4)	
Visiting relatives or friends or other people due to important matters				
Always	42 (9.9)	13 (30.9)	29 (69.1)	0.987
Not always	383 (90.1)	119 (31.1)	264 (68.9)	
Going to the markets/shops/offices/crowds				

Always	166 (39.1)	50 (30.1)	116 (69.9)	0.738
Not always	259 (60.9)	82 (31.7)	177 (68.3)	
Staying at home, except for essential matters				
Always	313 (73.7)	94 (30.1)	219 (69.9)	0.444
Not always	112 (26.3)	38 (33.9)	74 66.1)	

This study also explored seroprevalence based on the presence of selected chronic diseases and the history of comorbidities. Participants with a history of chronic conditions accounted for 22.6% of the total participants. Among those with comorbidities, seroprevalence for participants with obesity, diabetes mellitus, and hypertension was 50%, 40% and 35.4%, respectively.

Participants with a prior diagnosis of COVID-19 in the last six months accounted for 1 out of 132 seropositive cases. Seroprevalence among participants with no previous experience of COVID-19-related symptoms was found to be 31.1%. Seropositivity was observed in 14 participants from seven households, making family clusters account for 10.6% of the total positive cases.

Most of the participants in this study reported decreased social interactions and mobility during this period. Study participants stated that they attended fewer traditional and religious activities (90.8%), had reduced visits to relatives and friends (90.1%, decreased visits to markets (60.9%), and spent more time at home, except for essential tasks ((73.7%). If participants did leave home, they reported wearing masks (89.4%), regular washing of hands for 20 seconds with running water (80.9%) and maintaining physical distancing in public areas (64.5%). However, no significant differences were observed between individual preventive actions and the level of mobility with seropositivity status.

Seroprevalence of anti-SARS-CoV-2 based on the geographical distribution

The highest seroprevalence was observed in 3 semi-urban areas (Pajangan, Dlingo, Sanden). Most semi-urban areas have relatively higher seroprevalence than urban areas. However, we found no statistical significance. **(Fig 3).** The highest distribution of confirmed cases acquired from routine regional data reported higher cumulative cases in urban areas, such as Banguntapan, Bantul, Sewon, and Jetis.

Fig 3. Seroprevalence and Reported Cumulative Incidence of SARS-Cov-2 in April 2021 Based on District.

Risk factors associated with SARS-CoV-2 seropositivity

This study further explored the risk factors associated with the seropositivity of SARS-CoV-2, adjusting for age, sex, occupation, comorbidities, and vaccination status. A significant association was observed within specific age groups. The odds of SARS-CoV-2 seropositivity are higher in the age 55-64 (adjusted odds ratio [aOR]=3.79; 95% CI 1.46-9.85, p=0.006).

Females demonstrated higher seroprevalence. Nevertheless, no association was found between genders and seropositivity. Seropositivity also does not differ in occupation category (aOR=0.81; 95% CI 0.50-1.31, p>0.005) (Table 2).

Table 2. Factors Associated with SARS-Cov-2 Seropositivity in Bantul Regency (N=425).

Risk Factors	**ELISA+ (n=132)**	**Adjusted OR (95% CI)**	***p*-value**
Age group			
<=14	0	NA	NA
15-24	8	Reference	NA
25-34	8	0.93 (0.31-2.79)	0.893
35-44	33	2.71 (1.08-6.81)	0.034
45-54	32	2.67 (1.05-6.80)	0.040
55-64	31	3.79 (1.46-9.85)	0.006
65+	20	3.38 (1.27-9.00)	0.015
Sex			
Male	52	0.81 (0.50-1.31)	0.388
Female	80	Reference	NA
Occupation			
Unemployed/students/Housewives	52	Reference	NA
Professional/health worker	45	1.14 (0.64-2.00)	0.661
Daily worker/farmer	35	1.27 (0.70-2.31)	0.441
Comorbidities			
Hypertension	23	0.87 (0.48-1.57)	0.646
Obesity	4	2.46 (0.57-10.65)	0.229
Diabetes Mellitus	6	1.12 (0.38-3.31)	0.832
Vaccination Status			
Yes	14	Reference	NA
No	13	0.19 (0.05-0.68)	0.010

Comorbidities were presumed as one of the risk factors of SARS-CoV-2 seropositivity. The odds of SARS-CoV-2 seropositivity were higher in participants with obesity (aOR=2.46; 95% CI 0.57-10.65) and diabetes mellitus (aOR=1.12; 95% CI 0.38-3.31) than participants without each respective comorbidity. However, this finding is not statistically significant (p-value>0.05).

Although vaccination had not been made available to the general public during the data collection period, most participants (91.5%) were aware that the government would provide vaccinations in the future and stated their willingness (79.1%) to be vaccinated. However, a small proportion (19.1%) said they did not want to be vaccinated. Reasons for this included fear of adverse effects, concerns about the safety and effectiveness of the vaccines, and religious beliefs.

Table 3. Vaccine acceptance of the participants (N=425)

Variables	N = 425	Percentage
Aware that the government would provide the vaccination for the people		
Yes	389	91.5
No	36	8.5
Willing to get vaccinated if the government provide the vaccines		
Yes	336	79.1
No	81	19.1
Not decided	8	1.9

Discussion

This SARS-CoV-2 seroprevalence study found that the prevalence of IgG antibodies against SARS-CoV-2 was 31.1% in Bantul, significantly higher than the 11.4% seropositive which was found in a study in East Java in late 2020 [7]. On the other hand, surveillance data

of Bantul Regency DHO reported a considerably lower cumulative incidence of 1.1% during the study. This finding is in line with previous research suggesting that the estimates obtained from seroprevalence were 18.1 times higher than the corresponding cumulative incidence of COVID-19 infections, implying that confirmed cases are a poor indicator of the extent of the disease spread [19]. While antigen testing and Polymerase Chain Reaction (PCR) to monitor COVID-19 detect the presence of specific antigens or genetic material indicating current viral infection, serological tests for COVID-19 are used to detect antibodies that the body produces in response to the virus. Serological tests can detect antibodies in people who have been infected with COVID-19 in the past, even if they were asymptomatic or had mild symptoms. Those can help identify people who may have been infected but were never diagnosed. In population-level studies, serological testing is used to estimate the proportion of people who have been infected with COVID-19 in a given area.

Seroprevalence varies geographically, and previous research indicated that urban areas mainly reported a higher number of seropositivity [6]. The reported COVID-19 cases in the Bantul regency also showed a similar trend, in which more cases were reported in urban areas (Bantul, Banguntapan, Sewon, and Jetis). However, our study showed that the three highest seropositivities were observed in semi-urban areas (Pajangan, Dlingo and Sanden). Meanwhile, areas with the highest reported COVID-19 cases showed lower seropositivity. It suggests that the semi-urban areas were underreported and underdiagnosed due to access to diagnosis. These discrepancies can be explained by the limited and centralized diagnosis facilities when the study was conducted.

Urban areas with dense populations were impacted earlier at the pandemic's beginning. A previous study on epidemiological modelling predicted that COVID-19 spread faster in urban than rural areas [20]. Despite the rapid transmission, urban areas are usually supported

283 by better surveillance management, such as more accessible testing, tracing, and infection
284 control. Consequently, cases in urban areas were reported faster and earlier.

285 Meanwhile, rural areas usually have shortages in resources and workforces, which
286 affects their ability to detect, respond, prevent, and control infectious disease outbreaks [21,
287 22]. In this study, a large proportion of seropositivity was reported in participants with no prior
288 diagnosis of COVID-19, indicating that the infection might be undiagnosed due to a lack of
289 individual testing. Thus, this study suggests that the higher transmission observed in semi-
290 urban areas was lower access to health services, including lack of individual testing, resulting
291 in unreported positive cases.

292 Higher seroprevalence in this study was observed in females. However, no statistically
293 significant gender difference was reported. A previous study also suggested that SARS-CoV-
294 2 IgG/IgM dynamic is mainly affected by age and disease severity, not sex [23]. Higher
295 seropositivity was observed in the age group 55-64, and this finding supports many previous
296 studies that indicate older age as one of the risk factors for COVID-19.

297 Subsequently, this research found a portion of seropositivity among people who
298 frequently stayed home. Additionally, occupations requiring less mobility, such as housewives,
299 students, and the unemployed, also accounted for seropositivity. The research also discovered
300 a tenth of family clusters, suggesting that the transmission may have occurred at the household
301 level. Previous research contends that the primary mode of COVID-19 infection is through the
302 household spread. [24, 25].

303 Our study found no difference between high and low mobility, suggesting they were
304 already widespread community transmission, even in rural areas. Preventive measures did not
305 prevent getting an infection, which might be because it is challenging to do preventive
306 measures in household settings or practice preventive measures consistently outside. [26]

Surveillance and containment measures such as large-scale social restrictions and other anticipatory prevention tools become a priority to curb transmission, primarily focusing on areas with lower access to health services. Earlier in the pandemic, outbreak containment was concentrated in the dense urban areas, which may have reduced the number of cases. However, due to high mobility, the transmission slowly moved to semi-urban and rural communities. People living in rural communities might develop a false sense of security and take fewer precautions at the beginning than the urban communities [8,9].

One of the effective ways to prevent the fatalities caused by COVID-19 is through vaccination. Due to no vaccination programme being rolled out to the general population during the data collection period, it is reasonable that higher seroprevalence was found in unvaccinated groups. As this study explored the seroprevalence when most people were still unvaccinated, a better picture of the infection spread in Bantul was obtained since there was no implication from vaccine-induced antibodies.

Despite the findings, this study is not without limitations. Following a natural infection, antibody titers peak and begin to wane in various manners, with some in shorter duration [27]. However, this study did not consider the assay performance concerning the waning immunity. Thus it may underestimate the true prevalence. Furthermore, the analysis results only showed the association, not causality. Finally, as a cross-sectional study, this study only analyzed the variables at once and did not explore the seroprevalence changes over time. Therefore, future research is needed to conduct a periodic or longitudinal survey to determine the prevalence in the longer term.

Conclusion

This serosurvey demonstrated a higher seroprevalence than reported data in the same period. Based on the findings, it is strongly recommended that the local government strengthen

the surveillance and 3T (testing, tracing, and treatment) efforts by involving the task force at the neighbourhood community and village levels throughout Bantul Regency, particularly in areas with lower access to health services. Besides that, it needs to increase awareness and implementation of health protocols for high-mobility individuals to prevent transmission within the household. As the vaccination for the general population is being rolled out, it is crucial to provide adequate implementation information, including health resources and logistics. This study can be implemented in other areas, both at the district/city and provincial levels, to understand the seroprevalence of SARS-CoV-2 in Indonesia better. This study provides a district-level view of the extent of COVID-19 spread and a different approach to conducting serosurvey among diverse populations in various regions to fit the gaps in understanding COVID-19's spread globally.

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Conflict of interest

The authors declare no conflict of interest.

Supporting information

S1 Table. The response rate of each district.

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Figure 1

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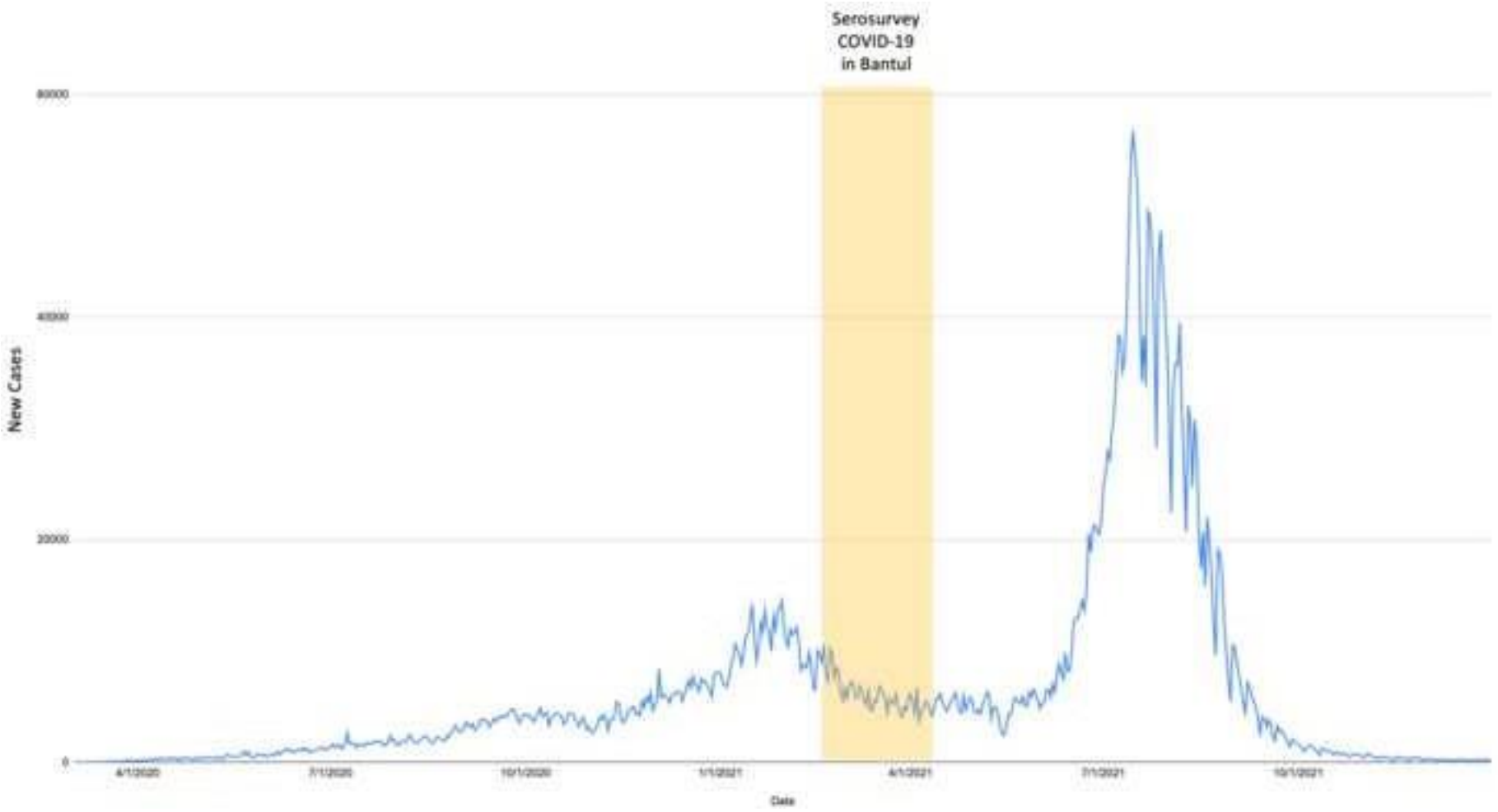

