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Mapping the temporal-spatial distribution of the Kwa-Zulu Natal SARS-CoV-2 epidemic: May 2020-September 2021; from the first to the third wave.

By

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DECLARATION

I, Dr Cherise Naicker, declare as follows:

1. The work described in this dissertation has not been submitted to UKZN or any other institution for the purposes of an academic qualification, whether by myself or any other party.

2. The research reported in this dissertation, except where otherwise indicated, is my original research.

3. The dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

4. Where other sources have been quoted:

a) Their words have been re-written, but the general information attributed to them has been referenced.

b) Where their exact words have been used, their writing has been placed inside quotation marks, and referenced.

5. My contribution to the project is that of the principal investigator. I have been involved in every aspect of the project including literature review, development of research protocol, data collection, critical review of the results and synthesis of the discussion.

6. The contribution of others to the project were as follows:

Dr Nokukhanya Msomi - Supervisor

Maanda Mudau – assistance with application of aspects of the geospatial analysis (Moran's I Spatial autocorrelation and Getis* Ord statistic)

Signed:  (Principal Investigator) Date: 14 September 2023

Signed:  (Supervisor) Date: 14 September 2023

This work is dedicated to my husband, Nimallen and my sons, Caiden and Aaron.

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TABLE OF CONTENTS

CHAPTER 2: MATERIALS AND METHODS

CHAPTER 3: RESULTS

CHAPTER 4: DISCUSSION AND CONCLUSION

APPENDICES

LIST OF ABBREVIATIONS

ACE-2 Angiotensin converting enzyme-2

AIDS Acquired immunodeficiency syndrome

BREC Biomedical Research Ethics Committee

CDC Centre for disease control

CDW Corporate data warehouse

CFR Case fatality rate

CT cycle threshold

DHIS District health information system

GIS Geographic information system

GISAID Global Initiative on Sharing All Influenza Data

HIV Human immunodeficiency virus

ICTV International Committee on taxonomy of viruses

KZN Kwa-Zulu Natal

LMIC Low-middle income countries

MAC Ministerial advisory committee

MERS-CoV Middle East respiratory syndrome coronavirus

MIS-C Multisystem inflammatory syndrome in children

NDoH national department of health

NGS-SA National Genomic surveillance for South Africa

NHLS National Health Laboratory service

NICD National institute of communicable diseases

PANGOLIN Phylogenetic Assignment of Named Global Outbreak Lineages

PCR Polymerase chain reaction

$\mathbf{R_0}$ Basic reproductive number

RBD Receptor Binding Domain

$\mathbf{R_e}$ Effective reproductive number

RT-PCR Reverse transcription polymerase chain reaction

SA South Africa

SARS-CoV-1 severe acute respiratory syndrome coronavirus 1

SARS-CoV-2 severe acute respiratory syndrome coronavirus 2

TB Tuberculosis

USA United states of America

VOC variants of concern

VOI Variant of interest

VUM Variant under monitoring

WHO World Health Organisation

LIST OF TABLES

LIST OF FIGURES

ABSTRACT:

Background:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in 2019 and has caused unparalleled morbidity and mortality. Like global trends, the South African epidemic waves were driven by variants of concern (VOC). In addition, early public restrictions characterized the pattern of spread of SARS-CoV-2 in South Africa.

Methods:

A retrospective database study was undertaken to identify patterns in temporal-spatial distribution of SARS-CoV-2 in Kwa-Zulu Natal (KZN) across the first three epidemic waves. ArcGIS® Pro was used to create thematic choropleth maps visualizing SARS-CoV-2 test positivity at sub-district level for each epidemic wave and inter-wave period. Moran's I statistic was used to determine spatial autocorrelation in SARS-CoV-2 test positivity. The Getis-Ord Gi* statistic was used to detect and map SARS-CoV-2 hotspots. The identified hotspots were further characterized with respect to mean cycle threshold (CT) value and predominant circulating SARS-CoV-2 strain.

Results:

The eThekwini district had high positivity rates (>25%) during all three epidemic waves. Across all districts, higher test positivity rates were seen during waves compared to the interwave periods. The highest positivity rates were seen in wave 2, with majority of the province having a positivity rate above 25%.

The hotspot analysis identified significant clustering in multiple study time periods. Further characterization of hotspots revealed that the mean CT values were lower during waves than post-wave periods. The predominantly circulating SARS-CoV-2 lineages in the province per hotspot corresponded with nationally detected variants as described in national genomic surveillance.

Conclusion:

Geospatial mapping of SARS-CoV-2 hotspots is a valuable tool for tracking epidemics and identifying areas for targeted responses and interventions. In this retrospective analysis, the KZN epidemic was observed to mirror national trends.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in late 2019 in Wuhan, China (1) and has since spread across the world, causing more than 760 million COVID-19 cases and almost 7 million reported deaths by July 2023 (2). The World Health Organization (WHO) made a declaration with respect to COVID-19 constituting a public health emergency of international concern in January 2020, and a pandemic situation in March 2020(1). The COVID-19 pandemic is still ongoing; however, despite the unremitting emergence of new SARS-CoV-2 variants, the case fatality rate (CFR) is declining. This could be associated with global COVID-19 vaccine rollout, natural immunity, improved medical treatment options as well as the declining virulence of SARS-CoV-2 variants. Despite continuous circulation of SARS-CoV-2 for more than three years, there is much we do not know and must learn about this highly adaptable respiratory virus.

1.1.1 Basic virology including International Committee on taxonomy of viruses *(ICTV) classification of Coronaviruses*

Coronaviruses are enveloped viruses enclosing unsegmented, positive stranded RNA genomes (Figure A) which are classified under the order *Nidovirales,* the family *Coronaviridae*, and the subfamily *Orthocoronavirinae. Orthocoronavirinae* is subdivided into 4 genera *Alphacoronavirus, Betacoronavirus, Gammacoronavirus* and *Deltacoronavirus.* SARS-CoV-2 belongs to a subgenus within the *Betacoronavirus* genus called *Sarbecoviruses.* Generally, coronaviruses cause minor to moderate respiratory and gastrointestinal infections in vertebrate hosts (3). Thus far, 7 human coronaviruses have been described. Of these, 4 coronaviruses cause an illness similar to the common cold, namely OC43, HKU1, 229E and NL63(3). In addition, there are 3 human coronaviruses causing severe respiratory conditions, severe acute respiratory syndrome coronavirus (SARS-CoV-1), Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-2(3).

