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Implementing SARS-CoV-2 routine surveillance in antenatal care in Zambia, 2021–2022: best practices and lessons learned

Tannia Tembo[1*], Elizabeth Heilmann[2,3], Bupe Mulenga Kabamba[4], Sombo Fwoloshi[5], Kalubi Kalenga[1], Felix Chilambe[5], Mpanji Siwinga[5], Marie-Reine Rutagwera[4], Maximillian Musunse[4], Chabu Kangale[4], Samuel Yingst[3], Ruchi Yadav[6], Theodora Savory[1], Julie R. Gutman[6], Izukanji Sikazwe[1], Lloyd B. Mulenga[5], Carolyn Bolton Moore[1,7†] and Jonas Z. Hines[3†]

Abstract

Background In Zambia, the true extent of SARS-CoV-2 infections is unknown because initial surveillance focused on patients with symptoms or severe disease. Antenatal sentinel surveillance had not been used to assess infection trends. The ANC COVID-19 surveillance study sought to determine SARS-CoV-2 seroprevalence and COVID-19 vaccine uptake among pregnant women. We provide insight into the study implementation, challenges encountered, best practices, and lessons learned.

Methods A repeated cross-sectional seroprevalence survey was implemented at 39 health facilities in four districts from September 2021 to September 2022. Pregnant women aged 15–49 years were enrolled at their first antenatal care visits. An electronic questionnaire gathered demographics and other COVID-19 related information from consenting participants. A dried blood sample was collected to detect IgG antibodies using a multiplex bead assay. Seropositive results were categorized as infection, infection and vaccination or infection based on anti-RBD and anti-nucleocapsid test results. Problems and their root causes were identified as they occurred. Practical problem-solving strategies were devised, implemented, and monitored to ensure that goals were accomplished.

Results In the primary analysis, 7% of the 9,221 samples collected from participants were not tested because they were missing. COVID-19 vaccine uptake of 9,111 pregnant women was assessed. Approximately 64% of participants were cumulatively seropositive for SARS-CoV-2 antibodies. Seroprevalence increased from 27.8% in September 2021 to 56.6% in July 2022. We observed an increase in vaccine coverage (0.5–27%) over time. Women aged 40–49 years old, without education and with prior COVID-19 infection were associated with higher vaccine uptake. The Delta variant of COVID-19 and the reallocation of health facilities between two partners delayed surveillance activities and increased the cost of implementation (e.g., the purchase of additional calibration and validation kits and DBS cards).

†Carolyn Bolton Moore and Jonas Z. Hines contributed equally to this work.

*Correspondence:
Tannia Tembo
Taniya.Tembo@cidrz.org

Full list of author information is available at the end of the article

Protocol deviations were attributed to the lack of experience in conducting research but, the district RAs repeatedly trained health facility staff to enhance their research knowledge.

Conclusions Incorporating SARS-CoV-2 surveillance into routine antenatal care is feasible and potentially sustainable when existing health system infrastructure, human resources, and surveillance systems are leveraged. Yet, careful planning is needed to anticipate implementation challenges and ensure high-quality data collection.

Keywords Prenatal care, COVID-19, SARS-CoV-2, Sentinel surveillance, Seroprevalence

Background

In Zambia, the Ministry of Health (MOH) and the National Public Health Institute (ZNPHI) conducted health facility-based routine surveillance of COVID-19 cases to monitor the epidemic [1]. Due to the large proportion of asymptomatic infections, testing limitations, and surveillance gaps, the true extent of SARS-CoV-2 infections was unknown and could have been greater than that detected passively through COVID-19 testing. Seroprevalence surveys can provide better estimates of SARS-CoV-2 exposure than official case counts [2]. During a population-based study in six districts in Zambia in July 2020, the ratio of reported cases to estimated infections ranged from 1:1012 (Livingstone District) to 1:21 (Nakonde District) [3].