Figure 2

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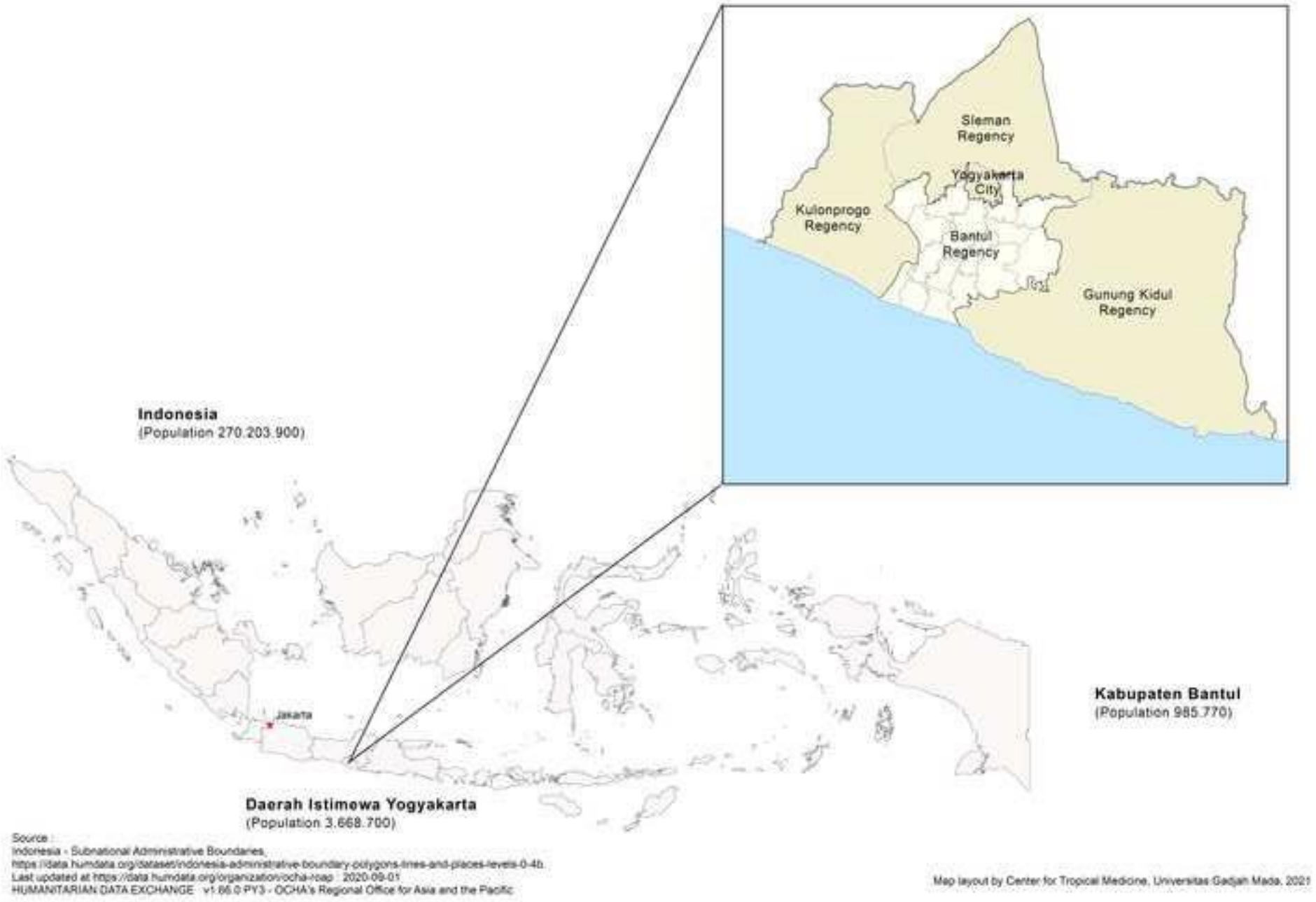

Figure 3

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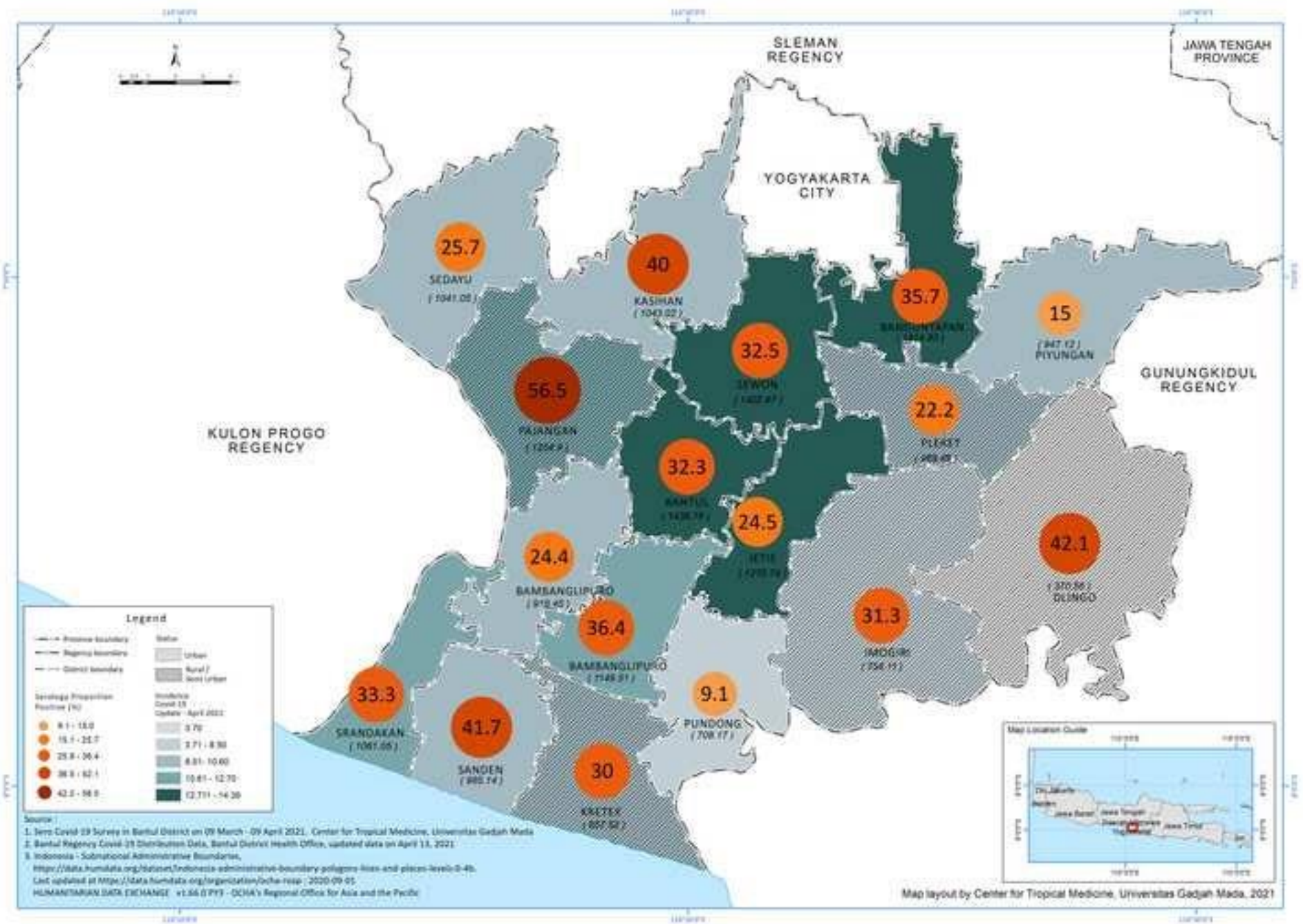

17 Januari 2023	Korespondensi review 2

29 Januari 2023	Perbaikan manuskrip sebagai respon review 2

Seroprevalence of SARS-CoV-2 and risk factors in Bantul Regency in March-April 2021, Yogyakarta, Indonesia

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Abstract

COVID-19 case counts in Indonesia inevitably underestimate the true cumulative incidence of infection due to limited diagnostic test availability, barriers to testing accessibility and asymptomatic infections. Therefore, community-based serological data is essential for understanding the true prevalence of infections. This study aims to estimate the seroprevalence of SARS-CoV-2 infection and factors related to the seropositivity in Bantul Regency, Yogyakarta, Indonesia. A cross-sectional study involving 425 individuals in 40 clusters was conducted between March and April 2021. Participants were interviewed using an e-questionnaire developed in the Kobo toolbox to collect information on socio-demographic, COVID-19 suggestive symptoms, history of COVID-19 diagnosis and COVID-19 vaccination status. A venous blood sample was collected from each participant and tested for immunoglobulin G (Ig-G) SARS-CoV-2 antibody titers using the enzyme-linked immunosorbent assay (ELISA). Seroprevalence was 31.1% in the Bantul Regency: 34.2% in semi-urban and 29.9% in urban villages. Participants in the 55-64 age group demonstrated the highest seroprevalence (43.7%; p=0.00), with a higher risk compared to the other age group (aOR= 3.79; 95% CI, 1.46-9.85, p<0.05). Seroprevalence in the unvaccinated participants was 29.9%. Family clusters accounted for 10.6% of the total seropositive cases. No significant difference was observed between seropositivity status, preventive actions, and mobility. Higher seroprevalence in semi-urban rather than urban areas indicates a gap in health services access. Surveillance improvement through testing, tracing, and treatment, particularly in areas with lower access to health services, and more robust implementation of health protocols are necessary.

Keywords: Indonesia; SARS-CoV-2; Seroprevalence; Urban; Semi-urban

Introduction

Coronavirus disease 2019 (COVID-19) is a respiratory illness caused by the newly discovered severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), leading to a global pandemic, including Indonesia. Since the first confirmed SARS-CoV-2 on March 2 2020 [1], Indonesia has experienced exponential growth of COVID-19 cases in 34 provinces. As of May 2 2021, the government of Indonesia reported 1.677.274 confirmed COVID-19 cases and 45.796 deaths [2] and became one of the countries with high cumulative and incidence cases of COVID-19. The number of COVID-19 cases in Indonesia has been

increasing rapidly since January, leading to the highest peak thus far, with provinces in Java making up 64% of the national cases. Two notable waves were observed from March 2020 to December 2021 **(Fig 1).** Based on the daily trend of COVID-19 cases, the first wave of COVID-19 cases was observed in January, with the highest number of reported cases reaching 14518 cases on January 16. After that, the cases fluctuated and reached the highest during the third wave in September 2022, with 56.757 recorded cases.

Fig 1. COVID-19 cases daily trend in Indonesia March 2020 – December 2021 (data source: https://ourworldindata.org/coronavirus/country/indonesia, accessed on October 4 2022)

However, these case counts inevitably underestimate the true cumulative incidence of infection because of limited diagnostic test availability, barriers to testing accessibility, and asymptomatic infections, not to mention underreporting [2]. As a consequence, the national prevalence of SARS-CoV-2 remains unknown.

The lack of clarity about the number of SARS-CoV-2 infections across Indonesia limits the Indonesian government's ability to plan appropriately, prepare and respond to this epidemic. Monitoring the incidence of newly diagnosed cases of severe COVID-19 and the case fatality rate is critical to address the demands on the healthcare system.

One of the epidemiological investigations used to determine the level of disease spread is to conduct a seroprevalence survey. According to the Centers for Disease Control and Prevention (CDC), this survey uses serological tests to detect antibodies in the blood, indicating an infection [3]. This test uses an enzyme-linked immunosorbent (ELISA) where the antigen used is purified SARS-CoV-2 S protein (without live virus). Population-based serological testing provides better estimates of the cumulative incidence of infection by complementing

diagnostic testing of acute illness and helping to inform the public health response to COVID-19. A seroprevalence study could also be a powerful tool to detect subclinical infections and improve policy-making in the country [4, 5]. 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 [2].