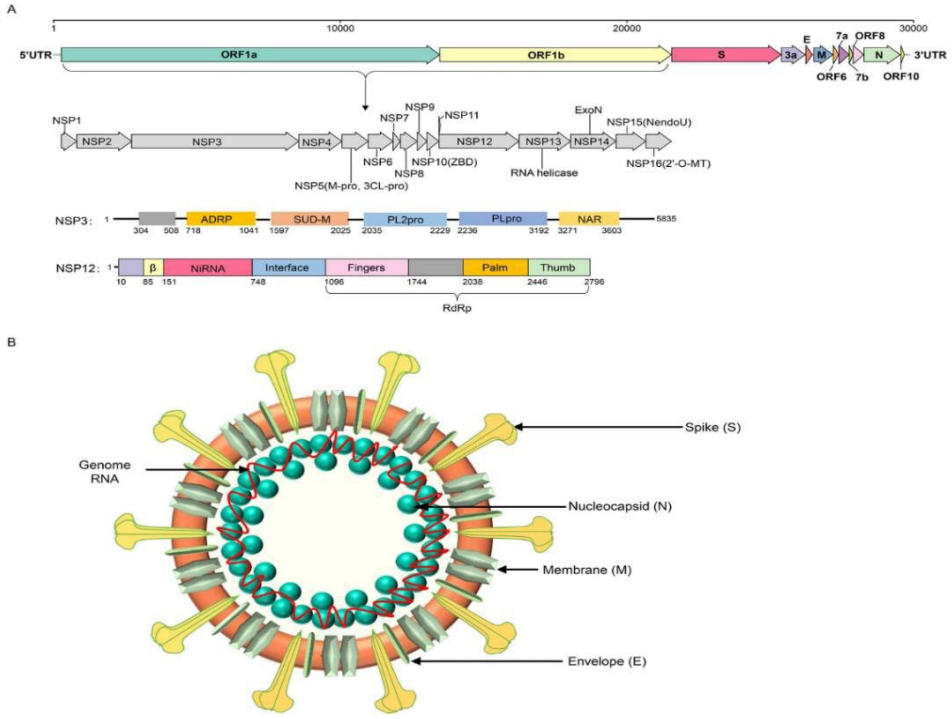

Figure A: Image of SARS-CoV-2 genome map(A) and structure(B).

Taken from: By Zhijian Zhou, Ye Qiu, and Xingyi Ge - Zhou, Z., Qiu, Y. & Ge, X. The taxonomy, host range and pathogenicity of coronaviruses and other viruses in the Nidovirales order. Animal Diseases 1, 5 (2021). https://doi.org/10.1186/s44149-021-00005-9

https://commons.wikimedia.org/w/index.php?curid=107217923 (Accessed on 05/04/2023)

1.1.2 SARS-CoV-2 classification methods used for describing genetic variability

The International Committee on Taxonomy of Viruses(ICTV) does not describe a general approach to defining viral genetic diversity below virus species level (4). In a quickly changing epidemic, such as SARS-CoV-2, a nomenclature system can allow for real-time tracking through commonly agreed upon terms to refer to viruses circulating in various regions. This assists in illuminating links between outbreaks that share similar viral strains. As such, there are 3 main SARS-CoV-2 nomenclature classification systems, namely PANGO, Nextclade and WHO. A dynamic lineage classification system described by Rambault *et al* (5), which makes use of the Phylogenetic Assignment of Named Global Outbreak Lineages (PANGOLIN) software suite, was used in this study. The PANGO nomenclature system suggests the presence of 2 main phylogenetic lineages: lineage A and lineage B (5). Genomic variability of SARS-CoV-2 has contributed to increased transmissibility and resulted in increased pathogenicity (6, 7, 8, 9, 10). WHO has classified SARS-CoV-2 variants according to their impact on transmissibility, pathogenicity as well as public health interventions. Variants are categorized as Variant of Interest (VOI), Variant of Concern (VOC) and Variant under Monitoring (VUM) (11). VOIs indicate SARS-CoV-2 variants that are associated with an increased number of cases and genomic changes that have been proven to affect transmission potential, virulence or diagnostic capabilities of current testing methods. VOCs designate variants that demonstrate a link with high transmissibility, increased virulence, treatment resistance or diagnostic detection failure. This uncomplicated naming convention was adopted as a means to simplify communication regarding these variants. In South Africa (SA), VOC have driven epidemic waves, Beta (B.1.351) in the second wave (12), Delta (B.1.617.2) in the third wave (13) and most recently with Omicron (B.1.1.529) accounting for the majority of new cases in the country's fourth wave (14). Whilst, Nextclade is a classification tool used to organize SARS-CoV-2 sequences according to their genetic relatedness. These clade names comprise the two-digit year of assignment followed by a letter indicating the order of assignment within the year.

1.1.3 COVID-19: A Global Health Challenge

COVID-19 brought with it an unmatched health crisis, the response to which required learning from previous epidemic and pandemic experiences, and quickly producing new evidence to guide control methods. The measures utilized in emergency responses to COVID-19 were based on lessons learnt from past epidemics, such as that caused by MERS-CoV and the previous pandemics caused by Influenza A (H1N1) viruses and SARS-CoV-1(15).

Although the CFR for SARS-CoV-2 is much lower than other respiratory virus outbreaks (16), COVID- 19 has caused an unprecedented number of deaths (2). This is due to the mode of viral transmission, which includes inhalation of air droplets and aerosol particles that contain the infectious virus (15). The risk of transmission is highest within one to two metres of an index case where the concentration of these droplets and particles is maximal (17). Additionally, SARS-CoV-2 infected asymptomatic and pre- symptomatic patients spread the virus and are therefore more likely to expose others, unknowingly, during the incubation period (17). As of February 2023, there were twelve countries reporting cumulative cases exceeding 10 million (2). Countries that have better diagnostic capabilities, higher population size and/or older populations tend to report more cases. Therefore, incidence rates per country may better reflect the vulnerability of a population to SARS-CoV-2 as well as testing coverage. European countries report the highest incidence rates (2), and this is likely due to the aging population and effective diagnostic capabilities.

Case fatality rates were higher in the early phase of the pandemic (2) and this was likely due to a lack of prior immunity, high viral virulence and a paucity of information on appropriate patient management. Mortality reporting differed between the various regions. When deaths are reported as cumulative deaths per 100 000 population – many European and American countries demonstrate high mortality rates (2). This likely relates to an aged population. When deaths are reflected as "Case Fatality Rates" (total reported deaths / total reported cases) - countries with the highest rates include those from the Eastern Mediterranean (2) which likely highlights the misdiagnosis of milder cases due to varying testing strategies.

The pathology associated with SARS-CoV-2/COVID-19 exhibits a vast spectrum of clinical presentations,

ranging from asymptomatic infections, to mild, moderate, and finally severe infections (18). Of note, however, the most common presentation was characterized by typical symptoms, including fever and cough (16, 18). Severe infections often require hospitalization to ensure assisted respiratory support and other medical treatments. The COVID-19 clinical continuum has been associated with risk factors such as gender and age. In addition, diabetes, cardiovascular disease, or diseases, or treatments impacting the immune system result in the greatest risk of serious disease and death (18). However, it is estimated that almost 80% of all infections remain unrecorded due to asymptomatic or very mild symptoms (19).

Age-related differences in the clinical manifestations of COVID-19 have been extensively described in the literature over the last 3 years (18, 19, 20, 21). Patients older than 60 years old tend to have atypical clinical pictures (22). The scientific consensus surrounding this involves the presence of typical geriatric syndromes (e.g. multi-morbidity, disability and immunosenescence) which can influence the clinical presentation of COVID-19 (21).

On the other hand of the age spectrum, children initially appeared to be spared, with reported cases mostly experiencing mild or no symptoms (23). United States of America (USA) reported cases in children under 12 years accounting for approximately 10% of total cases and fatality rates of about 0.01%(2). However, a clinical presentation that became typically associated with children was the multisystem inflammatory syndrome (MIS-C). MIS-C is an uncommon, severe post-infectious syndrome which was mainly described in people of Afro-Caribbean descent and in male individuals (23). The pathogenesis of this syndrome is multifactorial and remains to be elucidated (24).