Implementing seroprevalence and other field studies is resource-intensive and requires training, transportation, additional supplies, and focused data management. If implementing surveillance activities during ongoing community transmission of epidemics such as SARS-CoV-2, additional strategies for risk mitigation are required. In the absence of sufficient resources to conduct active surveillance in resource-limited settings like Zambia, personnel may experience more pronounced challenges during field work such as poor road networks and limited internet connectivity. Such challenges can be tackled when existing platforms for routine health services are leveraged, thereby reducing duplication of efforts toward service provision and/or study implementation.

The Antenatal Care (ANC) clinics were recognized as a potential platform for surveillance of SARS-CoV-2 antibodies that could provide key information into transmission trends in pregnant women in Zambia. Previously, pregnant women have served as a proxy for the healthy adult population in sentinel surveillance for routine testing of HIV and malaria [https://data.unaids.org/publications/irc-pub06/jc954-anc-serosurveys_guidelines_en.pdf]. Active surveillance can provide greater insight into the proportion of pregnant women who had previous exposure to SARS-CoV-2 enable the country to monitor changes in seroprevalence, and potentially identify increasing positivity rates. However, only a few African countries have leveraged routine ANC services to conduct SARS-CoV-2 surveillance during the 2020–2023 epidemic [4]. Between April and August 2020, SARS-CoV-2 seroprevalence among residual serum obtained from first ANC attendees in Ethiopia and South Africa ranged from 0 to 38% [5, 6]. In this study, we hypothesized that SARS-CoV-2 serologic testing during routine ANC could provide key information about the trends in COVID-19 infection and vaccine uptake among pregnant women in Zambia. The following discussion emerged from implementing the "*Assessing SARS-CoV-2 Seroprevalence during Routine Antenatal Care Visits in Zambia (*ANC COVID-19 Surveillance)". We revisit the implementation journey and describe the challenges encountered, best practices used to attain the best outcomes, successes realized, and lessons learned to inform similar surveys in similar settings.

Methods

With support from the U.S. Centers for Disease Control and Prevention (CDC), the Centre for Infectious Disease Research in Zambia (CIDRZ), and PATH, in collaboration with the MOH and ZNPHI, implemented the "*Assessing SARS-CoV-2 Seroprevalence during Routine Antenatal Care Visits in Zambia (*ANC COVID-19 Surveillance)" Study - a non-randomized assessment - to determine COVID-19 vaccine uptake and the feasibility of SARS-CoV-2 serologic testing among pregnant women attending first ANC visits as a surrogate for SARS-CoV-2 seroprevalence in the general population.

The CIDRZ study protocol was approved by the National Health Research Authority (NHRA) - Zambia's health research regulatory body - through an expedited review of COVID-19 related research. PATH sought approval from the University of Zambia Biomedical Research Ethics Committee (UNZABREC) to amend a pre-existing malaria surveillance protocol to include SARS-CoV-2 seroprevalence survey in Chadiza district.

Tasks completed during the implementation of the ANC COVID surveillance study stakeholder engagement

Following ethical approval of the CIDRZ and PATH study, protocols, data forms, and informed consent forms were shared with provincial and district health directors to obtain authorization for study implementation at the selected health facilities. Health facility staff were also

approached to schedule trainings and community sensitization meetings.

Study trainings and community sensitization meetings

Health care workers (HCWs) and community-based volunteers (CBVs) participated in 5-day trainings, which included practical sessions tailored to staff-specific (midwives and laboratory technicians) roles during the implementation. Facility staff were trained in the protocol, research ethics, data and sample collection and completed an assessment to determine levels of competence. They led community sensitization meetings at their respective health facilities with members of neighborhood health committees (NHCs). The study coordinator, data coordinator, and RAs were on hand to answer questions.