Seroprevalence varies geographically; the denser urban areas have higher seropositivity rates than rural areas [6]. A study in East Java, Indonesia, in the second semester of 2020 showed a higher prevalence in Surabaya (13.1%), which is an urban area, than in Jombang (9,9%), a rural area [7]. The epidemiological trend also implicates SARS-CoV-2 spread among rural communities only later in the epidemic wave [8, 9], which would require sound anticipatory interventions. A seroprevalence survey involving more diverse groups of people among urban and rural communities is necessary to grasp the overall picture of SARS-CoV-2 infection. Bantul Regency has become one area that contributes to many cases and leads to the high transmission of COVID-19 in the Yogyakarta Provinces. There was a lack of seroprevalence study during the time of the study. Therefore, this study aims to estimate the SARS-CoV-2 seroprevalence, seropositive risk factors, and COVID-19 vaccine acceptance in Bantul Regency, Yogyakarta.

Materials and methods

Study setting

Bantul Regency is located in the southern region of Yogyakarta Province, covering 506.85 km^2 **(Fig 2).** The regency consists of 17 sub-districts and 75 villages, 30% semi-urban.

Most adults are engaged in the non-formal sector, such as farming, trade and the service industry. [10]. The daily mobility of residents between districts to and from Bantul is high, which may increase the risk of disease transmission. [11].

Fig 2. Map of Bantul Regency, Special Region of Yogyakarta, Indonesia

Study design and sampling

A cross-sectional study was conducted from March to April 2021. The study population consisted of individuals who lived in the Bantul regency for at least six months, with 1,018,402 inhabitants. [10]. EpiInfo was used to calculate the sample size.

Sampling was determined using a multistage cluster random sample adopted from the WHO/EPI rapid survey. [12]. The calculated sample size was 414, which accounted for the estimated prevalence of 20%, 6% sampling error, a significance level of 0.05 with a design effect of 2 and a non-response rate of 15%. There were 40 clusters, with 11 participants per cluster. The cluster was based on the smallest administrative area called RT (*rukun tetangga* or household group). The RT consists of approximately 50-70 households.

We used systematic random sampling to select households at the selected cluster. In the selected households, we recruited all eligible household members, i.e. anyone who lived under the same building and resided in the study area for at least six months, with a minimum age of 5 years old.

Participants must meet the following inclusion criteria: residing in the study location at least six months before the survey commences, age of 5 years old or older, able to communicate verbally, give written consent to participate in the research or consent from parents/ guardians for respondents under 18 years old. Participants aged 13-17 were interviewed by their parents/guardians. However, for participants under 13 years old, interviews were conducted

with the parents. Meanwhile, the exclusion criteria include people with existing chronic illnesses (including immunocompromised individuals with a history of blood disorders and people with mental disorders.)

Data collection

A door-to-door visit was conducted to collect the primary data. Written informed consent was obtained from each study participant before data collection. Fifteen enumerators, including a phlebotomist, were involved in the data collection. Two supervisors were assigned to ensure the methodology and conduct the spot check. All study teams were involved in four training days regarding methods, data entry, phlebotomy and ethics in research.

Using an aseptic procedure, two millilitres of blood were drawn from a cubital vein with a disposable sterile syringe. The specimen was then kept in an EDTA tube. Before transportation, blood samples were placed in a cool box with an ice pack and transported within the same day to the laboratory. Blood samples were further stored in the refrigerator. Each participant's data included a unique identifier (barcode label) linked to their blood sample and data for tracking and confidentiality. The blood sample was transported, examined and stored in the Laboratory of Microbiology, Faculty of Medicine, Public Health, and Nursing UGM. Plasma was then tested for Ig-G anti-SARS-Cov2 procedures using (Human Anti-2019 n-CoV(N) IgG ELISA Kit V1.5 FineTest [13].

Risk factors information at household and individual levels were obtained using an electronic structured questionnaire developed in the KoBo toolbox [14, 15]. Variables included in the household questionnaire were socio-demographic information (age, gender, relationship with the head of household, and household income). Individual questionnaires were used to gather socio-demographic information (age, gender, highest education, occupation), COVID-19 vaccination status, previous diagnosis of COVID-19 and symptoms related to COVID-19

within the last six months, preventive actions taken, and mobility in the previous two weeks. The data manager validated data daily. A day after the data entry, the data manager sent the supervisor feedback and confirmation regarding completeness and data consistency.

Updated cumulative data on notified cases were obtained through the COVID-19 surveillance system conducted by Bantul District Health Office Bantul from March to April 2021. COVID-19 was defined as a symptomatic or asymptomatic person with a positive PCR result tested for SARS-CoV-2. Population numbers per sub-district were obtained through Bantul Statistical Bureau for calculating incidence per 1000 population [10]. In addition, data on urban-rural classification was obtained from the National Statistical Bureau [16].

Statistical analysis

Proportion and percentage describe the seroprevalence and socio-demographic characteristics of study participants. A map developed in ArcGIS spatially compared seroprevalence and incidence of notified cases by district and urban-rural status [17, 18]. Bivariate analysis was conducted to identify the association between presumed risk factors (age, gender, occupation, comorbidities, prevention taken and mobility over the last two weeks) and anti-SARS CoV-2 seropositivity. After considering collinearity, a multiple logistic regression was used to evaluate risks between seropositive and seronegative groups with adjustment for sex. The P-value would be considered statistically significant at $p < 0.05$. All statistical analysis was performed using STATA 14.0.

Ethics

The Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada approved the study (Ref: KE/1242/12/2020).

Written informed consent was obtained from adult respondents and parents of enrolled children. Confidentiality of information from the respondents was upheld with utmost care throughout data collection, processing and analysis for all data collected. Therefore, their names were included in the notes only for traceability and referral during the data analysis.

Results

Characteristics of study participants

A total of 425 people participated and were tested during the survey. The number of females (59.1%) is higher than that of males (40.9%); 47.2% of the participants were unemployed/students/housewives, and 72.5% lived in urban areas. The majority of the participants had no comorbidities and were not vaccinated.

The prevalence of SARS-CoV-2 seropositivity among the participants in this study was 31.1% (n=132/425). A significant difference was observed in the seroprevalence among age groups (p=0.000), with the highest proportion reported in the 55-64 age group (43.7%; n=31/71). Meanwhile, the under-15 years age group showed no seropositivity.

A significant difference was also observed among occupation groups (p=0.009). The highest seroprevalence was demonstrated by participants working as daily workers/farmers (37.2%), followed by professional/health workers (34.6%), and unemployed/students/housewives (26%). Seroprevalence did not differ between semi-urban and urban areas, even though we observed that semi-urban areas had higher seroprevalence (34.2%; n=40/117) than urban areas (29.9%; n=92/308). Females demonstrated higher seroprevalence; nevertheless, no association was found between genders and seropositivity. Seropositivity also does not differ in occupation category. (Table 1).

201 **Table 1. Distribution of Study Participants by Background Characteristics.**

Characteristic	Total	IgG-positive	IgG-negative	P-value
	n = 425	n=132	n=293	
Sex, n (%)				
Male	174 (40.9)	52 (29.9)	122 (70.1)	
Female	251 (59.1)	80 (31.9)	171 (68.1)	0.663
Age group, years, n (%)				
<=14	16 (3.8)	0 (0.0)	16 (100)	
15-24	52 (12.2)	8 (15.4)	44(84.6)	
25-34	51 (12.0)	8 (15.7)	43(84.3)	
35-44	90 (21.2)	33 (36.7)	57(63.3)	
45-54	92 (21.7)	32 (34.8)	60 (65.2)	
55-64	71 (16.7)	31 (43.7)	40 (56.3)	
65+	53 (12.5)	20 (37.7)	33 (62.3)	< 0.001
Occupation, n (%)				
Unemployed/students/Housewives	200 (47.2)	52 (26.0)	148 (74.0)	
Professional/health worker	130 (30.7)	45 (34.6)	85 (65.3)	0.009
Daily worker/farmer	94 (22.2)	35 (37.2)	59 (62.8)	
Residence set, n (%)				
Urban	308 (72.5)	92 (29.9)	216 (70.1)	
Semi-urban area	117 (27.5)	40 (34.2)	77 (65.8)	0.390
Smoking, n (%)				
Smoker	74 (18.1)	14 (18.9)	60(81.1)	
Non-smoker	335 (81.9)	118 (35.2)	217(64.8)	0.007
History of chronic disease, n (%)				
Yes	96 (22.6)	37 (38.5)	59 (61.5)	
No	329 (77.4)	95 (28.9)	234 (71.1)	0.072
Diabetes mellitus, n (%)				
Yes	15 (3.5)	6 (40.0)	9 (60.0)	
No	394 (92.5)	126 (32.0)	268 (68.0)	0.514
Hypertension, n (%)				

Yes	65 (15.9)	23 (35.4)	42 (64.6)	
No	344 (84.1)	109 (31.7)	235 (68.1)	0.559
Obesity, n (%)				
Yes	8 (1.9)	4 (50)	4 (50)	
No	401 (98.1)	128 (31.9)	273 (68.1)	0.279
Previous COVID-19 diagnosis, n (%)				
Yes	3 (0.7)	1 (33.3)	2 (66.7)	
No	422 (99.3)	131 (31.0)	291 (68.9)	1.000
COVID-19 symptoms n (%)				
Yes	155 (36.4)	44 (28.4)	111 (71.6)	
No	270 (63.4)	88 (32.6)	182 (67.4)	0.4
COVID-19 vaccination, n (%)				
Yes, at least one dosage	13 (3.1)	9 (69.2)	4 (30.8)	
Not yet	412 (96.9)	123 (29.9)	289 (70.2)	0.005
Preventive measures and mobility				
Wearing masks when going out				
Always	380 (89.4)	116 (30.5)	264 (69.5)	0.491
Not always	45 (10.6)	16 (35.6)	29 (64.4)	
Washing hands for at least 20 seconds with running water				
Always	344 (80.9)	106 (30.8)	238 (69.2)	0.822
Not always	81 (19.1)	26 (32.1)	55 (67.9)	
Maintain a physical distancing (1-2m) in the public area				
Always	274 (64.5)	82 (29.9)	192 (70.1)	0.497
Not always	151 (35.5)	50 (33.1)	101 (66.9)	
Mobility				
Attending invitations to traditional or religious activities (e.g. weddings, funerals)				
Always	39 (9.2)	14 (35.9)	25 (64.5)	0.493
Not always	386 (90.8)	118 (30.6)	268 (69.4)	
Visiting relatives or friends or other people due to important matters				
Always	42 (9.9)	13 (30.9)	29 (69.1)	0.987
Not always	383 (90.1)	119 (31.1)	264 (68.9)	
Going to the markets/shops/offices/crowds				

Always	166 (39.1)	50 (30.1)	116 (69.9)	0.738
Not always	259 (60.9)	82 (31.7)	177 (68.3)	
Staying at home, except for essential matters				
Always	313 (73.7)	94 (30.1)	219 (69.9)	0.444
Not always	112 (26.3)	38 (33.9)	74 66.1)	

This study also explored seroprevalence based on the presence of selected chronic diseases and the history of comorbidities. Participants with a history of chronic conditions accounted for 22.6% of the total participants. Among those with comorbidities, seroprevalence for participants with obesity, diabetes mellitus, and hypertension was 50%, 40% and 35.4%, respectively.

Participants with a prior diagnosis of COVID-19 in the last six months accounted for 1 out of 132 seropositive cases. Seroprevalence among participants with no previous experience of COVID-19-related symptoms was found to be 31.1%. Seropositivity was observed in 14 participants from seven households, making family clusters account for 10.6% of the total positive cases.

Most of the participants in this study reported decreased social interactions and mobility during this period. Study participants stated that they attended fewer traditional and religious activities (90.8%), had reduced visits to relatives and friends (90.1%, decreased visits to markets (60.9%), and spent more time at home, except for essential tasks ((73.7%). If participants did leave home, they reported wearing masks (89.4%), regular washing of hands for 20 seconds with running water (80.9%) and maintaining physical distancing in public areas (64.5%). However, no significant differences were observed between individual preventive actions and the level of mobility with seropositivity status.

Seroprevalence of anti-SARS-CoV-2 based on the geographical distribution

The highest seroprevalence was observed in 3 semi-urban areas (Pajangan, Dlingo, Sanden). Most semi-urban areas have relatively higher seroprevalence than urban areas. However, we found no statistical significance. **(Fig 3).** The highest distribution of confirmed cases acquired from routine regional data reported higher cumulative cases in urban areas, such as Banguntapan, Bantul, Sewon, and Jetis.

Fig 3. Seroprevalence and Reported Cumulative Incidence of SARS-Cov-2 in April 2021 Based on District.

Risk factors associated with SARS-CoV-2 seropositivity

This study further explored the risk factors associated with the seropositivity of SARS-CoV-2, adjusting for age, sex, occupation, comorbidities, and vaccination status. A significant association was observed within specific age groups. The odds of SARS-CoV-2 seropositivity are higher in the age 55-64 (adjusted odds ratio [aOR]=3.79; 95% CI 1.46-9.85, p=0.006).

Females demonstrated higher seroprevalence. Nevertheless, no association was found between genders and seropositivity. Seropositivity also does not differ in occupation category (aOR=0.81; 95% CI 0.50-1.31, p>0.005) (Table 2).

Table 2. Factors Associated with SARS-Cov-2 Seropositivity in Bantul Regency (N=425).