1.1.4 Vaccines and Therapeutics

In order to limit SARS-CoV-2 transmission, several recommendations for the use of non- pharmaceutical control measures have been advised, including the use of facemasks, social distancing and travel restrictions. However, these measures have limited success, and preventive vaccine development quickly became a priority in order to achieve herd immunity and halt the ongoing epidemic. An ideal vaccine must encourage both cellular and neutralizing antibody responses against SARS-CoV-2 without inducing vaccine-enhanced clinical disease. The required percentage of immune individuals needed to achieve effective herd immunity is estimated to be at least 80% of the population – this refers to the estimated proportion of the population with adequate immunity that is sufficient to interrupt transmission, either from natural infection or vaccination. COVID-19 vaccine-related efforts and development have been remarkable, and are particularly encouraging when considering the availability of vaccines for other global health problems. As of February 2023, 65.5% of the world's population were fully vaccinated and approximately 71% had received at least a single dose of COVID-19 vaccine (2) (Figure B). Of note, global vaccine coverage was disproportionate, which is clearly highlighted for Africa in Figure B. Vaccine coverage was better in South Africa when compared to other parts of Africa. Despite substantial impact on hospitalizations and deaths, restrictions in global access to COVID-19 vaccines implied that many individuals remained at risk. By May 2022, roughly 74% of the population from high-income countries had received an adequate COVID-19 vaccine course compared to <40% of individuals in Low-middle income countries (LMIC), and only 13% of people from low-income countries (25). Governance over the fair distribution of vaccine resources is needed in order to combat pandemics, and priority should be given to high risk groups, like the elderly, when vaccines are limited. Even amongst vaccinated individuals, concerns remain regarding the durability of protection and effectiveness of current vaccines against emerging SARS-CoV-2 variants and sub-variants.

The shortcomings of COVID-19 vaccines underscore the need for effective treatment options. Accordingly, the WHO makes recommendations for various new antivirals, immune modulators (e.g.

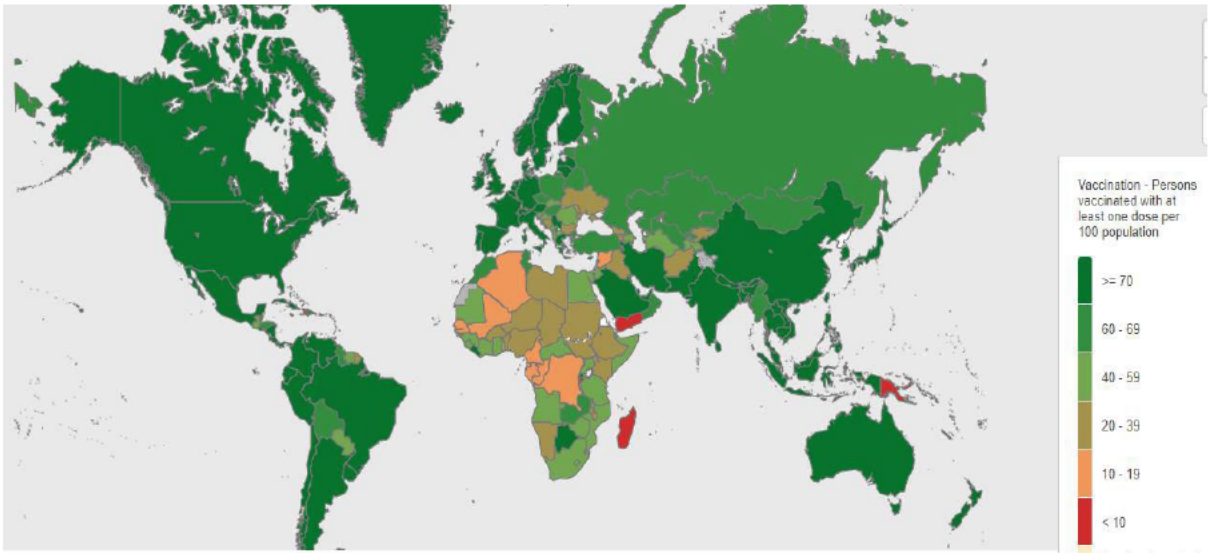

Figure B: World map showing individuals who have been vaccinated with at least one dose per 100 population as at 01 March 2023 (Taken from: https://covid19.who.int/) Accessed on 01/03/2023.

IL-6 inhibitors; JAK inhibitors) and other repurposed drugs for use in specific severity categories of COVID-19 (26).

1.1.5 SARS-CoV-2 genetic variability

Viruses undergo genetic evolution as an adaptation technique which may alter viral characteristics including tropism, pathogenicity and transmissibility (27, 28, 29). Sarbecoviruses are of particular concern, as two different species have caused recent human outbreaks. The main mechanism of adaptation of sarbecoviruses are changes in the receptor binding domain of the spike surface glycoprotein (27, 29). This results in an improved ability of the virus to bind to the host cellular receptor on epithelial cells in the gastrointestinal and respiratory tract. This process occurs as a result of high genetic diversity combined with frequent recombination events during circulation within the natural reservoir (e.g. bats) (29). These recombination events allow for more efficient transfer of virus to humans directly or via an intermediate host (27). Although human infectivity depends on many factors, the ability to bind to human angiotensin converting enzyme-2 (ACE-2) receptors has proven to be a key factor (27).

The Alpha (B.1.1.7) variant was first described in the United Kingdom on 14 December 2020, whilst the Beta (501Y.V2) variant was initially reported in South Africa on 18 December 2020 (10). The Delta variant first emerged in India in October 2020 (13). This variant was described as being highly infectious, and up to 60% more transmissible than the Alpha variant, exhibiting a higher hospital admission risk than previously reported variants (20). The Delta variant was also associated with escape of immune responses targeting the Receptor Binding Domain (RBD) of the spike protein (30). Vaccine efficacy was also reduced by this VOC, but improved with additional vaccine doses (30). The next VOC seen was Omicron which was first described in specimens collected from both Botswana and South Africa in November 2021(31). The Omicron spike protein was shown to have approximately 30 mutations (32). Notably, this variant spread quickly across the world and surpassed the Delta variant as the dominant SARS-CoV-2 variant (32). Omicron is highly contagious and exhibits a higher reproduction number, allowing the variant to easily spread globally (31, 32). In addition, the Omicron variant displayed tropism for the upper respiratory tract with markedly lower virulence (31). This resulted in less severe illness than previously reported SARS-CoV-2 variants (32). Omicron was able to escape immune responses generated from previous vaccinations or infections with SARS-CoV-2, causing a greater number of breakthrough infections than previously described variants (32).

Alpha, Beta, Gamma, Delta as well as the Omicron parent lineage (B.1.1.529) are considered VOCs that are no longer in circulation (33). WHO has demoted BA.2, BA.4 and BA.5 from the SARS-CoV-2 VOC list in March 2023, as these parental lineages are no longer in circulation (11). The WHO is currently tracking VOIs, XBB.1.5 and XBB.1.16, along with other VUMs as well as their descendent lineages (33). The VUMs include BA.2.75, CH.1.1, BQ.1, XBB, XBB.1.9.1, XBB.1.9.2, XBB.2.3 and BA.2.86(33). As the COVID-19 epidemic evolves, new SARS-CoV-2 variants and sub-variants may emerge. Therefore, ongoing research is imperative to investigate the effect of newer variants on infection, transmission, and interactions with natural and/or vaccine-induced immunity. In addition, promotion for development of updated vaccines against novel SARS-CoV-2 variants is needed.