Study design, study setting, and participant sampling

Repeated cross-sectional seroprevalence surveys were conducted at 39 purposefully selected health facilities in Chadiza, Chipata, Chongwe, and Lusaka districts from September 2021 to September 2022. Community Based Volunteers provided information about the ANC COVID Surveillance study to pregnant women during group health education. We used a facility-specific sampling interval based on historic monthly mean ANC attendance a year before the study was implemented to select eligible participants. However, some facilities transitioned to a convenience sampling approach to recruit the expected number of participants due to inadequate staff and fluctuating ANC attendance. A district Research Assistant (RA) obtained written informed consent from pregnant women aged 15–49 years before enrollment. Pregnant women below the age of 18 provided their own informed consent as they were considered emancipated minors as per law in Zambia. Routine ANC services and study procedures were conducted simultaneously.

Sample collection, transfer, and SARS-CoV-2 serologic testing

Using a finger prick or venipuncture, a dry blood spot (DBS) sample was collected on filter paper for a SARS-CoV-2 antibody test at the same time as when a sample was drawn for routine HIV, hepatitis B, and syphilis tests. Samples were immediately labeled with a sticker preprinted with a unique study Identification (ID). At the beginning of implementation, samples from Chipata, Chongwe, and Lusaka districts were transported via the existing HIV viral load sample courier system directly to the centralized testing laboratory at the University Teaching Hospital (UTH). Later, samples were transported to the central laboratory by respective RAs. Chadiza samples were transported to the National Malaria Elimination Centre (NMEC) laboratory by PATH study personnel. Serologic testing was done using the Tetracore FlexImm Array SARS-CoV-2 Human IgG Antibody Test (Tetracore Inc, Rockville, MD, USA) on the MAGPIX platform (Luminex Corp, Austin, TX, USA) [7]. Four controls were incorporated into every test plate and three different SARS-CoV-2 antigens were used in the multiplex assay (receptor binding domain (RBD) of the spike-1 protein, nucleocapsid protein (NP), and anN-RBD fusion protein). Sample analysis was batched, and up to 20% of samples were re-run for quality assurance [https://doi.org/10.1111/irv.13143].

For the primary analysis, seroprevalence was estimated as the number of participants with a positive test result by the number of participants with a valid SARS-CoV-2 test result by district and month. Seroprevalence was adjusted based on assay sensitivity (89.8%) and specificity (100%) from an independent test [10]. A trend analysis compared SARS-CoV-2 seroprevalence and district-specific COVID-19 confirmed cases provided by the ZNPHI [11]. Based on the decision tree developed by Duarte et al. [12], a sub-analysis of target-specific assay results and COVID-19 vaccination status grouped antibody responses into no evident response (anti-RBD positive, vaccinated, anti-N negative) or vaccination and infection (anti-RBD positive, vaccinated, anti-N positive). A detailed account of the serologic test and results analysis is described by Heilmann et al. [13]

Data collection and analysis

The study questionnaire was programmed using Open Data Kit (ODK - get ODK Inc., San Diego, CA, USA) and administered to participants via a tablet, but paper-based questionnaires were available as backup. The questionnaire collected demographic data, HIV, hepatitis B and syphilis test results, and SARS-CoV-2 exposure. COVID-19 vaccinations were self-reported but verified from vaccination cards if participants had them available on the day of data collection. To assess risky behavior, the questionnaire gathered information about participants' inter and intra-district travel, engagement in social activities, and use of personal protective clothing during the pandemic. Data were reviewed for accuracy and completeness while the participant was still at the health facility and before forms were uploaded to a central server. A paper screening and eligibility log was filled out by the midwife and reviewed by the district RA at the health facility, weekly. To maintain confidentiality and maintain linkage between individual paper questionnaire (if used), laboratory requisition form, and DBS cards, participants' records were de-identified and allocated a unique 16-digit study identification numbers were printed on adhesive labels in triplicate for linkage between each participant's DBS card, hard copy questionnaire (if used), and consent form. We used "R" and Stata to analyze

the seroprevalence data and vaccination uptake data, respectively.