Risk Factors	**ELISA+ (n=132)**	**Adjusted OR (95% CI)**	***p*-value**
Age group			
<=14	0	NA	NA
15-24	8	Reference	NA
25-34	8	0.93 (0.31-2.79)	0.893
35-44	33	2.71 (1.08-6.81)	0.034
45-54	32	2.67 (1.05-6.80)	0.040
55-64	31	3.79 (1.46-9.85)	0.006
65+	20	3.38 (1.27-9.00)	0.015
Sex			
Male	52	0.81 (0.50-1.31)	0.388
Female	80	Reference	NA
Occupation			
Unemployed/students/Housewives	52	Reference	NA
Professional/health worker	45	1.14 (0.64-2.00)	0.661
Daily worker/farmer	35	1.27 (0.70-2.31)	0.441
Comorbidities			
Hypertension	23	0.87 (0.48-1.57)	0.646
Obesity	4	2.46 (0.57-10.65)	0.229
Diabetes Mellitus	6	1.12 (0.38-3.31)	0.832
Vaccination Status			
Yes	14	Reference	NA
No	13	0.19 (0.05-0.68)	0.010

Comorbidities were presumed as one of the risk factors of SARS-CoV-2 seropositivity. The odds of SARS-CoV-2 seropositivity were higher in participants with obesity (aOR=2.46; 95% CI 0.57-10.65) and diabetes mellitus (aOR=1.12; 95% CI 0.38-3.31) than participants without each respective comorbidity. However, this finding is not statistically significant (p-value>0.05).

Although vaccination had not been made available to the general public during the data collection period, most participants (91.5%) were aware that the government would provide vaccinations in the future and stated their willingness (79.1%) to be vaccinated. However, a small proportion (19.1%) said they did not want to be vaccinated. Reasons for this included fear of adverse effects, concerns about the safety and effectiveness of the vaccines, and religious beliefs.

Table 3. Vaccine acceptance of the participants (N=425)

Variables	N = 425	Percentage
Aware that the government would provide the vaccination for the people		
Yes	389	91.5
No	36	8.5
Willing to get vaccinated if the government provide the vaccines		
Yes	336	79.1
No	81	19.1
Not decided	8	1.9

Discussion

This SARS-CoV-2 seroprevalence study found that the prevalence of IgG antibodies against SARS-CoV-2 was 31.1% in Bantul, significantly higher than the 11.4% seropositive which was found in a study in East Java in late 2020 [7]. On the other hand, surveillance data

of Bantul Regency DHO reported a considerably lower cumulative incidence of 1.1% during the study. This finding is in line with previous research suggesting that the estimates obtained from seroprevalence were 18.1 times higher than the corresponding cumulative incidence of COVID-19 infections, implying that confirmed cases are a poor indicator of the extent of the disease spread [19]. While antigen testing and Polymerase Chain Reaction (PCR) to monitor COVID-19 detect the presence of specific antigens or genetic material indicating current viral infection, serological tests for COVID-19 are used to detect antibodies that the body produces in response to the virus. Serological tests can detect antibodies in people who have been infected with COVID-19 in the past, even if they were asymptomatic or had mild symptoms. Those can help identify people who may have been infected but were never diagnosed. In population-level studies, serological testing is used to estimate the proportion of people who have been infected with COVID-19 in a given area.

Seroprevalence varies geographically, and previous research indicated that urban areas mainly reported a higher number of seropositivity [6]. The reported COVID-19 cases in the Bantul regency also showed a similar trend, in which more cases were reported in urban areas (Bantul, Banguntapan, Sewon, and Jetis). However, our study showed that the three highest seropositivities were observed in semi-urban areas (Pajangan, Dlingo and Sanden). Meanwhile, areas with the highest reported COVID-19 cases showed lower seropositivity. It suggests that the semi-urban areas were underreported and underdiagnosed due to access to diagnosis. These discrepancies can be explained by the limited and centralized diagnosis facilities when the study was conducted.

Urban areas with dense populations were impacted earlier at the pandemic's beginning. A previous study on epidemiological modelling predicted that COVID-19 spread faster in urban than rural areas [20]. Despite the rapid transmission, urban areas are usually supported

by better surveillance management, such as more accessible testing, tracing, and infection control. Consequently, cases in urban areas were reported faster and earlier.

Meanwhile, rural areas usually have shortages in resources and workforces, which affects their ability to detect, respond, prevent, and control infectious disease outbreaks [21, 22]. In this study, a large proportion of seropositivity was reported in participants with no prior diagnosis of COVID-19, indicating that the infection might be undiagnosed due to a lack of individual testing. Thus, this study suggests that the higher transmission observed in semi-urban areas was lower access to health services, including lack of individual testing, resulting in unreported positive cases.

Higher seroprevalence in this study was observed in females. However, no statistically significant gender difference was reported. A previous study also suggested that SARS-CoV-2 IgG/IgM dynamic is mainly affected by age and disease severity, not sex [23]. Higher seropositivity was observed in the age group 55-64, and this finding supports many previous studies that indicate older age as one of the risk factors for COVID-19.

Subsequently, this research found a portion of seropositivity among people who frequently stayed home. Additionally, occupations requiring less mobility, such as housewives, students, and the unemployed, also accounted for seropositivity. The research also discovered a tenth of family clusters, suggesting that the transmission may have occurred at the household level. Previous research contends that the primary mode of COVID-19 infection is through the household spread. [24, 25].

Our study found no difference between high and low mobility, suggesting they were already widespread community transmission, even in rural areas. Preventive measures did not prevent getting an infection, which might be because it is challenging to do preventive measures in household settings or practice preventive measures consistently outside. [26]

Surveillance and containment measures such as large-scale social restrictions and other anticipatory prevention tools become a priority to curb transmission, primarily focusing on areas with lower access to health services. Earlier in the pandemic, outbreak containment was concentrated in the dense urban areas, which may have reduced the number of cases. However, due to high mobility, the transmission slowly moved to semi-urban and rural communities. People living in rural communities might develop a false sense of security and take fewer precautions at the beginning than the urban communities [8,9].

One of the effective ways to prevent the fatalities caused by COVID-19 is through vaccination. Due to no vaccination programme being rolled out to the general population during the data collection period, it is reasonable that higher seroprevalence was found in unvaccinated groups. As this study explored the seroprevalence when most people were still unvaccinated, a better picture of the infection spread in Bantul was obtained since there was no implication from vaccine-induced antibodies.

Despite the findings, this study is not without limitations. Following a natural infection, antibody titers peak and begin to wane in various manners, with some in shorter duration [27]. However, this study did not consider the assay performance concerning the waning immunity. Thus it may underestimate the true prevalence. Furthermore, the analysis results only showed the association, not causality. Finally, as a cross-sectional study, this study only analyzed the variables at once and did not explore the seroprevalence changes over time. Therefore, future research is needed to conduct a periodic or longitudinal survey to determine the prevalence in the longer term.

Conclusion

This serosurvey demonstrated a higher seroprevalence than reported data in the same period. Based on the findings, it is strongly recommended that the local government strengthen

the surveillance and 3T (testing, tracing, and treatment) efforts by involving the task force at the neighbourhood community and village levels throughout Bantul Regency, particularly in areas with lower access to health services. Besides that, it needs to increase awareness and implementation of health protocols for high-mobility individuals to prevent transmission within the household. As the vaccination for the general population is being rolled out, it is crucial to provide adequate implementation information, including health resources and logistics. This study can be implemented in other areas, both at the district/city and provincial levels, to understand the seroprevalence of SARS-CoV-2 in Indonesia better. This study provides a district-level view of the extent of COVID-19 spread and a different approach to conducting serosurvey among diverse populations in various regions to fit the gaps in understanding COVID-19's spread globally.

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Conflict of interest

The authors declare no conflict of interest.

Supporting information

S1 Table. The response rate of each district.

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7 Maret 2023	**Korespondensi review 3**

12 April 2023	**Perbaikan manuskrip sebagai respon review 2**

COVER LETTER
Response to Reviewers

Dear Editor,

We are grateful to you and the reviewers for reading our manuscript, offering insightful suggestions, and allowing us to submit the revised draft of the manuscript. Your valuable and insightful comments led to possible improvements in the current version. The authors thoroughly evaluated the feedback and made extensive revisions as a result. We are hopeful that the manuscript will meet your high standards. The authors welcome any further constructive comments.

Below we provide the point-by-point responses. All modifications in the manuscript have been highlighted in yellow.

Sincerely,
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Response to Reviewers

Reviewer #3

Reviewer #3: Generally, the paper is well written though there are some sections that are written in present tense instead of past tense. There are sections where singular instead of plural is used e.g. Result instead of Results. Since this is not a scientific issue, the paper should be published.

Response: Thank you for your affirmation of the content of our paper. We carefully checked through the valuable suggestions and have incorporated the changes in the revised manuscript.

Reviewer #4

Reviewer #4: The study by Riris Andono Ahmad et al. aimed to estimate the SARS-CoV-2 seroprevalence, seropositivity risk factors, and COVID-19 vaccine acceptance in Bantul Regency, Yogyakarta. The study is relevant and conducted following an appropriate methodology. Furthermore, enough information is provided to allow for its replication. However, although the authors have incorporated suggestions from prior reviewers to strengthen the manuscript, further corrections would still be appropriate.

Therefore, we suggest that the authors revise the manuscript extensively for language clarity and grammatical errors to facilitate the reading.

Response: Thank you for your valuable suggestions to improve the quality of our manuscript. We have checked and incorporated each comment as suggested in the revised manuscript.

Abstract

- Lines 40-41: The interpretation of statistically significant differences in seropositivity between individual preventive actions and mobility categories should be revised. Indeed, there is not a statistically significant difference between the categories. Still, the way it is stated may confuse the readers, as it currently reads like preventive actions were compared to mobility.

  Response: Thank you for raising this issue. We noted and apologized that the statement may confuse the readers. Therefore, we revised the sentence into,*"No significant difference was observed between seropositivity status and preventive actions as well as mobility"* in lines 40-41.

Materials and methods:

- Did participants under 18 respond on their own regarding risk factors, preventive actions, and mobility? Or were their responses provided by a parent or guardian? Please include this clarification in the manuscript.

Response: Participants aged 13-17 were interviewed by their parents/guardians. However, for participants under 13 years old, interviews were conducted with the parents. We have included this in the manuscript line 125-126.

Results

- Lines 199-200: Please revise the statement of non-statistically significant differences between urban and semi-urban areas. The way the sentence currently reads may need to be clarified for the readers.

  Response: Thank you so much for your valuable input. We have changed this sentence into "*Seroprevalence did not differ between semi-urban and urban areas, even though we observed that semi-urban areas had higher seroprevalence (34.2%; n=40/117) than urban areas (29.9%; n=92/308)'* in the manuscript line 198-202.

  Lines 221-222: Same comment regarding individual preventive actions and mobility.

  Response: Thank you for this comment. We have changed this sentence into *"Most of the participants in this study reported decreased social interactions and mobility during this period. Study participants stated that they attended fewer traditional and religious activities (90.8%), had reduced visits to relatives and friends (90.1%, decreased visits to markets (60.9%), and spent more time at home, except for essential tasks ((73.7%). If participants did leave home, they reported wearing masks (89.4%), regular washing of hands for 20 seconds with running water (80.9%) and maintaining physical distancing in public areas (64.5%). However, no significant differences were observed between individual preventive actions and the level of mobility with seropositivity status."* in line 216-223.

  Line 226: Same comment regarding urban and semi-urban areas.

  Response: Thank you for your valuable advice. We have changed this sentence to "*The highest seroprevalence was observed in 3 semi-urban areas (Pajangan, Dlingo, Sanden). Most semi-urban areas have relatively higher seroprevalence than urban areas. However, we found no statistical significance."* in line 226-228.

- Lines 24-241: The interpretation of odds of Sars-Cov-2 seropositivity in different categories of gender and occupation needs to be revised.

  Response: Thank you for this comment. We have revised it accordingly: "*Females demonstrated higher seroprevalence, nevertheless, no association was found between genders and seropositivity. Seropositivity also does not differ in occupation category."* in the manuscript line 240-241.

- Line 277: Limited diagnosis?

  Response: Yes, as mentioned in the discussion section of the manuscript, semi-urban areas have limited access to health services, such as diagnosis facilities that were limited in these areas.

Reviewer #5

Reviewer #5: This article describes a prospective seroprevalence survey for SARS-CoV-2 that was conducted in Indonesia in 2021. Thus, it contributes to the broader knowledge base on methodologies for tracking and monitoring a viral pandemic. However, within the paper there are numerous sections that are confusing and could obscure the interpretation of the data and the conclusions. I have made multiple recommended edits and revisions, using the line monitoring numbers of Revision 2, in order to improve clarity on the methodologies used for the interpretation of results.

In addition to these recommended changes, the paper would be more valuable if it contained a brief discussion of why this methodology was chosen from a broader set of epidemiological tools for tracking SARS-CoV-2. Specifically, how does the described methodology compare to other sampling approaches including unlinked anonymous testing of available sera samples (prenatal blood, donors for transfusions, military recruits, hospital admissions, etc) or through the use of wastewater monitoring.

Specific comments/corrections follow, based on the REVISION 2 version and the corresponding Line Numbering.

Response: We would like to thank the reviewer for the valuable suggestions to improve the clarity of the writing of our manuscript. Based on the comments and suggestions, we have extensively revised the manuscript.

ABSTRACT:

- The first sentence (Line 24-25) needs to be replaced with, "COVID-19 case counts in Indonesia inevitably underestimate the true cumulative incidence of infection due to limited diagnostic test availability, barriers to testing accessibility and asymptomatic infections."

  Response: Thank you for the feedback. We have revised the sentence accordingly in lines 24-26.

- In (Line 36), delete the sentence, "A significant difference was reported in the age group."

  Response: Thank you for the comment. We have deleted the sentence as suggested.