SARS-CoV-2 could become endemic in regions with suboptimal vaccine coverage and therefore pose a risk to global eradication efforts. Furthermore, knowledge of transmission dynamics in zoonotic hosts and enhanced surveillance of zoonotic diseases are necessary to eradicate SARS-CoV-2 and prevent further spill over events.

1.1.6 An African Perspective

COVID-19 posed a particular challenge for the African continent because of the high prevalence of other communicable diseases, namely human immunodeficiency virus (HIV)/ acquired immunodeficiency syndrome (AIDS), Tuberculosis (TB), cholera, and malaria as well as a disproportionate burden of poverty (34).

SARS-CoV-2 was introduced to Africa by air travelers coming from countries with high community transmission rates, with initial cases being reported from Nigeria, Egypt and South Africa between mid-February and early March 2020(35). Throughout the pandemic, Africa accounted for a relatively small share of reported cases and deaths – corresponding to under 5% of the global burden (2). However, data from sero-prevalence and autopsy studies in certain African countries suggest that the correct case counts and deaths may be markedly higher than the reported statistics (36, 37). Early public control measures including, international travel restrictions, quarantine for returning travelers and internal lockdown measures were utilized by many African countries in order to limit community spread of the virus and allow for preparation of healthcare services. Initially, the epidemic was heterogeneous, with higher cases reported from North and Southern Africa and less from other regions (38).

Genomic surveillance has been at the frontline of the African COVID-19 response (38). Timeous implementation of SARS-CoV-2 sequencing by several laboratories in Africa allowed for genomic data from the initial imported cases to be collected and shared timeously. Genomic surveillance has been integral for monitoring the evolution of SARS-CoV-2 and detection of emerging variants in Africa (38). In South Africa, the National Genomic Surveillance Network, facilitated the detection of the B.1.351 (501Y.V2) (12), which was subsequently designated as a VOC (Beta) by the WHO. Analysis of the genetic diversity and lineage dynamics in Africa revealed a pattern that reflects multiple imports of virus from the rest of the world over time (38). Wilkinson *et al* also showed Africa's contribution to international spread of the virus, mainly to Europe, Asia and North America (38). The early phase of the pandemic was dominated by lineage B.1. which was introduced multiple times into Africa. After its recognition in South Africa, B.1.351 quickly spread and became the dominant lineage detected in Africa.

Following implementation of travel restrictions, the number of detectable viral introductions into Africa decreased followed by sustained community transmission with the occasional import from a neighboring country, either by road or rail (38, 39).

The spread of SARS-COV-2 on the African continent has been dominated by VOCs and VOIs which likely had implications on vaccine rollout. In addition, the slow rollout of vaccines was a concern in an environment with many circulating VOCs and VOIs.

Vaccine nationalism in global north countries resulted in sluggish vaccine rollout in Africa (25, 40). Furthermore, vaccine hesitancy, fuelled by misinformation and conspiracy theories, also threatened COVID-19 vaccine coverage in the global south (41). In addition, the discovery of numerous COVID-19 variants raised doubt about the efficacy of available vaccines and further impacted on vaccine acceptance. Vaccination occurred in a stepwise approach, beginning with priority groups and then the general population, usually oldest to youngest. Vaccine coverage in Africa has been estimated to be 27.5% of the population who are fully vaccinated (2), with only Mauritius and Liberia achieving coverage greater than the recommended target (2).

Despite the suboptimal vaccine coverage, Africa has contributed a relatively smaller proportion of both COVID-19 cases and deaths (37). Africa's lessons learnt from this pandemic underscore the importance of developing country-specific strategies to roll-out therapeutic and preventive measures as the "one size fits all" approach is unlikely to work in our setting.

1.1.7 National trends

SA's first COVID case was reported on 5 March 2020 from a traveler who had recently returned from Italy (42). Thereafter, imported cases and their close contacts dominated the newly identified cases in the country. A nosocomial outbreak in KZN also contributed to early cases (43, 44). SA is responsible for most SARS-CoV-2 cases in Africa. As of February 2023, SA had cumulative SARS-CoV-2 case counts of approximately 4 million and around 100 000 COVID-19 related deaths (2). This contributed a mere 0.6% to global case counts and just under 1.4% to global deaths. Although, caution must be exercised when interpreting these figures, as global excess mortality studies have indicated that the pooled global excess mortality was 104.84 per 100,000 persons, with lower-income countries bearing most of the burden of excess deaths during the pandemic (45). Excess mortality data has shown that far more people died in South Africa during the COVID-19 pandemic than the reported COVID-19 deaths (46, 47)

The SARS-CoV-2 pandemic in SA was characterized by periods of peak infections with multiple major epidemic waves (12). The SARS-CoV-2 epidemiological profile in SA has been influenced significantly by government responses, including the declaration of a national state of disaster and implementation of lockdown measures. Lockdown measures involved international travel restrictions, closure of schools, introduction of a mandatory daily curfew, as well as restricted movement of non-essential personnel. SA instituted temperature checking and COVID-19 symptom screening at all country ports of entry. The government also promoted non-pharmaceutical measures such as frequent handwashing, mandatory use of facemasks in public places, avoidance of social gatherings and isolation procedures for symptomatic individuals. In the absence of a vaccine or prior immunity, the aim of the government response was to decelerate the rate of community transmission in order to allow healthcare facilities to prepare and improve capacity to deal with the anticipated influx of patients, and to ultimately decrease COVID-19 related mortality. A key prevention tool was the rollout of COVID-19 vaccines. South Africa decided to prioritize vaccination of healthcare workers and then to administer vaccines to the general population in descending order of age in a stepwise fashion. As of February 2023, more than 38 million vaccine doses had been administered in SA, with a provincial vaccine coverage rate ranging between 40 and 60 percent (48). The 60+ age category had the highest vaccine coverage (66.7%), whilst the 12 to 17-year age category had the lowest vaccine coverage (34.8%) (48).

Similar to global trends, the South African epidemic waves were driven by VOCs and there is evidence to suggest that these variants were also responsible for epidemic waves at a local level (49).

1.1.8 Diagnostic testing for SARS-CoV-2

Optimal management of the COVID-19 epidemic requires adequate diagnostic tools. The purposes of testing include ascertaining a diagnosis to link patient to appropriate clinical care, to initiate the necessary infection control measures as well as for surveillance. The rapid development of diagnostic tests for SARS-CoV-2 was unprecedented and was facilitated by the prompt sequencing and public sharing of the SARS-CoV-2 genome. This allowed for protocol development for the detection of SARS-CoV-2 by real time reverse transcription - polymerase chain reaction (RT-PCR), which has been globally implemented (50). Consideration of cycle threshold (CT) values is important when interpreting RT-PCR results. CT values represent the amplification cycle number where the target gene exceeded a threshold value (51). CT values are therefore inversely proportional to viral load and can provide an indirect method of estimating the amount of viral RNA in a sample (51). Lower CT values are also found to be associated with increased infectivity (51).