Results

A summary of study activities and achievements is shown in Table 1. Detailed results are available elsewhere [13, 14]. Initially, the study trained 113 facility staff and oriented 870 NHC members. Of the 9,221 samples drawn from the participants, 8,558 (92.8%) samples were tested by serology. At least 7% of the remaining samples had either gone missing or could not be linked to questionnaire data, hence their exclusion from the primary analysis. At least 8,304 (97.0%) had valid antibody results, 63.8% of which were SARS-CoV-2 seropositive. Seropositivity was highest in Lusaka and lowest in Chadiza. In the secondary analysis, only 2.2% of women reported having COVID-19 before the study was implemented. Less than 5% reported being in close contact with a suspected or confirmed COVID-19 case within or outside their household. Approximately half of the participants reported or were observed to be wearing a face mask during the ANC visit, while 96.7% had received an HIV test as compared to less than half who were tested for syphilis (40.2%) and hepatitis B (11.7%) [13].

COVID-19 vaccine uptake among pregnant women was at 0.5% at the beginning of the study but increased to 27% by September 2022. When point estimates were compared by district, uptake varied between 2.8% and 17.4% in September 2021 and 21.8% and 29% in September 2022. Of the 3,789 participants who reported never being offered a COVID-19 vaccine, 35.3% said they would not take it because of safety concerns and vaccine effects [14].

Solving implementation challenges using best practices

Division of study districts between implementing partners and reduced number of implementing health facilities

This study's protocol was modeled after the malaria surveillance project funded by the National Malaria Elimination Program and PATH's Program for the Advancement of Malaria Outcomes [15]. Consequently, PATH expanded its existing malaria surveillance study to include SARS-CoV-2 in Chadiza dstrict, while CIDRZ implemented study activities in Chipata, Chongwe, and Lusaka districts. This approach required the revision of the study questionnaire and retraining of staff to enable them to undertake malaria and ANC COVID-19 surveillance simultaneously. This approach also required close coordination and collaboration between the two partners to ensure that study processes were implemented similarly across the 39 health facilities. All participants were

Table 1 Study outputs and outcomes

Activity	District	Output	
		Planned	Achieved
Study site selection	Chadiza	10 public health care facilities	10 public health care facilities
	Chipata	10 public health care facilities	10 public health care facilities
	Chongwe	10 public health care facilities	10 public health care facilities
	Lusaka	9 public health care facilities 1 private health care facility	9 public health care facilities
Stakeholder engagement	Chadiza, Chipata, Chongwe, and Lusaka	2 Provincial Health Directors 4 District Health Directors 4 MNCH Coordinators 4 Laboratory Scientists	2 Provincial Health Directors 4 District Health Directors 4 MNCH Coordinators 4 Laboratory Scientists
Study trainings	Chadiza,	156 Midwives, Phlebotomy staff and CBVs	28 staff[†]
	Chipata		32 staff
	Chongwe		27 staff
	Lusaka		26 staff
Community sensitization meetings	Chadiza, Chipata, Chongwe and Lusaka	40 Community sensitization meetings 30 NHC members	39 Community sensitization meetings 30 NHC members
Participant recruitments	Chadiza	2,600 participants	1,755 participants[§]
	Chipata	2,600 participants	2,396 participants
	Chongwe	2,600 participants	2,527 participants
	Lusaka	2,600 participants	2,332 participants
Samples tested	Chadiza	2,600 samples	[§ 1],755 samples
	Chipata	2,600 samples	2,161 samples
	Chongwe	2,600 samples	2,455 samples
	Lusaka	2,600 samples	2,187 samples

[†]Another 30 staff were retrained after incorporation of the ANC COVID-surveillance protocol into the existing malaria surveillance pilot. [§]Participant recruitment ended in July 2022, MNCH: maternal, newborn, and child health; CBV: community-based volunteer; NHC: neighborhood health committee

enrolled from 39 out of the 40 health facilities that participated in the survey. The only private hospital that was included in the survey withdrew its participation before study implementation began due to the perceived additional time required to perform study procedures and the costs associated with implementing the study. The sample framework was recalculated to ensure the estimated sample size was achieved from the 39 participating health facilities.