- In Line 40 and 41, rewrite the sentence as follows, "No significant difference was observed between preventive actions of an individual, nor their level of mobility, with seropositivity status" I do not understand the intent of the sentence, "This study observed a discrepancy with COVID-19 confirmed cumulative incidence data reported in the same time period (11 out of 100 population), indicating silent transmission may have occurred within the community." Can you rephrase this sentence in order to clarify the meaning or delete it. In the final sentence of the abstract (Line 46) the word "implementation" should be singular not plural.

  Response: Thank you for the valuable suggestion. We have rewritten the sentence as a suggestion in lines 39-41. We apologize for the lack of clarity in the sentence *"This study*

observed a discrepancy…", to improve the understanding, we have deleted the sentence as suggested. We have changed the word "implementation" into singular in line 44.

MAIN ARTICLE:

- In the INTRODUCTION section, in line 53, rewrite the sentence as follows, "Since the first confirmed case of SARS-CoV 2 on March 2 2020, Indonesia has experience exponential growth of COVID-19 cases in 34 provinces" Indonesia has 38 provinces, was there no data available from 4 provinces?? In line 56 replace "incidence" with "incident."

  Response: Thank you for noting this. The Indonesian government expanded the provinces of Papua from two to six provinces in late 2022. Therefore, the COVID-19 data during the study were part of Papua and West Papua provinces.

- In Lines 57 to 62 the authors are presenting COVID-19 data from another data source. It's not clear whether they are presenting case reporting per day or per month. Can the authors clarify and revise these sentences?

  Response: We apologize for the lack of clarity on this. The data source presents daily reported data, and we observed the peak between months. We have changed the sentence into, "*Based on the daily trend of COVID-19 cases, the first wave of COVID-19 cases was observed in January with the highest reported cases reaching 14518 cases on January 16."* in lines 57-58.

- In Line 73, replace "Counting" with "Monitoring" and in Line 74 delete the phrase, "...as it is necessary..."

  Response: Thank you for your input. We have incorporated it in the revised manuscript line 72.

- In Line 78 replace "apply" with "conduct.." In the next sentence, can you clarify that you are referring to the United States CDC?

  Response: Thank you for your suggestion. We have incorporated the changes in the revised manuscript. Yes, we refer to the US CDC.

MATERIALS AND METHODS

- Line 105 ends with "...30% semi-urban..... Is there something missing from this sentence?

  Response: Thank you for pointing this out. The sentence is correct. Our apologies for not placing the period after the sentence. We have revised this in the manuscript line 104.

- In Line 106, revise the sentence to state< Most adults are engaged in the non-formal sector, such as farming trade, and the service industry "

  Response: Thank you for your comments. We have revised the sentence as suggested in lines 105-106.

- In Line 108 rewrite sentence to state, "The daily mobility of residents between districts to and from Bantul is high which may increase the risk of disease transmission."

  Response: Thank you for your comment. We have revised the sentence as suggested in lines 106-107.

- In Lines 113 to 114, rewrite the sentence to state, "The study population consisted of individuals who lived in the Bantul regency for a minimum of six months, with a total of 1,018,40 inhabitants."

  Response: Thank you for your suggestion. We have revised it accordingly in lines 112-114.

- In Line 116, rewrite the sentence "Sampling was determined using a multistage cluster random sample adopted from the WHO/EPI rapid survey."

  Response: Thank you for your input. We have revised it accordingly in lines 115-116.

- In line 126, rewrite the sentence to state, "Participants must meet the following inclusion criteria: residing in the stud location at least six months before the survey commences. "

  Response: Thank you for your input. We have revised it accordingly in lines 125-126.

- In Lines 129 to 131, rewrite the sentence to state, "Meanwhile, the exclusion criteria include people with existing chronic illness (including immunocompromised individuals with a history of blood disorders and people with mental disorders.)

  Response: Thank you for your input. We have revised it accordingly in lines 130-132.

- in Line 134, rewrite the sentence as " A door to door visit was conducted. .... "

  Response: Thank you for your comment. We have revised the sentence as suggested in line 135.

- In Line 139, rewrite the sentence as " Using aseptic procedures, two milliliters of blood. "

  Response: Thank you for your comment. We have revised the sentence as suggested in line 140.

- In Line 149, can you provide a footnote, giving a brief reference to the KoBo toolbox?

  Response: Thank you for your suggestion. We have added the reference to the KoBo toolbox as well as the usage of the KoBo toolbox in the earlier studies in line 150.

[14] Dizon, Timothy John R et al. “Experiences in Using KoBo Collect and KoBo Toolbox in a Cross-Sectional Dog Population and Rabies Knowledge and Practices Household Survey in the Philippines.” *Studies in health technology and informatics* vol. 290 (2022): 1082-1083. doi:10.3233/SHTI220278

[15] https://www.kobotoolbox.org/about-us/software

- In Line 151, rewrite the sentence, "Individual questionnaires were used to gather socio-demographic information. "

  Response: Thank you for your input. We have revised it accordingly in lines 152-153.

- In Line 159 to 161, rewrite the sentence as, "COVID-19 was defined as a symptomatic or asymptomatic person with a positive PCR result, tested for SARS-CoV-2"

  Response: Thank you for your input. We have revised it accordingly in lines 160-161.

- In Line 166 please provide a footnote for "ArcGIS".

  Response: Thank you for your suggestion. We have added the reference of ArcGIS as well as its usage in the earlier studies in lines 168-169.

  [17] Zamani, Mohammad et al. "Seroprevalence of SARS-CoV-2 antibody among urban Iranian population: findings from the second large population-based cross-sectional study." *BMC public health* vol. 22,1 1031. 23 May. 2022, doi:10.1186/s12889-022-13464-7

  [18] https://www.esri.com/en-us/arcgis/products/arcgis-online/overview

- In Line 190 to 191, rewrite the sentence as, "The majority of the participants had no comorbidities and were not vaccinated."

  Response: Thank you for your suggestion. We have revised it accordingly in lines 189-190.

- In Line 199 rewrite the sentence, "Seroprevalence did not differ between semi-urban and urban areas, even though we observed that semi-urban areas had higher seroprevalence (34.2%; n=40/117) than urban areas (29.9%; n=92/308."

  Response: Thank you for your suggestion. We have revised it accordingly in lines 198-202.

- In Lines 207 to 213, revise to read as follows, "This study also explored seroprevalence based on the presence of selected chronic diseases and the history of comorbidities. Participants with a history of chronic conditions accounted for 22.6% of the total participants. Among those with comorbidities, seroprevalence for participants with obesity, diabetes mellitus, and hypertension was 50%, 40% and 35.4% respectively.

  Response: Thank you for your suggestion. We have revised it accordingly in lines 206-210.

- The paragraph covered in lines 216 to 222 needs to be completely revised to read as follows: "Most of the participants in this study reported decreased social interactions and mobility during this time period. Study participants stated that they attended fewer traditional and religious activities (90.8%), had decreased visits to relatives and friends (90.1%, decreased visits to markets (60.9%), and spent more time at home, except for essential tasks ((73.7%). If participants did leave home, they reported wearing masks (89.4%), regular washing of hands for 20 seconds with running water (80.9%) and maintaining physical distancing in public areas (64.5%) However, no significant

differences were observed between individual preventive actions and the level of mobility with seropositivity status."

Response: Thank you for your suggestion. We have revised it accordingly in lines 216-223.

- In the section, SEROPREVALENCE OF ANTI-SARS-COV-2 BASED ON GEOGRAPHICAL DISTRIBUTION, lines 224 to 230, the authors are comparing geographic data from this study with routine regional data that was already being collected. I found that the conclusions about urban and semi-urban seropositive rates to be confusing. Can the authors review and revise this short paragraph?

  Response: Thank you for this comment. To improve clarity, we have revised the sentence: "*Highest seroprevalence was observed in 3 semi-urban areas (Pajangan, Dlingo, Sanden), and most of the semi-urban areas have relatively higher seroprevalence compared to urban areas. However, we found no statistical significance. The highest distribution….”* in lines 226-228.

- In the section, RISK FACTORS ASSOCIATED WITH SARS-CoV-2, in lines 236 to 241, please revise the second sentence to read, "A significant association was observed within specific age groups."

  Response: Thank you for your suggestion. We have revised it accordingly in lines 237-238.

- In line 250, the sentence should be in past tense. Replace "are" with "were."

  Response: Thank you for your input. We have revised it accordingly in line 246.

- Revise the sentence in Lines 254 to 256 to read as follows: "Although vaccination had not been made available to the general public during the time period of the data collection, most participants (91.5%) were aware that the government would provide vaccinations in the future and stated their willingness (79.1%) to be vaccinated. A small proportion (19.1%) stated that they did not want to be vaccinated. Reasons for this, included fear of adverse effects, concerns about safety and effectiveness of the vaccines, and religious beliefs.

  Response: Thank you for your input. We have revised it accordingly in lines 250-255.

DISCUSSION

needs major revisions throughout. I have attempted to rewrite selected sentences. However, there are entire sections that need to be reconsidered and rewritten. I have identified these.

- The first paragraph (lines 262 to 270) should be revised to: "This SARS-CoV-2 seroprevalence study found that the prevalence of IgG antibodies against SARS-CoV-2 was 31.1% in Bantul, which is significantly higher than the 11.4% seropositive which was found in a study in East Java in late 2020 (7)."

  Response: Thank you for your suggestion. We have revised it accordingly in lines 259-263.

- The next sentence, which begins with "The discrepancy " does not make any sense to me. Can the authors rewrite the second half of this paragraph which discusses cumulative incidence numbers? I would also recommend deleting the sentence about vaccinations targeting health care workers in this paragraph. Not sure it is relevant here.

  Response: Thank you for your comment. We have revised accordingly into *"the other hand, surveillance data of Bantul regency DHO reported considerably lower cumulative incidence of 1.1% cumulative period during the time of study.*" in lines 261-263.

- The second, third and fourth paragraphs (Lines 271 to 291) attempt to identify the relationship between geographic location (urban, semi-urban, rural, etc) and seropositivity. However in explaining these differences, the authors seem to confuse the correlation between diagnosis/testing with variable infection control efforts. It's not clear from these sections whether the authors believe that the higher seropositivity rates in semi urban areas were due to better testing or to poor infection control efforts including warning the public about high transmission in these areas.

  Response: Thank you for highlighting this issue. This paragraph highlights the discrepancies between reported cases, with the majority from urban areas, while in our study, we found that higher seroprevalence was observed in semi-urban areas, more when community-wide mobility restrictions were enforced in these areas. Thus, we think that the discrepancy between the reported cases and the study is not because of the infection control efforts but due to access to the diagnosis facility and recording and reporting system.

- The paragraph from line 292 to 296 is ok.

  Response: Thank you.

- The paragraph from line 297 to 303 is extremely confusing and needs to be rewritten. It attempts to look at occupation and whether someone primarily stays at home and links this with seropositivity.

  Response: Thank you for your suggestion. We have revised it into *"Subsequently, this research discovered a portion of seropositivity among people who frequently stayed home. Additionally, occupations requiring less mobility, such as housewives, students, and the unemployed, also accounted for seropositivity. The research also discovered a tenth of family clusters, suggesting that the transmission may have occurred at the household level.*

Previous research contends that the primary mode of COVID-19 infection is through the household spread" in lines 300-305.

- The paragraph from line 304 to 310 examines levels of mobility and whether prevention methods are being used. However, it contradicts an earlier statement in the paper that use of standard prevention methods seemed to have no impact on seropositivity rate.

  Response: Thank you for raising this issue. To improve the clarity, we have revised the sentence: "*Our study found no difference between high and low mobility, suggesting they were already widespread community transmission, even in rural areas. Preventive measures did not prevent getting an infection, which might be because it is difficult to do preventive measures at household settings or practice preventive measures consistently outside."* in lines 306-310.

- The paragraph from line 311 to 317 covers an important topic. However please rewrite the first sentence as, "Surveillance and containment measures such as large scale social restrictions and other anticipatory prevention tools become a priority to curb transmission, primarily focusing on areas with lower access to health services."

  Response: Thank you for your suggestion. We have revised it accordingly in lines 311-313.

- The next paragraph (lines 318 to 324) is very confusing. At the beginning, it states that vaccination programs were just being rolled out. However, earlier in the paper and at the end of this paragraph, it states that vaccinations were not available to the general public, particularly in semi urban and rural locations during the time of this study. Please clarify.

  Response: COVID-19 vaccinations in Indonesia are rolled out in stages, which during the time of the study have only targeted health workers, the elderly and essential public workers. Vaccination to the general public was started in late April. Therefore, the majority of the participants in this study have not received COVID-19 vaccination yet.

- The final paragraph in the Discussion (Lines 324 to 330) is also confusing. It notes the weaknesses of the serological testing as it relates to determining incidence/prevalence and potential false negatives due to waning immunoglobulin levels. Do the authors have any recommendations as to how to address these issues in future studies?

  Response: Thank you for your input. We have added the recommendations in lines 329-331, "*future research is needed to conduct a periodic survey or longitudinal survey to determine the prevalence in the longer term.*"

- Finally, per my comments at the beginning, it would be helpful if the authors could provide a brief discussion as to how this prospective serological survey compares with other accepted methods for monitoring the SARS=CoV-2 pandemic.