1.1.8.1 The evolving SARS-CoV-2 testing strategy in South Africa

The country's testing strategy was established by National Department of Health (NDoH) based on advice from the National Institute of Communicable Diseases (NICD) initially, and later the Ministerial Advisory committee for COVID-19. Initially, the testing criteria was restrictive in order to avoid overburdening the healthcare and laboratory testing services with test requests from "worried-well" individuals. The case definition included an epidemiologic criterion in order to detect imported cases (17). Following detection of a COVID-19 case, further testing for contact tracing would be carried out (17). Following the detection of the first case in SA, testing increased. At the start, most cases were detected by private health sector laboratories with limited testing in the public sector. To some extent, the strict criteria for testing facilitated community transmission and this was quickly broadened to include symptomatic individuals without a travel history. This facilitated active case finding via a community-led screening and testing programme. The presence of two or more signs or symptoms was a prompt for referral for SARS-CoV-2 testing. This strategy increased the testing coverage, although the positivity rate remained below 10%(52).

However, due to global test kit shortages, the laboratory testing services quickly became overwhelmed and turnaround times were substantially increased. This delay was obstructive to interrupting community transmission via active case finding and isolation of contacts as results were often available near the end of the recommended isolation period. The supply of alternate rapid testing technologies was also strained, and this did not allow for its widespread use for diagnosis in SA. Provinces with low positivity rates engaged in pooled sample testing to increase throughput and decrease demand for kits and consumables (52). As the lockdown measures were relaxed, positivity rates increased which excluded the utility of pool testing. The focus of testing then shifted to hotspot identification, which involved investigation of case clusters in order to mitigate their spread. As testing demand increased beyond available supply, prioritisation of target populations for testing was needed. The ministerial advisory committee (MAC) group categorised population groups according to testing priority (*see Appendix Table C) (53).* The rationale for "high priority" was to decrease turnaround times in order to maximize the clinical relevance of the test result. The reasoning behind the "medium priority" group was to limit the number of outbreaks and preserve essential services. Testing in the "low priority" group was only suggested if testing capabilities were not under strain. If testing capabilities were stretched, individuals in this group were advised to isolate if symptomatic, or quarantine if they were a close contact of a confirmed case, regardless of test results. Whilst this approach assisted with rationing of diagnostic kits, it may have failed to adequately address community transmission.

As the peak of the first wave had passed, the testing priorities shifted to symptomatic hospitalized patients, high risk elective surgical admissions and suspected COVID-19 related deaths with limited contact tracing. This testing approach continued for the rest of the study period. Rapid antigen testing

was introduced in December 2020 and was reserved for symptomatic individuals and screening at ports of entry.

As the epidemic progressed, the national testing strategy needed to adjust to meet the evolving demands and availability of diagnostic kits.

1.1.9 Geospatial mapping for surveillance of infectious diseases

The introduction of Geographic Information Systems (GIS) has allowed for the prospect of examining the interplay between the health attributes of a population and both human and environmental characteristics. It has been suggested that a new discipline of "spatial epidemiology" has emerged (54), which involves quantifying and describing geographic variability in disease, especially with respect to differences in environmental exposures at a low scale.

Geospatial analysis involves the use of geographic co-ordinate information to describe objects or features in relation to each other. Advances in information technology and spatial features resulted in a tool which has many public health applications, including:

1. Assessing the health of a population by analysing disease rates and other health statistics by geographic location.
2. Using the differences in health and health service usage for the purpose of resource allocation.
3. Investigation of time trends in disease at a local level.
4. Exploring the spatial distribution of health care facilities and referral patterns to assist with healthcare planning.
5. Studies of variation in health interventions and outcomes for the optimization of health services.
6. Surveillance of communicable diseases.
7. Review of recognized environmental hazards (e.g. pollution).
8. Probing of disease clusters in order to understand patterns of spread in a specific region.
9. To inform distribution of limited resources in order to curb outbreaks.

GIS involves the combination of complex algorithms, spatial analysis, geo-statistics and modelling, which makes it a significant tool for illuminating disease patterns. The use of GIS can provide dynamic maps which aid understanding the geographic distribution of diseases. This assists with investigation of case frequency, spatial cluster of diseases and disease association with environmental factors. Clustering comprises the study of spatial-temporal patterns related to the spread of communicable diseases. In addition, it assists with recognizing factors associated with heterogeneous geographical distribution which might be helpful in clarifying the diseases' spread mechanism. Thus, clustering enables timely prevention and control measures and appropriate resource allocation to limit transmission of infectious diseases.

Another useful geospatial tool is "hotspot analysis" or "risk mapping". Risk mapping involves assigning a probability value to the occurrence of a specified condition which may indicate a degree of unusual aggregation in spatial data. The spatial clustering of high values is known as a hotspot, whereas the collection of low values is known as a cold-spot.

This technology has been successfully used to illustrate the epidemiology related to COVID-19 and to depict variable severity by geographic region (55, 56, 57).

1.2 Study Rationale

SARS-CoV-2/COVID-19 has overwhelmed many countries globally and Africa was the last continent to be affected by SARS-CoV-2. COVID-19 reached Africa via importation from Asia, Europe, and America (35), and therefore it's onset in most African countries was much later than other continents.

Africa was anticipated to be the most at-risk continent to SARS-CoV-2/COVID-19, largely due to the low access to quality healthcare and a large immunocompromised population owing to high prevalence of communicable diseases as well as the generally poor socio-economic status (58). Many African countries employed early lockdown measures as a COVID-19 response in order to flatten the curve as a means to compensate for already over-burdened healthcare systems (59). During the early phases of the pandemic, many developing countries had low COVID-19 associated fatality, indicating that the relatively younger age structure of these countries might have shielded them against the severe outcomes of this disease (37). More recently, however, it has become evident that the perceived differences in mortality may have been misrepresentative, rather reflecting poor reporting systems which lead to inaccurate accounts of COVID-19 deaths (37).

SA was amongst the first countries to report SARS-COV-2 cases in Africa (38) and experienced the first peak of SARS-CoV-2 infections in early July 2020, followed by a second wave that peaked in early January 2021(60, 61). SA has the largest COVID-19 epidemic in Africa with cumulative SARS-CoV-2 cases of 4 076 463 with a total of 102,595 COVID-19 related deaths as of July 2023(2). SA implemented prevention strategies in the form of early lockdown restrictions and public vaccinations programs. The vaccination drives targeted healthcare workers initially and progressed in a stepwise fashion, according to age, from June 2021. The early public restrictions characterized the pattern of spread of SARS-CoV-2 in SA. The epidemic in SA can be described as having two phases: one related to early travel-related introductions, the other being the periods of peak infections (12). South African dominant lineages have been described (B.1.1.5.4, B.1.1.5.6 and C.1) which accounted for approximately 42% of all infections during the first wave in South Africa (62). C.1 was identified early in the South African epidemic and was described as the most widespread lineage in SA by the end of August 2020(62). The timing and scale of the first and second waves varied between the provinces in SA (60, 61). In order to track the evolution of SARS-CoV-2 in SA, the Network for Genomic Surveillance in South Africa (NGS-SA), a consortium of genomics and bioinformatics scientists who work with diagnostic and reference laboratories to generate and analyze data in a timeous manner, was formed in May 2020. This collaboration of five large NHLS Virology laboratories attached to academic sequencing centres allowed for the rapid generation and analysis of sequence data to inform regional and national responses (63).