Delayed implementation due to the delta variant of SARS-CoV-2

Field activities were initiated in June 2021 before Zambia experienced a third wave of COVID-19 caused by the Delta variant, the most severe to affect Zambia (Table 2) [16]. To contain its increasing spread, the MOH announced and enforced strict COVID-19 mitigation measures including prohibiting in-person workshops from 16th June 2021 [17]. The NHRA temporarily suspended research activities that were deemed high risk for researchers and participants during this period of intense SARS-CoV-2 transmission [18]. These restrictions impacted and delayed study implementation. Community sensitization meetings in Chipata District were halted for three months while participant enrollment did not begin until September 2021 (CIDRZ) and October 2021 (PATH).

In August 2021, the NHRA lifted the ban on the implementation of research activities and only when the study demonstrated adequate COVID-19 risk mitigation procedures (e.g., wearing of masks, hand hygiene, Infection Prevention and Control (IPC), SARS-CoV-2 testing and treatment, and COVID-19 vaccination) did the study-related activities recommence. All potential study staff were screened for SARS-CoV-2 symptoms using an MOH screening checklist and tested with a rapid antigen test before registration for the training. This helped reduce the likelihood of trainings being at the core of SARS-CoV-2 transmission. Staff who tested positive were referred to a health facility for a confirmatory nucleic acid amplification test (NAAT) and did not participate in the training. Additionally, training sessions were limited to 30 participants to accommodate social distancing.

Task-shifting between routine ANC services and study implementation

The study leveraged existing resources (e.g., MOH staff and infrastructure) in the ANC clinics to reduce implementation costs, enhance the acceptability of the study, and counteract hesitancy around COVID-19 activities. Nurse-midwives were selected from a limited pool of staff at implementing health facilities and were offered 30 Zambian Kwacha (approximately 1.85 USD) per participant enrolled to reinforce their participation in activity implementation (which is common for studies in Zambia). They continued to provide routine ANC services and were still entitled to permissible vacation time and study leave.

Although facility staff were trained in research ethics, some had difficulties adhering to research and protocol requirements during implementation. Consequently, the study reported several protocol deviations, such as the recruitment of a minor or the consenting of an illiterate participant, both in the absence of a witness. To mitigate the impact of staff absences and enhance their adherence to research ethics and study protocol, the district RAs conducted repeated onsite orientations for health facility staff (including those who had not been trained before). They also conducted supervision visits during which they provided support for participant recruitment when health facility staff were unavailable.

Mislabeled and missing samples

At the beginning of the study implementation, the CIDRZ and PATH had differently designed participant IDs. This proved to be problematic when the data sets from both implementing partners needed to be consolidated. To enable uniform barcode scanning IDs across health facilities, CIDRZ reprogrammed the electronic questionnaire, redesigned the labels, and printed them in triplicate to cater for the DBS card, laboratory requisition, and informed consent form (and malaria RDT form for PATH). All previously generated study documentation (i.e., screening and recruitment logs, DBS cards, and laboratory forms) were relabeled with the newly designed barcodes.

At the beginning of the study, blood samples drawn from participants recruited in the 29 CIDRZ health facilities were distinctively labeled and packaged in the ANC clinic before they were batched with the Early Infant Diagnosis (EID) samples from the Antiretroviral Therapy (ART) clinics at the same health facility. They were transported to central hubs using the national HIV viral load sample courier system. Thereafter, samples were transferred to the EID laboratory at the UTH for testing. This resulted in 6.2% (450 of the 7,255) missing samples and needed the RAs to physically scrutinize and count the DBS cards from the ANC and ART clinics to recover. When data issues were identified at the CIDRZ-supported health facilities, the transportation of samples was assigned to the district RAs. They checked the DBS cards for sample and data collection accuracy and completeness before transporting them to the CIDRZ and UTH central laboratories for testing and archiving. Samples collected from the Chadiza health facilities were transported to the NMEC laboratory for testing. The process of verifying the number of DBS samples received from implementing facilities was slow and hampered by the