  Response: Thank you so much for your valuable suggestion. We have briefly introduced the comparison between serological testing and other COVID-19 testing method in lines 266-273. *"While antigen testing and Polymerase Chain Reaction (PCR) to monitor COVID-19 detect the presence of specific antigens or genetic material indicating current viral infection, serological tests for COVID-19 are used to detect antibodies that the body produces in response to the virus. Serological tests can detect antibodies in people who*

have been infected with COVID-19 in the past, even if they were asymptomatic or had mild symptoms. Those can help identify people who may have been infected but were never diagnosed. In population-level studies, serological testing is used to estimate the proportion of people who have been infected with COVID-19 in a given area."

CONCLUSION

The CONCLUSION section is fine.

Response: Thank you.

25 Mei 2023	**Manuskrip Accepted**

AMANDMENT APPROVAL

The Ethical Committee of Research in Medical Health, Faculty of Medicine, Public Health, and Nursing, has carefully reviewed the protocol entitled:

SeroSurvey Berbasis Populasi untuk Menentukan Prevalensi dan Faktor Risiko Infeksi Virus SARS CoV-2 Provinsi Yogyakarta, Indonesia, 2020

Reference Number of Ethical Approval Letter	:	KE/FK/1378/EC 30 Desember 2020
Name of Principal Investigator	:	dr. Riris Andono Ahmad, MPH., Ph.D.
Name of Institution	:	Faculty of Medicine, Public Health, and Nursing Universitas Gadjah Mada

And approved the submitted amendment of document :

Title of the Research Protocol	:	SeroSurvey Berbasis Populasi untuk Menentukan Prevalensi dan Faktor Risiko Infeksi Virus SARS CoV-2 Provinsi Yogyakarta, Indonesia, 2021
Document(s) Approved and version	:	1. Study Protocol version Amandment 2021 2. Information for Subjects version Amandment 2021 3. Informed consent form version Amandment 2021 4. Kuesioner version Amandment 2021

Yogyakarta, 15 MAR 2021

Prof. dr. Madarina Julia, Sp.A(K)., MPH., Ph.D.
Panel's Chairperson

P.S: This letter uses signature scan of the panel's chairperson and Secretary of the Ethics Committee. The hardcopy official letter with authority's signature will be issued when it is possible and are kept as an archive of the Ethics Committee	Validation number : 604f2b10bcaad (http://komisietik.fk.ugm.ac.id/validasi)

2 Juni 2023	Perbaikan manuskrip sebagai respon review 4

Dr. Catherine Kyobutungi
Editor-in-Chief
PLOS Global Public Health

April 24, 2022

Dear Editor,

We wish to submit an original research article entitled **"Seroprevalence of SARS-CoV-2 and Risk Factors in Bantul Regency, Yogyakarta, Indonesia**" for consideration by PLOS Global Public Health.

The reported cases of COVID-19 in Indonesia inevitably underestimate the true cumulative incidence of infection due to limited barriers in testing and asymptomatic infections. The absence of a reasonable estimate of the number of infections poses a challenge in estimating this need. A seroprevalence survey involving more diverse groups of people among urban and semi-urban communities is necessary to grasp the overall picture of SARS-CoV-2 infection. Bantul regency has become one area that contributes to a high number of cases and leads to the high transmission of COVID-19 in Yogyakarta Province. Therefore, this study aims to estimate the seroprevalence of COVID-19 and the risk factors in Bantul Regency, Yogyakarta.

This study indicates a discrepancy with COVID-19 confirmed cumulative incidence data reported in the same period, suggesting that silent transmission might have occurred within the community. Furthermore, a higher seroprevalence was observed in semi-urban areas than urban areas, indicating a gap in access to health services, especially in testing, tracing, and treating COVID-19 cases. This finding will be beneficial to assist policymakers in surveillance improvement, particularly in areas with lower access to health services.

We believe the findings presented in this study will be of interest to PLOS Global Public Health readers. Thus, we sincerely hope you would consider this paper for publication.

We confirm that this work is original and has not been published elsewhere, nor it is currently under consideration for publication elsewhere. We have no conflicts of interest to disclose.

Sincerely,
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PLOS GLOBAL PUBLIC HEALTH

RESEARCH ARTICLE

Seroprevalence of SARS-CoV-2 and risk factors in Bantul Regency in March-April 2021, Yogyakarta, Indonesia

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Abstract

COVID-19 case counts in Indonesia inevitably underestimate the true cumulative incidence of infection due to limited diagnostic test availability, barriers to testing accessibility and asymptomatic infections. Therefore, community-based serological data is essential for understanding the true prevalence of infections. This study aims to estimate the seroprevalence of SARS-CoV-2 infection and factors related to the seropositivity in Bantul Regency, Yogyakarta, Indonesia. A cross-sectional study involving 425 individuals in 40 clusters was conducted between March and April 2021. Participants were interviewed using an e-questionnaire developed in the Kobo toolbox to collect information on socio-demographic, COVID-19 suggestive symptoms, history of COVID-19 diagnosis and COVID-19 vaccination status. A venous blood sample was collected from each participant and tested for immunoglobulin G (Ig-G) SARS-CoV-2 antibody titers using the enzyme-linked immunosorbent assay (ELISA). Seroprevalence was 31.1% in the Bantul Regency: 34.2% in semi-urban and 29.9% in urban villages. Participants in the 55–64 age group demonstrated the highest seroprevalence (43.7%; p = 0.00), with a higher risk compared to the other age group (aOR = 3.79; 95% CI, 1.46–9.85, p<0.05). Seroprevalence in the unvaccinated participants was 29.9%. Family clusters accounted for 10.6% of the total seropositive cases. No significant difference was observed between seropositivity status, preventive actions, and mobility. Higher seroprevalence in semi-urban rather than urban areas indicates a gap in health services access. Surveillance improvement through testing, tracing, and treatment, particularly in areas with lower access to health services, and more robust implementation of health protocols are necessary.

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Introduction

Coronavirus disease 2019 (COVID-19) is a respiratory illness caused by the newly discovered severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), leading to a global pandemic, including Indonesia. Since the first confirmed SARS-CoV-2 on March 2 2020 [1], Indonesia has experienced exponential growth of COVID-19 cases in 34 provinces. As of May 2 2021, the government of Indonesia reported 1.677.274 confirmed COVID-19 cases and 45.796 deaths [2] and became one of the countries with high cumulative and incidence cases of COVID-19. The number of COVID-19 cases in Indonesia has been increasing rapidly since January, leading to the highest peak thus far, with provinces in Java making up 64% of the national cases. Two notable waves were observed from March 2020 to December 2021 **(Fig 1).** Based on the daily trend of COVID-19 cases, the first wave of COVID-19 cases was observed in January, with the highest number of reported cases reaching 14518 cases on January 16. After that, the cases fluctuated and reached the highest during the third wave in September 2022, with 56.757 recorded cases.

However, these case counts inevitably underestimate the true cumulative incidence of infection because of limited diagnostic test availability, barriers to testing accessibility, and asymptomatic infections, not to mention underreporting [2]. As a consequence, the national prevalence of SARS-CoV-2 remains unknown.

The lack of clarity about the number of SARS-CoV-2 infections across Indonesia limits the Indonesian government's ability to plan appropriately, prepare and respond to this epidemic. Monitoring the incidence of newly diagnosed cases of severe COVID-19 and the case fatality rate is critical to address the demands on the healthcare system.

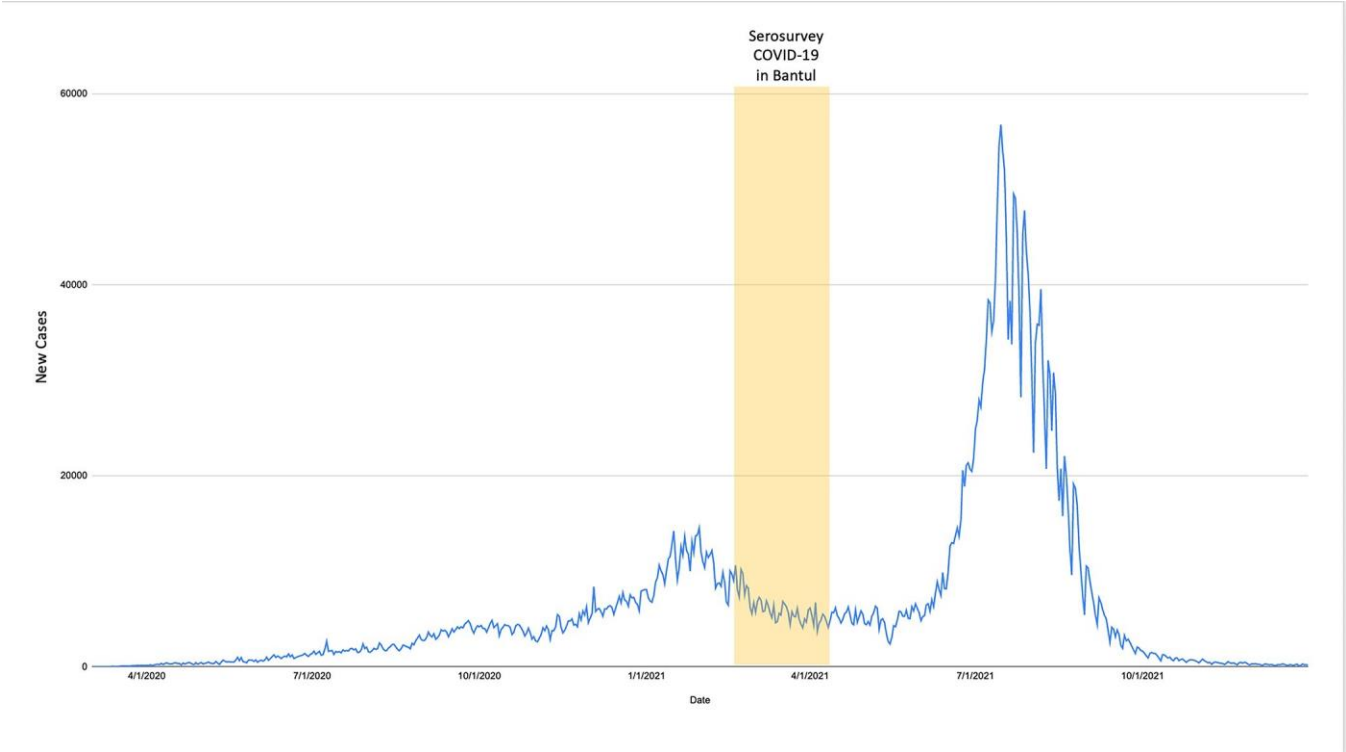

Fig 1. COVID-19 cases daily trend in Indonesia March 2020 –December 2021 (data source: https://ourworldindata.org/coronavirus/country/indonesia, accessed on October 4 2022).

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One of the epidemiological investigations used to determine the level of disease spread is to conduct a seroprevalence survey. According to the Centers for Disease Control and Prevention (CDC), this survey uses serological tests to detect antibodies in the blood, indicating an infection [3]. This test uses an enzyme-linked immunosorbent (ELISA) where the antigen used is purified SARS-CoV-2 S protein (without live virus). Population-based serological testing provides better estimates of the cumulative incidence of infection by complementing diagnostic testing of acute illness and helping to inform the public health response to COVID-19. A seroprevalence study could also be a powerful tool to detect subclinical infections and improve policy-making in the country [4,5]. 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 [2].

Seroprevalence varies geographically; the denser urban areas have higher seropositivity rates than rural areas [6]. A study in East Java, Indonesia, in the second semester of 2020 showed a higher prevalence in Surabaya (13.1%), which is an urban area, than in Jombang (9,9%), a rural area [7]. The epidemiological trend also implicates SARS-CoV-2 spread among rural communities only later in the epidemic wave [8,9], which would require sound anticipatory interventions. A seroprevalence survey involving more diverse groups of people among urban and rural communities is necessary to grasp the overall picture of SARS-CoV-2 infection. Bantul Regency has become one area that contributes to many cases and leads to the high transmission of COVID-19 in the Yogyakarta Provinces. Therefore, this study aims to estimate the SARS-CoV-2 seroprevalence, seropositive risk factors, and COVID-19 vaccine acceptance in Bantul Regency, Yogyakarta.

Materials and methods

Study setting

Bantul Regency is located in the southern region of Yogyakarta Province, covering 506.85 km^2 **(Fig 2).** The regency consists of 17 sub-districts and 75 villages, 30% semi-urban. Most adults are engaged in the non-formal sector, such as farming, trade and the service industry [10]. The daily mobility of residents between districts to and from Bantul is high, which may increase the risk of disease transmission [11].

Study design and sampling

A cross-sectional study was conducted from March to April 2021. The study population consisted of individuals who lived in the Bantul regency for at least six months, with 1,018,402 inhabitants [10]. EpiInfo was used to calculate the sample size.

Sampling was determined using a multistage cluster random sample adopted from the WHO/EPI rapid survey [12]. The calculated sample size was 414, which accounted for the estimated prevalence of 20%, 6% sampling error, a significance level of 0.05 with a design effect of 2 and a non-response rate of 15%. There were 40 clusters, with 11 participants per cluster. The cluster was based on the smallest administrative area called RT (*rukun tetangga* or household group). The RT consists of approximately 50–70 households.

We used systematic random sampling to select households at the selected cluster. In the selected households, we recruited all eligible household members, i.e. anyone who lived under the same building and resided in the study area for at least six months, with a minimum age of 5 years old.