Geographically, KZN is located on the east coast of South Africa and hosts the country's two busiest ports, namely Durban and Richard's Bay. KZN is divided into 11 health districts with eThekwini being mostly urban whilst many of the other districts are generally rural. KZN is characterized by heterogeneity in its demography, geography, economy, culture and disease patterns. This diversity can be appreciated even at a health district level and may have contributed to the local COVID-19 epidemic response.

KZN reported the first SARS-CoV-2 case in SA and since then has reported over 740 000 cases with approximately 60 000 deaths (48). The epidemic in KZN was characterized by the early nosocomial outbreak in April 2020 which accounted for 14% of infections in KZN and 45% of national deaths at the time (43). KZN has frequently remained amongst the three biggest contributors to the total COVID-19 cases in SA (64). During the first wave of the pandemic, uMkhanyakude district reported the leading number of COVID-19 cases whilst in the second wave eThekwini metro health district reported the highest number of infections (64, 65).

The need to define specific geographic distributions of SARS-CoV-2 cases becomes of practical importance to define transmission networks, disease burden as well as to evaluate the effectiveness of local public health response measures. In addition, understanding the genomic distribution of SARS-CoV-2 at a country level has implications for both diagnostic, therapeutic and vaccine development. The COVID-19 pandemic has underscored the role of surveillance in the timely development of diagnostics (50), vaccine development, monitoring viral evolution(66), transmissibility, and virulence,

illuminating transmission dynamics (42), as well as, assessing the overall infection prevention and control measures (43). From a South African public health standpoint, being able to trace viral dynamics at a local level is crucial and would allow for tailor-made diagnostic and prevention approaches both for current and future outbreak response measures. SA's limited use of GIS during the COVID-19 epidemic included the development and maintenance of a provincial health dashboard as a reliable source of up-to-date information (67).

This research attempts to address the paucity of geospatial data related to the burden of COVID-19 as well as the pattern of spread of SARS-COV-2 in Kwa-Zulu Natal. Insight into the transmission dynamics specific to a region is crucial for laboratory and medical resource allocation in order to assist with outbreak preparedness for future epidemics.

1.3 Study aim and objectives

1.3.1 Aims:

To describe basic epidemiologic data of the COVID-19 outbreak in KZN across the first three epidemic waves.

To illustrate the geographic distribution of SARS-CoV-2 in KZN across the first three epidemic waves.

To identify SARS-CoV-2 hotspots in KZN across the first three epidemic waves.

1.3.2 Objectives:

1.3.2.1 Primary Objectives:

COVID-19 positivity rates per sub-district (from NHLS labs) in KZN were geographically represented using geospatial software in order to identify hotspot areas.

SARS-CoV-2 hotspots were further characterised with respect to mean CT value and predominant circulating sequence

1.3.2.2 Secondary Objectives:

Mean CT values for SARS-CoV-2 PCRs performed during and in between KZN epidemic waves were determined.

The clade distribution of SARS-CoV-2 within the identified hotspot areas were examined using the whole genome sequenced COVID-19 specimens from the Spatial and genomic monitoring of COVID-19 cases in South Africa study [BREC/00001510/2020].

1.4 Conclusion:

This research intends to map the geospatial and temporal distribution of the COVID-19 epidemic in KZN, across its 11 health districts, from May 2020 to September 2021. This data can be used to inform transmission dynamics and epidemic readiness for prospective outbreaks.

CHAPTER 2

MATERIALS AND METHODS

2.1 Study Design

A retrospective descriptive study was undertaken to identify patterns in the geographic and temporal distribution of SARS-CoV-2 cases in KZN across the first three epidemic waves. This is a sub-study from the parent protocols "Spatial and genomic monitoring of COVID-19 cases in South Africa" [BREC/00001510/2020] and Research Applications of the Department of Virology Laboratory Database [BCA 143/09].

2.2 Ethics statement

This database study used SARS-CoV-2 PCR positives that were tested in a public sector(NHLS) laboratory. The sequencing of a subset of COVID-19 positive specimens was carried out as a sub-study from a protocol that was approved by University of Kwa-Zulu-Natal Biomedical Research Ethics Committee (Spatial and genomic monitoring of COVID-19 cases in South Africa study [BREC/00001510/2020]). Patient consent was not required for the genomic surveillance as decided by the research ethics committee. This study has ethics approval by University of Kwa-Zulu-Natal Biomedical Research Ethics Committee [BREC/00002405/2021].

2.3 Data and data analysis

2.3.1 Definitions:

The Basic reproductive number (R_0) represents the number of secondary cases that result from an index case in a population of susceptible individuals only. Whilst, effective reproductive number (R_e) is the estimated number of secondary cases per infectious case in a population consisting of both susceptible and non-susceptible people (68). R_e can be estimated by the product of R_0 and the fraction of the host population that is at risk (69). An epidemic wave was defined as per NICD, which uses the South African Resurgence plan (unpublished), which describes a wave as the period from when COVID-19 weekly incidence is greater than or equal to 30 cases per 100 000 persons until the weekly incidence falls below 30 cases per 100 000 persons (70). An interwave period was defined as the time between epidemic waves. An epidemiologic week was defined using USA Centre for Disease Control(CDC) Morbidity and Mortality Weekly report definition (Sunday-Saturday).

2.3.2 Epidemiological data:

We analysed COVID-19 case counts by applying for access to the National Health Laboratory Services (NHLS) Corporate Data Warehouse (CDW) from May 2020 to September 2021. The KZN COVID-19 dashboard was used to obtain cumulative COVID-19 testing data. Results from 1 048 576 tests were included in our analysis.

The NICD COVID-19 weekly epidemiologic and COVID-19 special public health surveillance reports were used to review national and provincial epidemiologic trends.

2.3.3 SARS-CoV-2 PCR metadata and database management:

This database study was conducted in the Discipline of Virology, University of Kwa-Zulu-Natal and the NHLS, which is located in Durban, South Africa. The data that was used was obtained from patient specimens (community screening, inpatients, outpatients, and healthcare workers) that tested in a NHLS laboratory (public health sector) for SARS-CoV-2 by RT-PCR method during the time period between May 2020 to September 2021.

Metadata received from CDW is anonymized as each specimen is assigned a system generated unique episode number. This data was cleaned and sorted by filtering for positive results per health district. Duplicates were removed by searching for and eliminating all duplicate unique identifiers. Healthcare facilities (GPS co-ordinates) where specimens were collected were used as a proxy for patient locater information. Healthcare facilities were categorized into levels of care namely, primary healthcare clinics,

district, regional and tertiary hospitals. Data was analyzed by filtering for various parameters, including collection date, health district, level of care, age and mean CT value.