Table 2 ANC COVID-19 surveillance timeline

Activity		2021												2022												2023					
		Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Planning and Initiation	Finalize research question																														
	Design research																														
	Finalize sampling plan																														
	Prepare protocol																														
	Develop and code questionnaire																														
	Informed consent translations																														
	Develop database																														
	Pretest/pilot data tools																														
Execution	Stakeholder engagement																														
	HCW and CBV trainings																														
	Community sensitizations																														
	Participant enrolment																														
	Data collection																														
	Sample testing/ retesting																														
Monitoring	QA/QC																														
	Data cleaning																														
	Reporting																														
	Data analysis																														
Close out	Abstract presentation																														
	Manuscript writing																														
	Dissemination																														

unavailability of permanent study staff at the UTH laboratory. To prevent further loss of samples, in March 2022, the RAs were tasked with documenting all collected samples in a study laboratory logbook and physically transporting them to the CIDRZ laboratory for entry into the LIMS.

Data collection

Study data were collected and stored on different servers since data were gathered by different implementing partners. The Chadiza dataset was to be shared monthly by PATH for review by the CIDRZ study team. However, due to the remote location of some health facilities in Chadiza District and the resulting poor cellular network connection, there were often delays in uploading completed questionnaires to the PATH server and, consequently, delayed submission to CIDRZ. The delays in dataset sharing impacted the cleaning, merging, query resolution, and reporting of the data. Using alternative Internet sources through mobile phone connectivity helped to alleviate some of the Internet accessibility challenges.

Sample testing with infrequently serviced MAGPIX machines

DBS samples were tested using the Tetracore FlexImmArray SARS-CoV-2 Human IgG Antibody Test and processed using Luminex MAGPIX machines. While multiple MAGPIX machines were available in Zambian laboratories before study implementation, they were infrequently serviced and required maintenance before they could be used to process samples drawn from this study's participants. This servicing issue was not known until April 2022. The study sought assistance from laboratory engineers in South Africa to maintain the machines. Prior assessment of equipment and service records and earlier engagement of maintenance staff may have circumnavigated this complication and could have prevented delays with sample processing. An additional unforeseen cost for the purchase of calibration and validation kits was incurred because a proportion of the samples needed to be retested for quality assurance purposes.

Due to international travel restrictions, UTH and PATH laboratory technicians were initially oriented on the assay protocol virtually. Subsequently, a laboratory scientist traveled to Zambia from the CDC headquarters in Atlanta to provide hands-on technical assistance. The wait for practical training and supervision by a laboratory expert resulted in a delay in the availability of laboratory results since the testing of samples only started one year after the study implementation started. This delay impeded the study objective to monitor survey results in near real-time for new COVID-19 waves.

Inconsistent supply of DBS cards for sample collection

Since this study was integrated into routine ANC services, health facility staff ordered DBS cards for SARS-CoV-2 sample collection and infant HIV at the same time through the MOH district laboratories. In February 2022, the MOH began to implement a phased roll-out of point of care (POC) testing for the EID of HIV (which no longer relied on DBS cards) in health facilities in Lusaka District. Consequently, the MOH reduced the quantity of DBS cards procured. CIDRZ and PATH incurred unanticipated costs for the procurement of additional DBS cards for their supported health facilities.

Myths and misconceptions about the SARS-CoV-2 infection and vaccinations

This study was conducted during the 3rd and 4th waves (Delta and Omicron, respectively) of COVID-19 in Zambia. There was still much stigma and mistrust around SARS-CoV-2 infection and COVID-19 vaccination, which had only become available in Zambia in April 2021. Vaccine awareness and education efforts were in their infancy and uptake was poor. Rumors around the vaccine causing infertility, vaccine effects on pregnancy, and even death were commonplace [19].

Added to this was wariness around COVID-19 testing using nasal swabbing as the technique was new to most and had not previously been used widely in Zambia for disease diagnosis. Although our study did not utilize nasal swabs, the use of DBS came with its challenges as some people were hesitant to allow their blood to be drawn. These myths and misconceptions were addressed during group talks and individually when consenting participants. Over time, the MOH embarked upon widespread vaccine campaigns and worked actively to correct the misinformation.