Participants must meet the following inclusion criteria: residing in the study location at least six months before the survey commences, age of 5 years old or older, able to

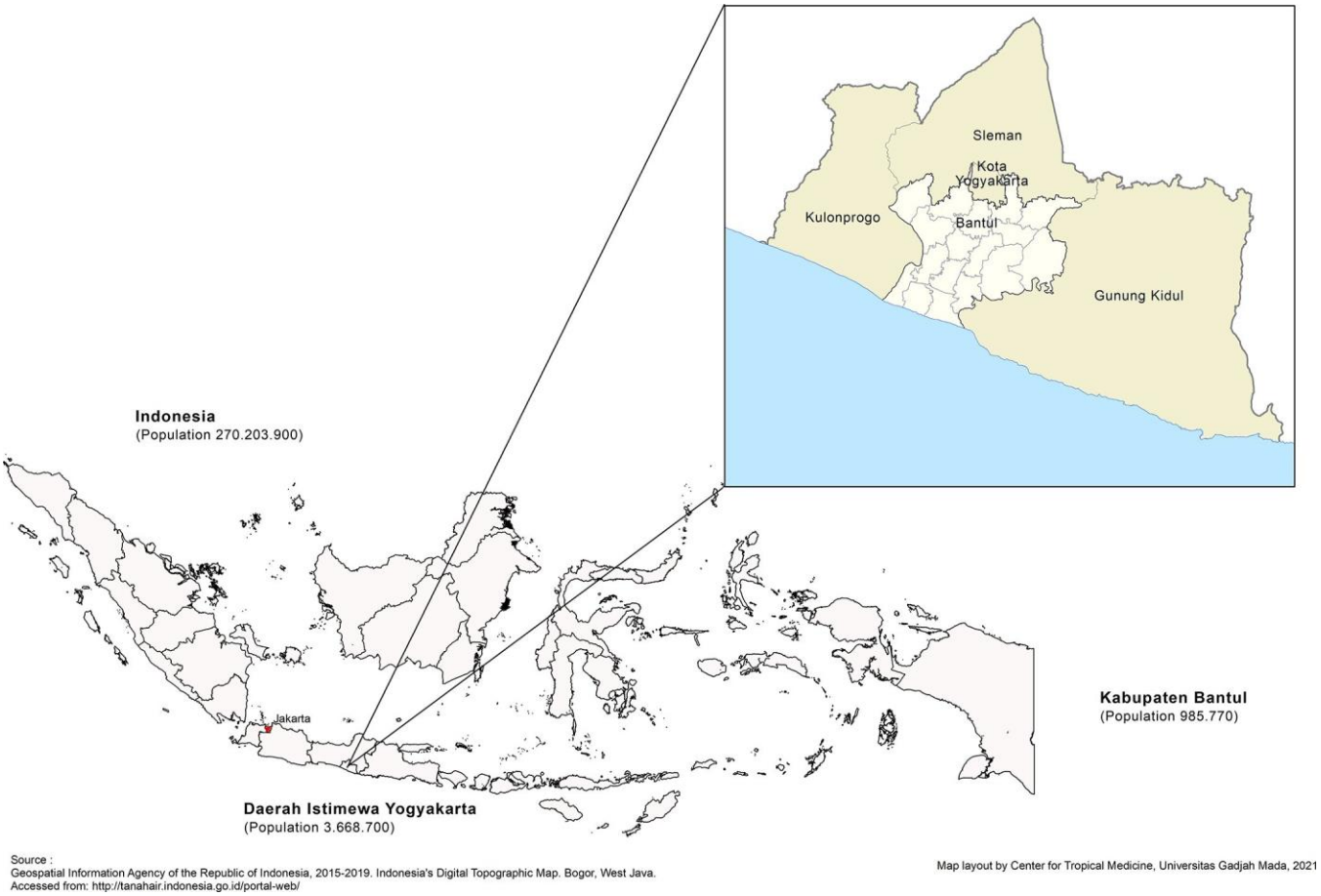

Fig 2. Map of Bantul Regency, Special Region of Yogyakarta, Indonesia.

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communicate verbally, give written consent to participate in the research or consent from parents/ guardians for respondents under 18 years old. Participants aged 13–17 were interviewed by their parents/guardians. However, for participants under 13 years old, interviews were conducted with the parents. Meanwhile, the exclusion criteria include people with existing chronic illnesses (including immunocompromised individuals with a history of blood disorders and people with mental disorders).

Data collection

A door-to-door visit was conducted to collect the primary data. Written informed consent was obtained from each study participant before data collection. Fifteen enumerators, including a phlebotomist, were involved in the data collection. Two supervisors were assigned to ensure the methodology and conduct the spot check. All study teams were involved in four training days regarding methods, data entry, phlebotomy and ethics in research.

Using an aseptic procedure, two millilitres of blood were drawn from a cubital vein with a disposable sterile syringe. The specimen was then kept in an EDTA tube. Before transportation, blood samples were placed in a cool box with an ice pack and transported within the same day to the laboratory. Blood samples were further stored in the refrigerator. Each participant's data included a unique identifier (barcode label) linked to their blood sample and data for tracking and confidentiality. The blood sample was transported, examined and stored in

the Laboratory of Microbiology, Faculty of Medicine, Public Health, and Nursing UGM. Plasma was then tested for Ig-G anti-SARS-Cov2 procedures using (Human Anti-2019 n-CoV (N) IgG ELISA Kit V1.5 FineTest [13].

Risk factors information at household and individual levels were obtained using an electronic structured questionnaire developed in the KoBo toolbox [14,15]. Variables included in the household questionnaire were socio-demographic information (age, gender, relationship with the head of household, and household income). Individual questionnaires were used to gather socio-demographic information (age, gender, highest education, occupation), COVID-19 vaccination status, previous diagnosis of COVID-19 and symptoms related to COVID-19 within the last six months, preventive actions taken, and mobility in the previous two weeks. The data manager validated data daily. A day after the data entry, the data manager sent the supervisor feedback and confirmation regarding completeness and data consistency.

Updated cumulative data on notified cases were obtained through the COVID-19 surveillance system conducted by Bantul District Health Office Bantul from March to April 2021. COVID-19 was defined as a symptomatic or asymptomatic person with a positive PCR result tested for SARS-CoV-2. Population numbers per sub-district were obtained through Bantul Statistical Bureau for calculating incidence per 1000 population [10]. In addition, data on urban-rural classification was obtained from the National Statistical Bureau [16].

Statistical analysis

Proportion and percentage describe the seroprevalence and socio-demographic characteristics of study participants. A map developed in ArcGIS spatially compared seroprevalence and incidence of notified cases by district and urban-rural status [17,18]. Bivariate analysis was conducted to identify the association between presumed risk factors (age, gender, occupation, comorbidities, prevention taken and mobility over the last two weeks) and anti-SARS CoV-2 seropositivity. After considering collinearity, a multiple logistic regression was used to evaluate risks between seropositive and seronegative groups with adjustment for sex. The P-value would be considered statistically significant at $p < 0.05$. All statistical analysis was performed using STATA 14.0. Raw data used for analysis is provided in (S1 Data).

Ethics

The Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada approved the study (Ref: KE/1242/12/2020). Written informed consent was obtained from adult respondents and parents of enrolled children. Confidentiality of information from the respondents was upheld with utmost care throughout data collection, processing and analysis for all data collected. Therefore, their names were included in the notes only for traceability and referral during the data analysis.

Results

Characteristics of study participants

A total of 425 people participated and were tested during the survey. See 1 File. The number of females (59.1%) is higher than that of males (40.9%); 47.2% of the participants were unemployed/students/housewives, and 72.5% lived in urban areas. The majority of the participants had no comorbidities and were not vaccinated.

The prevalence of SARS-CoV-2 seropositivity among the participants in this study was 31.1% (n = 132/425). A significant difference was observed in the seroprevalence among age

groups (p = 0.000), with the highest proportion reported in the 55–64 age group (43.7%; n = 31/71). Meanwhile, the under-15 years age group showed no seropositivity.

A significant difference was also observed among occupation groups (p = 0.009). The highest seroprevalence was demonstrated by participants working as daily workers/farmers (37.2%), followed by professional/health workers (34.6%), and unemployed/students/housewives (26%). Seroprevalence did not differ between semi-urban and urban areas, even though we observed that semi-urban areas had higher seroprevalence (34.2%; n = 40/117) than urban areas (29.9%; n = 92/308). Females demonstrated higher seroprevalence; nevertheless, no association was found between genders and seropositivity. Seropositivity also does not differ in occupation category (Table 1).

This study also explored seroprevalence based on the presence of selected chronic diseases and the history of comorbidities. Participants with a history of chronic conditions accounted for 22.6% of the total participants. Among those with comorbidities, seroprevalence for participants with obesity, diabetes mellitus, and hypertension was 50%, 40% and 35.4%, respectively.

Participants with a prior diagnosis of COVID-19 in the last six months accounted for 1 out of 132 seropositive cases. Seroprevalence among participants with no previous experience of COVID-19-related symptoms was found to be 31.1%. Seropositivity was observed in 14 participants from seven households, making family clusters account for 10.6% of the total positive cases.

Most of the participants in this study reported decreased social interactions and mobility during this period. Study participants stated that they attended fewer traditional and religious activities (90.8%), had reduced visits to relatives and friends (90.1%, decreased visits to markets (60.9%), and spent more time at home, except for essential tasks ((73.7%). If participants did leave home, they reported wearing masks (89.4%), regular washing of hands for 20 seconds with running water (80.9%) and maintaining physical distancing in public areas (64.5%). However, no significant differences were observed between individual preventive actions and the level of mobility with seropositivity status.

Seroprevalence of anti-SARS-CoV-2 based on the geographical distribution

The highest seroprevalence was observed in 3 semi-urban areas (Pajangan, Dlingo, Sanden). Most semi-urban areas have relatively higher seroprevalence than urban areas. However, we found no statistical significance **(Fig 3).** The highest distribution of confirmed cases acquired from routine regional data reported higher cumulative cases in urban areas, such as Banguntapan, Bantul, Sewon, and Jetis.

Risk factors associated with SARS-CoV-2 seropositivity

This study further explored the risk factors associated with the seropositivity of SARS-CoV-2, adjusting for age, sex, occupation, comorbidities, and vaccination status. A significant association was observed within specific age groups. The odds of SARS-CoV-2 seropositivity are higher in the age 55–64 (adjusted odds ratio [aOR] = 3.79; 95% CI 1.46–9.85, p = 0.006).

Females demonstrated higher seroprevalence. Nevertheless, no association was found between genders and seropositivity. Seropositivity also does not differ in occupation category (aOR = 0.81; 95% CI 0.50–1.31, $p > 0.005$) (Table 2).

Comorbidities were presumed as one of the risk factors of SARS-CoV-2 seropositivity. The odds of SARS-CoV-2 seropositivity were higher in participants with obesity (aOR = 2.46; 95% CI 0.57–10.65) and diabetes mellitus (aOR = 1.12; 95% CI 0.38–3.31) than participants without each respective comorbidity. However, this finding is not statistically significant (p-value$>$0.05).

Table 1. Distribution of study participants by background characteristics.

Characteristic	Total	IgG-positive	IgG-negative	P-value
	n = 425	n = 132	n = 293	
Sex, n (%)				
Male	174 (40.9)	52 (29.9)	122 (70.1)	0.663
Female	251 (59.1)	80 (31.9)	171 (68.1)	
Age group, years, n (%)				
< = 14	16 (3.8)	0 (0.0)	16 (100)	< 0.001
15–24	52 (12.2)	8 (15.4)	44(84.6)	
25–34	51 (12.0)	8 (15.7)	43(84.3)	
35–44	90 (21.2)	33 (36.7)	57(63.3)	
45–54	92 (21.7)	32 (34.8)	60 (65.2)	
55–64	71 (16.7)	31 (43.7)	40 (56.3)	
65+	53 (12.5)	20 (37.7)	33 (62.3)	
Occupation, n (%)				
Unemployed/students/Housewives	200 (47.2)	52 (26.0)	148 (74.0)	0.009
Professional/health worker	130 (30.7)	45 (34.6)	85 (65.3)	
Daily worker/farmer	94 (22.2)	35 (37.2)	59 (62.8)	
Residence set, n (%)				
Urban	308 (72.5)	92 (29.9)	216 (70.1)	0.390
Semi-urban area	117 (27.5)	40 (34.2)	77 (65.8)	
Smoking, n (%)				
Smoker	74 (18.1)	14 (18.9)	60(81.1)	0.007
Non-smoker	335 (81.9)	118 (35.2)	217(64.8)	
History of chronic disease, n (%)				
Yes	96 (22.6)	37 (38.5)	59 (61.5)	0.072
No	329 (77.4)	95 (28.9)	234 (71.1)	
Diabetes mellitus, n (%)				
Yes	15 (3.5)	6 (40.0)	9 (60.0)	0.514
No	394 (92.5)	126 (32.0)	268 (68.0)	
Hypertension, n (%)				
Yes	65 (15.9)	23 (35.4)	42 (64.6)	0.559
No	344 (84.1)	109 (31.7)	235 (68.1)	
Obesity, n (%)				
Yes	8 (1.9)	4 (50)	4 (50)	0.279
No	401 (98.1)	128 (31.9)	273 (68.1)	
Previous COVID-19 diagnosis, n (%)				
Yes	3 (0.7)	1 (33.3)	2 (66.7)	1.000
No	422 (99.3)	131 (31.0)	291 (68.9)	
COVID-19 symptoms n (%)				
Yes	155 (36.4)	44 (28.4)	111 (71.6)	0.4
No	270 (63.4)	88 (32.6)	182 (67.4)	
COVID-19 vaccination, n (%)				
Yes, at least one dosage	13 (3.1)	9 (69.2)	4 (30.8)	0.005
Not yet	412 (96.9)	123 (29.9)	289 (70.2)	
Preventive measures and mobility				
Wearing masks when going out				
Always	380 (89.4)	116 (30.5)	264 (69.5)	0.491
Not always	45 (10.6)	16 (35.6)	29 (64.4)	