2.3.4 Geospatial and Statistical analysis:

COVID-19 positive results were sorted into health districts as per testing facility information obtained from the CDW dataset. The NICD proposed definition for a COVID-19 epidemic wave was used for this retrospective analysis. The provinces experienced the beginning and ending of the waves at different epidemiologic weeks. The first and second KZN epidemic wave lasted approximately eight weeks and as such eight weeks of data was chosen to create the corresponding thematic maps. Whilst the third KZN epidemic wave lasted thirteen weeks, and matching data was chosen to map this wave. All data between the previously described epidemic waves were used to map the interwave periods. The geographic coordinates of healthcare facilities listed in the CDW data were obtained from the District Health Information System (DHIS). A Point-in-polygon spatial query was used to assign clinics to relevant sub-districts using ArcGIS Pro (Environmental Systems Research Institute: Redlands, CA, USA). Data was aggregated to facility, sub-district and district level, and then merged with geographical data and saved into shape files. Thematic choropleth maps visualising SARS-CoV-2 PCR positivity at sub-district and district-level were created for each wave that occurred during the study period as well as time points between waves in order to adequately depict the timeline of the KZN epidemic.

Moran's I statistic in ArcGIS Pro was used to determine spatial autocorrelation in SARS-CoV-2 test positivity and prevalence. Moran's I is a global measure of disease clustering and quantifies the degree of similarity in disease rates between regions (geographic features) that are described as neighbours. The statistic can have values ranging from -1 to +1. A value below zero indicates negative spatial autocorrelation
i.e. neighbouring regions tend to have rate that are dissimilar (dispersed pattern), while a value above zero indicates positive spatial autocorrelation i.e. neighbouring regions tend to have rates that are similar (clustering). A value of zero indicates that the geographic pattern of disease is random (71).

The Gertis-Ord Gi* statistic was used to detect and map hotspots of SARS-CoV-2. The statistic measures the concentration of a spatially distributed variable; it detects regions with high attribute (test positivity) values that are surrounded by regions with high attribute values (potential hotspots) or areas with low attribute values surrounded by areas with low attribute values (potential cold-spots)(54). Thereafter the local sum for a feature and its neighbours is compared proportionally to the sum of all features; when the local sum is very different from the expected local sum, and therefore the difference is too large to be the result of random chance, a statistically significant z-score results. For statistically significant positive z-scores, the larger the z-score is, the more intense the clustering of high values (hot spot). For statistically significant negative z-scores, the smaller the z-score is, the more intense the clustering of low values (cold spot)(71, 72). Both spatial autocorrelation analysis and hotspot analysis were conducted using the spatial statistics toolbox in ArcGIS Pro. Spatial relationships between districts and sub-districts were described with inverse distance weights.

2.3.5 Mean CT analysis:

Average CT values per specimen were calculated using a function in Microsoft Excel. Thereafter mean CT values were calculated per district per wave as well as the interwave periods within the study timeline. Mean CT values were plotted on a standard deviation graph to assess variability per district for all study time points.

2.3.6 Testing Coverage and Positivity Rate calculations:

Testing coverage for KZN was calculated by taking the total SARS-CoV-2 PCR tests done over the population estimates per 100 000. This was performed for each epidemic wave and interwave period. The positivity rate was calculated by taking the total positive SAR-CoV-2 PCR results divided by total tests done per epidemic wave and interwave period.

2.3.7 National Testing strategies and Public Health Restriction measures:

This information was obtained from reports uploaded by the MAC on the NDoH COVID-19 online resource and news portal website (https://sacoronavirus.co.za/).

2.3.8 SARS-CoV-2 clade analysis:

A subset of these specimens were submitted for genomic surveillance as part of a sequencing project to describe the temporal distribution of SARS-CoV-2 clades in "hotspot" areas in KZN over the study Period (Spatial and genomic monitoring of COVID-19 cases in South Africa study [BREC/00001510/2020] Principal Investigator – Professor T de Oliveira). These sequences were subsequently deposited into Global Initiative on Sharing All Influenza Data (GISAID), a global open access database. Sequences circulating with the highest frequency within the areas corresponding to the identified hotspots were retrieved from GISAID.

2.3.9 Characterization of SARS-CoV-2 hotspot areas:

Data corresponding to the time points with hotspot areas was analyzed in order to detect any characteristics that were associated with SARS-CoV-2 hotspots. Parameters analyzed included location (health district/sub-district), age, level of care, mean CT value as well as the circulating SARS-CoV-2 sequence with the highest frequency.

2.4 Study significance

This study is important as it describes the geospatial and epidemiologic characteristics of SARS-CoV-2 epidemic in KZN which can be used for future outbreak preparedness. The findings of this study can shed light on possible characteristics of SARS-CoV-2 hotspots – this information can be used for policy makers for distribution of resources amidst an outbreak.

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Discussion

CHAPTER 3

RESULTS

3.1 Demographic and Laboratory findings:

This study analysed 1 048 575 SARS-COV-2 PCR results from public sector laboratories from May 2020 to September 2021. Analysis of the demographic data (Table 1) of the SARS-CoV-2 positive cases in KZN revealed that age category 19 – 59 years had the highest PCR positivity rate across all health districts during the study period. The age category under 12 years old had an average positivity rate of 2.4% across health districts. Over 80-year-old age group had positivity rates of less than 1% in all health districts. Females had higher positivity rates than males in all 11 health districts in KZN. Overall, eThekwini contributed the largest proportion of positive cases for the study period. The highest positivity rate for the study period was seen in Harry Gwala district.

3.2 Spatial analysis to identify SARS-CoV-2 clustering:

The Moran's I spatial auto-correlation statistic for SARS-CoV-2 positivity rate in KZN showed that statistically significant clustering occurred in all time periods except Pre-Wave 1 and Post-wave 3 (Supplementary data – Table B). The hotspot analysis identified various hot and cold-spots which differed from wave to wave (Figure 4). Further evaluation of the identified hotspots (Table 2) revealed hotspots across different sub-districts during each time point except for Emadlangeni which accounted for the hotspots seen in both Wave 1 and Wave 3. The mean CT value across hotspots seen during wave periods were lower (CT 26) than during post-wave periods (CT 29). The mean CT value across hotspots seen in Wave 1 and 3 were two CT values less than the mean CT seen from hotspots during wave 2. The mean CT value from hotspots during Post-wave 1 was almost three CT values lower than that of Post-wave 2. The most frequently detected strain identified during Wave 1 was B.1.1.54 and accounted for 23.7% of the specimens sent for sequencing. B.1.1.448 made up 26.7% of the sequenced specimens for the post-wave 1 period. B.1.351 accounted for all the sequenced specimens during Wave 2 and 78.2% during the post-wave 2 time point. During Wave 3, the majority sequence identified was AY.45 (76.4%).

3.3 Kwa-Zulu Natal SARS-CoV-2 epidemic timeline:

The timeline of the KZN SARS-CoV-2 epidemic (Figure 1) depicts the effective reproductive number (R_e) and case count contributions per district for the first three waves of the KZN epidemic. Positivity rate as well as testing coverage for the province per epidemic wave and interwave period is also represented on Figure 1. The SA Alert levels are also indicated on Figure 1 and are further described in the Supplementary data in Appendix 6. The largest proportion of positive cases across all three epidemic waves was contributed by eThekwini health district. In addition, Ilembe, King Cetshwayo and Umgungundlovu made up a significant number of cases in the first and third epidemic wave, whilst Ilembe, King Cetshwayo, Umgungundlovu and Ugu contributed significantly in the second wave. The R_e increased above 1 during the time periods that coincided with epidemic waves. Both the testing coverage and the positivity rate were greater during epidemic waves than during interwave periods.