Lessons learned

Adequate implementation preparations

Implementers endeavored to identify and engage local partners and stakeholders before and during implementation to encourage community ownership and acceptability of the study. Community engagement entailed discussing the importance of the study, inclusion and exclusion criteria, and informed consent. The aim was to ensure that the community understood the integration of study activities (including the pre-existing ANC-cased malaria surveillance) into routine ANC services, leading to the improved quality of the study and its impact on various communities. Since surveillance systems are only as good as the data they produce, incorporating lessons from prior studies and ensuring stakeholder buy-in, collaboration, and support was critical for ensuring activities were acceptable, sustainable, and successful. At the initial proposal writing stage, the identification of

stakeholders was meticulous to ensure that a comprehensive list was developed.

Integration of study activities into pre-existing surveillance platforms

This protocol was integrated into the ANC-based malaria surveillance study protocol that was being implemented by PATH in Chadiza district. Integration required consistent communication with the district health offices and health facility staff, retraining of staff, use of job aids, careful monitoring of workloads and leave schedules, and meticulous data collection. Our study demonstrated that integration of COVID-19 surveillance into an already established service delivery system is feasible and more sustainable since study activities ride on existing structures. A similar approach could be adopted for other diseases of public health importance. Laboratory equipment must be frequently maintained to avoid unexpected delays in sample processing and results availability.

Ensuring data completeness and integrity from the beginning of the study

Before study implementation, all available records and data collection tools were reviewed and piloted to assess usability and allow for adaptation and identification of potential challenges. Daily probing of study data did not only help to identify inaccuracies and inconsistencies but also ensured that data queries were addressed promptly, making data fit for its intended use. Continuous quality assurance checks saved money by reducing expenses associated with fixing bad data.

While using tablets for direct data collection added to costs and required additional training of staff, maintenance of tablets, and adequate security, it provided a means of reviewing data in real-time and correcting any identified issues. Nevertheless, hard copies of questionnaires were needed as a backup in the event of malfunctioning tablets, highlighting that preparing for device and other technological failures is important to consider at the planning stage.

Buffer budgeting and absorbing unanticipated expenditures

Despite careful planning, the study still ran into unanticipated challenges. Additional funds were required to compensate for delays in enrolling participants. The study incurred additional costs for extending staff contracts, purchasing additional buffer stock of DBS cards, and unanticipated calibration and validation kits to process the samples. Even though providing nominal rewards or incentives added to costs, it encouraged staff engagement and participation and fostered accountability for deliverables. Maintaining a budget buffer that was rapidly accessible was key to maintaining study momentum and avoiding unnecessary delays and interruptions.

Using CBVs to ease the burden on health facility staff

MOH staff are already burdened with a high patient-nurse ratio, particularly in rural areas. Recruiting CBVs to assist with some of the study procedures, such as conducting health education, screening and selecting potential study participants, obtaining consent, and administering the questionnaire reduced the additional workload of the study on the professional staff.

Strengths and limitations of this study

A major strength of our study is that this study was successfully integrated SARS-CoV-2 surveillance into routine antenatal care visits, leading to reduced implementation costs and resources required. The monitoring of implementation through multiple in-person and virtual platforms facilitated early detection and prompt resolution of challenges. An additional strength was that study implementation took place during the 3rd and 4th waves of COVID-19 in Zambia, and mitigation strategies implemented can serve as a model should additional COVID-19 waves hit Zambia or different disease outbreaks occur.

One notable limitation is that the study did not provide testing services for the serologic test at the health facility. This would have facilitated real-time laboratory testing and quickened results turnaround time, thereby enhancing outbreak detection. Additionally, the number of missing samples and costs associated with transporting samples to a central laboratory would have been reduced. Consequently, laboratories in remote health facilities would have enhanced their capacity to provide similar testing capacity. Secondly, staff were also unable to assess the potential for early outbreak detection due to delays in laboratory testing. While retrospective analysis still adds value to understanding COVID-19 epidemic dynamics, our results could not be used for real-time decision-making. Lastly, even though pregnant women have been used as a healthy general population proxy for other diseases (e.g., HIV and malaria), it is not known if they are representative of the general population for SARS-CoV-2.