(*Continued*)

Table 1. (Continued)

Characteristic	Total	IgG-positive	IgG-negative	P-value
	n = 425	n = 132	n = 293	
Washing hands for at least 20 seconds with running water				
Always	344 (80.9)	106 (30.8)	238 (69.2)	0.822
Not always	81 (19.1)	26 (32.1)	55 (67.9)	
Maintain a physical distancing (1-2m) in the public area				
Always	274 (64.5)	82 (29.9)	192 (70.1)	0.497
Not always	151 (35.5)	50 (33.1)	101 (66.9)	
Mobility				
Attending invitations to traditional or religious activities (e.g. weddings, funerals)				
Always	39 (9.2)	14 (35.9)	25 (64.5)	0.493
Not always	386 (90.8)	118 (30.6)	268 (69.4)	
Visiting relatives or friends or other people due to important matters				
Always	42 (9.9)	13 (30.9)	29 (69.1)	0.987
Not always	383 (90.1)	119 (31.1)	264 (68.9)	
Going to the markets/shops/offices/crowds				
Always	166 (39.1)	50 (30.1)	116 (69.9)	0.738
Not always	259 (60.9)	82 (31.7)	177 (68.3)	
Staying at home, except for essential matters				
Always	313 (73.7)	94 (30.1)	219 (69.9)	0.444
Not always	112 (26.3)	38 (33.9)	74 66.1)	

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Although vaccination had not been made available to the general public during the data collection period, most participants (91.5%) were aware that the government would provide vaccinations in the future and stated their willingness (79.1%) to be vaccinated. However, a small proportion (19.1%) said they did not want to be vaccinated (Table 3). Reasons for this included fear of adverse effects, concerns about the safety and effectiveness of the vaccines, and religious beliefs.

Discussion

This SARS-CoV-2 seroprevalence study found that the prevalence of IgG antibodies against SARS-CoV-2 was 31.1% in Bantul, significantly higher than the 11.4% seropositive which was found in a study in East Java in late 2020 [7]. On the other hand, surveillance data of Bantul Regency DHO reported a considerably lower cumulative incidence of 1.1% during the study. This finding is in line with previous research suggesting that the estimates obtained from seroprevalence were 18.1 times higher than the corresponding cumulative incidence of COVID-19 infections, implying that confirmed cases are a poor indicator of the extent of the disease spread [19]. While antigen testing and Polymerase Chain Reaction (PCR) to monitor COVID-19 detect the presence of specific antigens or genetic material indicating current viral infection, serological tests for COVID-19 are used to detect antibodies that the body produces in response to the virus. Serological tests can detect antibodies in people who have been infected with COVID-19 in the past, even if they were asymptomatic or had mild symptoms. Those can help identify people who may have been infected but were never diagnosed. In population-level studies, serological testing is used to estimate the proportion of people who have been infected with COVID-19 in a given area.

Seroprevalence varies geographically, and previous research indicated that urban areas mainly reported a higher number of seropositivity [6]. The reported COVID-19 cases in the

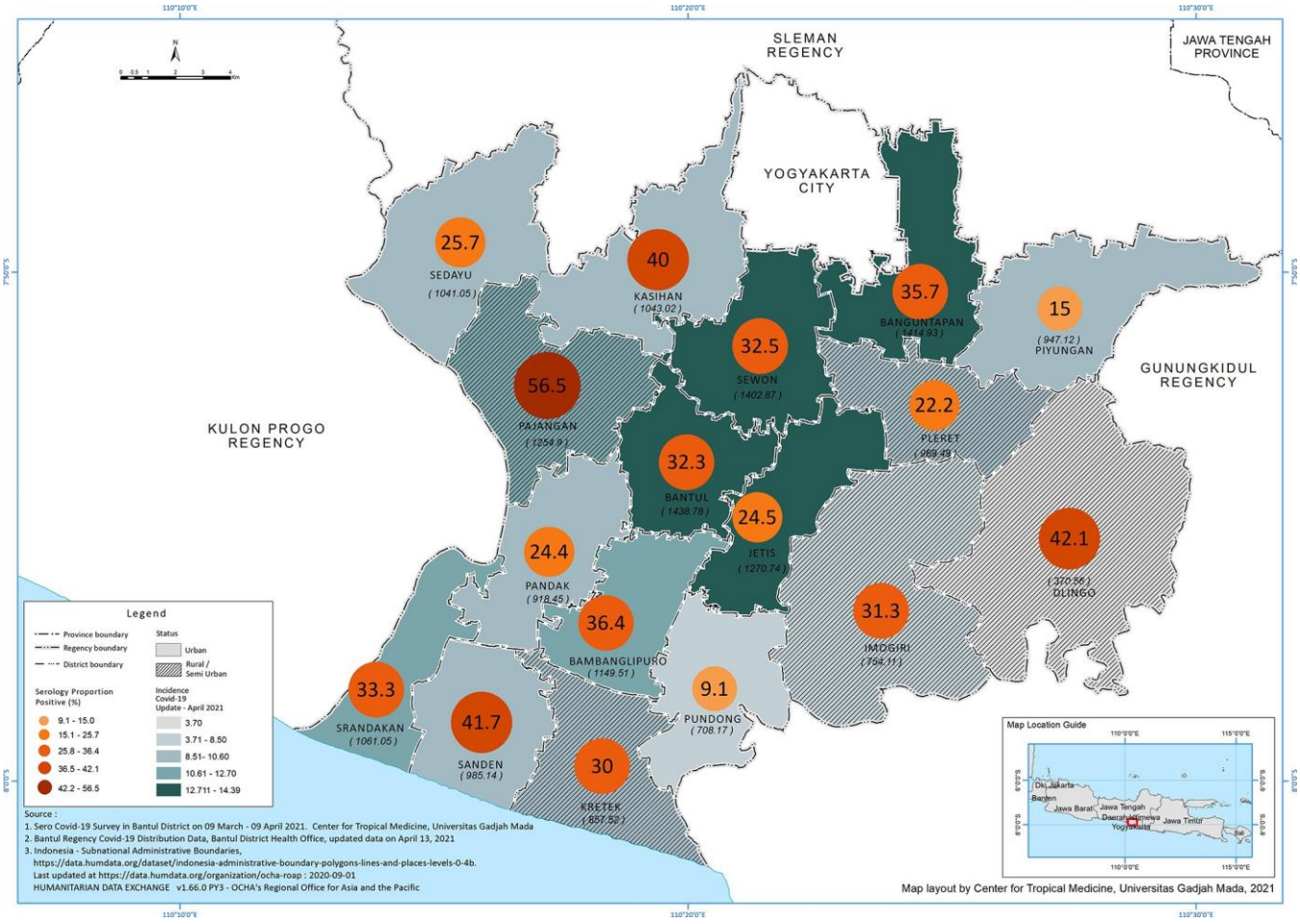

Fig 3. Seroprevalence and reported cumulative incidence of SARS-Cov-2 in April 2021 Based on District.

https://doi.org/10.1371/journal.pgph.0000698.g003

Bantul regency also showed a similar trend, in which more cases were reported in urban areas (Bantul, Banguntapan, Sewon, and Jetis). However, our study showed that the three highest seropositivities were observed in semi-urban areas (Pajangan, Dlingo and Sanden). Meanwhile, areas with the highest reported COVID-19 cases showed lower seropositivity. Thus, this study suggest that the higher transmission observed in semi-urban areas was due to lower access to health services, including the lack individual testing, resulting in unreported positive cases. These discrepancies can be explained by the limited and centralized diagnosis facilities when the study was conducted.

Urban areas with dense populations were impacted earlier at the pandemic's beginning. A previous study on epidemiological modelling predicted that COVID-19 spread faster in urban than rural areas [20]. Despite the rapid transmission, urban areas are usually supported by better surveillance management, such as more accessible testing, tracing, and infection control. Consequently, cases in urban areas were reported faster and earlier.

Meanwhile, rural areas usually have shortages in resources and workforces, which affects their ability to detect, respond, prevent, and control infectious disease outbreaks [21,22]. In this study, a large proportion of seropositivity was reported in participants with no prior diagnosis of COVID-19, indicating that the infection might be undiagnosed due to a lack of individual testing. Thus, this study suggests that the higher transmission observed in semi-urban

Table 2. Factors associated with SARS-Cov-2 seropositivity in Bantul Regency (N = 425).

Risk Factors	ELISA+ (n = 132)	Adjusted OR (95% CI)	*p*-value
Age group			
< = 14	0	NA	NA
15–24	8	Reference	NA
25–34	8	0.93 (0.31–2.79)	0.893
35–44	33	2.71 (1.08–6.81)	0.034
45–54	32	2.67 (1.05–6.80)	0.040
55–64	31	3.79 (1.46–9.85)	0.006
65+	20	3.38 (1.27–9.00)	0.015
Sex			
Male	52	0.81 (0.50–1.31)	0.388
Female	80	Reference	NA
Occupation			
Unemployed/students/Housewives	52	Reference	NA
Professional/health worker	45	1.14 (0.64–2.00)	0.661
Daily worker/farmer	35	1.27 (0.70–2.31)	0.441
Comorbidities			
Hypertension	23	0.87 (0.48–1.57)	0.646
Obesity	4	2.46 (0.57–10.65)	0.229
Diabetes Mellitus	6	1.12 (0.38–3.31)	0.832
Vaccination Status			
Yes	14	Reference	NA
No	13	0.19 (0.05–0.68)	0.010

https://doi.org/10.1371/journal.pgph.0000698.t002

areas was lower access to health services, including lack of individual testing, resulting in unreported positive cases.

Higher seroprevalence in this study was observed in females. However, no statistically significant gender difference was reported. A previous study also suggested that SARS-CoV-2 IgG/IgM dynamic is mainly affected by age and disease severity, not sex [23]. Higher seropositivity was observed in the age group 55–64, and this finding supports many previous studies that indicate older age as one of the risk factors for COVID-19.

This research found a portion of seropositivity among people who primarily stayed at home. Additionally, occupations requiring less mobility, such as housewives, students, and the unemployed, also accounted for seropositivity. The research also discovered a tenth of family clusters, suggesting that the transmission may have occurred at the household level. Previous research contends that the primary mode of COVID-19 infection is through the household spread [24,25].

Table 3. Vaccine acceptance of the participants (N = 425).

Variables	N = 425	Percentage
Aware that the government would provide the vaccination for the people		
Yes	389	91.5
No	36	8.5
Willing to get vaccinated if the government provide the vaccines		
Yes	336	79.1
No	81	19.1
Not decided	8	1.9

https://doi.org/10.1371/journal.pgph.0000698.t003

Our study found no difference between high and low mobility, suggesting they were already widespread community transmission, even in rural areas. Preventive measures did appear to prevent acquiring an infection, which might be because it is challenging to do preventive measures in a household setting or to practice preventive measures consistently outside [26].

Surveillance and containment measures such as large-scale social restrictions and other anticipatory prevention tools become a priority to curb transmission, primarily focusing on areas with lower access to health services. Earlier in the pandemic, outbreak containment was concentrated in the dense urban areas, which may have reduced the number of cases. However, due to high mobility, the transmission slowly moved to semi-urban and rural communities. People living in rural communities might develop a false sense of security and take fewer precautions at the beginning than the urban communities [8,9].

One of the most effective ways to prevent fatalities caused by COVID-19 is through vaccination. Due to the lack of a vaccination program for general populations during the data collection period, it is reasonable that higher seroprevalence was found in unvaccinated groups. As this study explored the seroprevalence when most people were still unvaccinated, a better picture of the infection spread in Bantul was obtained since there was no implication from vaccine-induced antibodies.

Despite the findings, this study has the following limitations. Following a natural infection, antibody titers peak and begin to wane in various manners, with some in shorter duration [27]. However, this study did not consider the assay performance concerning the waning immunity. Thus it may underestimate the true prevalence. Furthermore, the analysis results only showed the association, not causality. Finally, as a cross-sectional analysis, this study only analyzed the variables at one point of time and did not explore the seroprevalence changes over a longer period. Therefore, future research is needed to conduct a periodic or longitudinal survey to determine the prevalence in the longer term.

Conclusion

This serosurvey demonstrated a higher seroprevalence than reported data in the same period. Based on the findings, it is strongly recommended that the local government strengthen the surveillance and 3T (testing, tracing, and treatment) efforts by involving the task force at the neighbourhood community and village levels throughout Bantul Regency, particularly in areas with lower access to health services. Besides that, it needs to increase awareness and implementation of health protocols for high-mobility individuals to prevent transmission within the household. As vaccination program for the general population are being rolled out, it is crucial to provide adequate implementation information, including health resources and logistics support. This study can be implemented in other areas, both at the district/city and provincial levels, to understand the seroprevalence of SARS-CoV-2 in Indonesia better. This study provides a district-level view of the extent of COVID-19 spread and a different approach to conducting serosurvey among diverse populations in various regions to fit the gaps in understanding COVID-19's spread globally.

Supporting information

S1 Table. The response rate of each district.
(PDF)

S1 Data. Data of Seroprevalence of SARS-CoV-2 in Yogyakarta.
(XLSX)

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