Conclusions

Our study demonstrated that implementing routine SARS-CoV-2 surveillance in ANC is feasible and potentially sustainable. Potential implementation challenges can be mitigated by careful planning and clear communication and reporting strategies. Engaging MOH staff to implement activities can be cost-saving and enhance the acceptability of the study among ANC attendees but should be weighed against the risk that overburdening staff may have on data quality. Frequent and layered

monitoring is needed to ensure the completeness of data and the fidelity of implementation. Antenatal care clinics provided a relatively easy venue to access pregnant women and monitor SARS-CoV-2 prevalence. The best practices and lessons learned from this study can be adopted for disease surveillance and still provide valuable insights into waning immunity among populations in resource-limited settings. However, researchers must bear in mind that the role of seroprevalence studies may evolve as pandemics continue to evolve. However, they may still be valuable for providing insight into waning immunity in populations.

Abbreviations

ANC	Antenatal Care
CDC	U.S. Centers for Disease Control and Prevention
CIDRZ	Centre for Infectious Disease Research in Zambia
CBVs	Community Health Workers
COVID-19	Coronavirus disease 2019
DBS	Dried Blood Spot
DHDs	District Health Directors
EID	Early Infant Diagnosis
IPC	Infection Prevention and Control
LIMS	Laboratory Information Management System
MNCH	Maternal Newborn and Child Health
MOH	Ministry of Health
NAAT	Nucleic Acid Amplification Test
NHCs	Neighborhood Health Committees
NHRA	National Health Research Authority
NP	Nucleocapsid Protein
ODK	Open Data Kit
PHDs	Provincial Health Directors
POC	Point of Care
PPE	Personal Protective Equipment
RA	Research Assistant
RBD	Receptor Binding Domain (RBD)
MNCH	Maternal, Newborn, and Child Health
SARS-CoV-2	Severe acute respiratory coronavirus type 2
UTH	University Teaching Hospital
UNZABREC	University of Zambia Biomedical Research Ethics Committee
ZNPHI	Zambia National Public Health Institute

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Author contributions
The study protocol was developed by JH, JRG, IS, EH, TT, JG, CK, MM, BK, LM, SF and MS. BK and TT provided oversight for activity Implementation was supervised. Study data was managed by BK, KK, MR and TT. The initial draft of this manuscript was prepared by TT and reviewed by CBM. Subsequent drafts were reviewed by EH, JH, JRG, BK, MR, CK, MM, SF, FC, SY, RY, IS, TS, and LM. All authors proofread and approved the final version of the manuscript.

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Data availability
Not applicable (no datasets were generated and/or analyzed).

Declarations

Ethics approval and consent to participate
CIDRZ obtained ethics approval directly from the NHRA as there was a concession and expedited review of COVID-19-related studies. PATH obtained approval for the SARS-CoV-2 amendment to its original malaria surveillance protocol from NHRA and UNZABREC. This study was reviewed by CDC human research protection procedures and was determined to be research, but CDC investigators did not interact with human subjects or have access to identifiable data or specimens for research purposes.

Consent for publication
Not applicable (no individual details, images, or videos).

Competing interests
The authors declare no competing interests.

Author details
[1]Centre for Infectious Disease Research in Zambia (CIDRZ), P.O Box 34681, Lusaka, Zambia
[2]Public Health Institute, Oakland, CA, USA
[3]U.S. Centers for Disease Control and Prevention, Lusaka, Zambia
[4]PATH, Lusaka, Zambia
[5]University Teaching Hospital, Lusaka, Zambia
[6]U.S. Centers for Disease Control and Prevention, Atlanta, GA, USA
[7]University of Alabama at Birmingham, Tuscaloosa, USA

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