3.4 SARS-CoV-2 PCR mean cycle threshold analysis:

The SARS-CoV-2 PCR mean CT value per district per epidemic wave and interwave periods is demonstrated in Figure 2. The mean CT value was lower during epidemic waves (CT 26.9) and higher in between epidemic waves (CT 31). The greatest variability in mean CT value was seen in eThekwini, whilst the least variability was seen in Ugu, Umgungundlovu and Zululand health districts. The mean CT value was lower during Wave 2 than during Wave 3.

3.5 Heat map analysis depicting SARS-CoV-2 PCR positivity:

SARS-CoV-2 PCR test positivity across KZN sub-districts during and between epidemic waves are depicted in heat maps in Figure 3. Test positivity rates are higher during wave periods than during interwave periods. The highest positivity rates were seen in wave 2 with the majority of the province having a positivity rate above 25%. Ethekwini had high positivity rates (>25%) during all three epidemic waves. The positivity rates seen during Post-wave 1 were the highest from the interwave periods.

3.6 Kwa-Zulu Natal SARS-CoV-2 hotspot mapping analysis:

The cluster analysis illustrating both hot and cold-spots in KZN over the study period is shown in Figure 4. The hotspots are described in detail in Table 2. Cold-spots are noted during Post-wave 1, Wave 2 and Wave 3. Cold-spots occurred in Uthukela in both the Post-wave 1 and Wave 2 time period and in Umgungundlovu during Post-wave 1 and Wave 3. An additional cold-spot for Wave 3 was identified in the Ilembe health district.

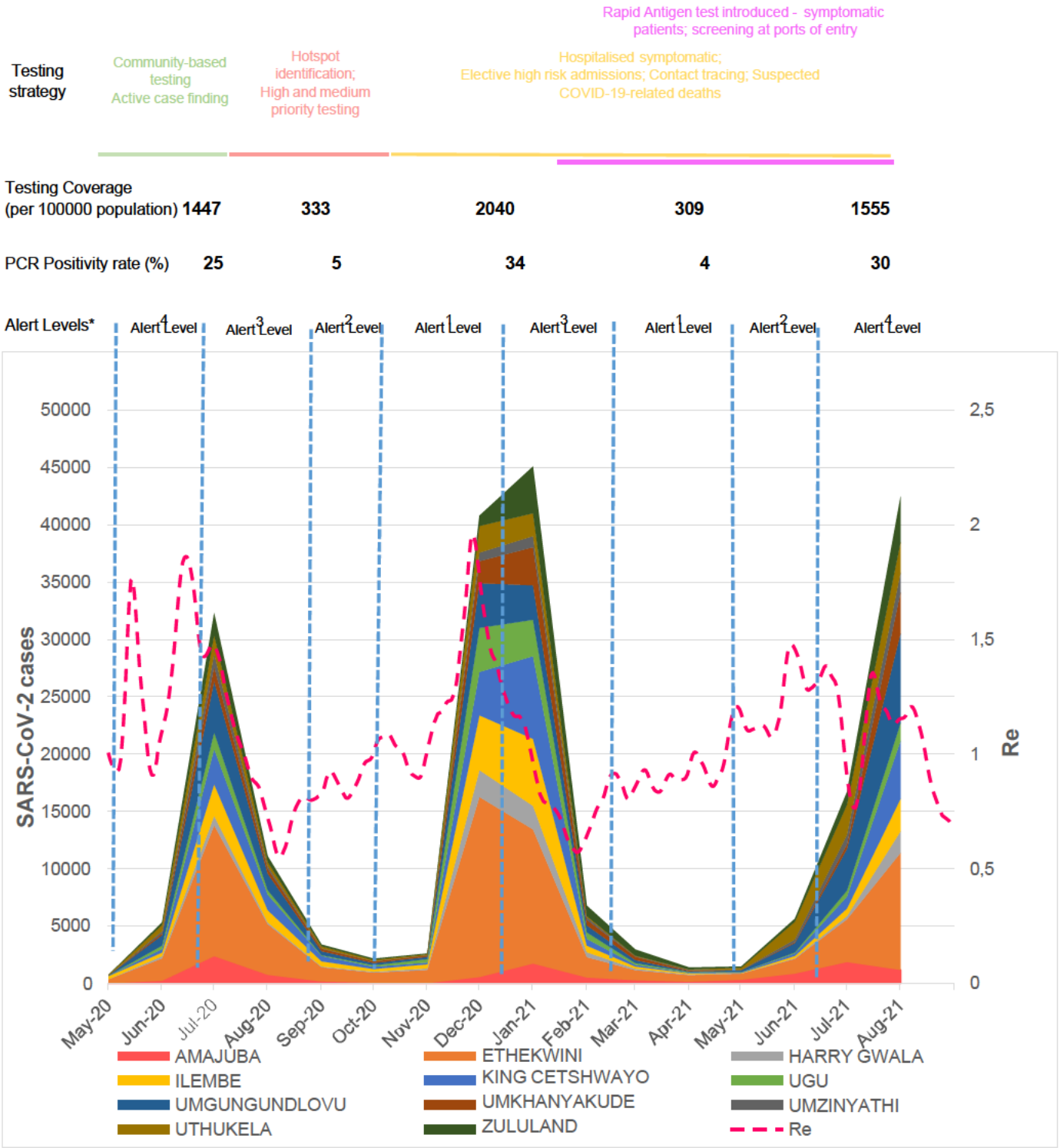
Table 1: Demographic data of SARS-CoV-2 positive cases in KZN per health district (May 2020 – September 2021)

	eThekwini		Ugu		Harry Gwala		Umgungundlovu		Ilembe		Umzinyathi		Uthukela		Amajuba		Zululand		King Cetshwayo		Umkhanyakude	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Age																						
<12	5 105	1,6	1 046	1,9	770	2,2	2 846	1,9	2 697	2,6	1072	3,2	2 117	2,7	1299	2,9	804	2,8	2 967	2,3	1 401	2,3
12-18	4 268	1,3	1 054	1,9	1 179	3,3	2 945	2,0	2 470	2,3	1 310	3,9	2 408	3,0	1 329	3,0	1 059	3,6	3 349	2,6	2 302	3,8
19-59	48 876	14,9	8 253	14,8	5 472	15,4	17 731	12,1	13 317	12,6	3 915	11,7	8 894	11,2	6 889	15,6	3 419	11,7	14 203	10,9	7 690	12,8
60-80	8 836	2,7	2 146	3,8	1 225	3,4	2 897	2,0	2 089	2,0	607	1,8	1 526	1,9	1 477	3,3	549	1,9	2 636	2,0	1 107	1,8
>80	873	0,3	364	0,7	175	0,5	302	0,2	219	0,2	79	0,2	198	0,2	167	0,4	81	0,3	414	0,3	184	0,3
unknown	5 056	1,5	1152	2,1	709	2,0	1 563	1,1	1 088	1,0	375	1,1	561	0,7	186	0,4	681	2,3	2 260	1,7	1 155	1,9
Gender																						
M	26 345	8,0	4 525	8,1	3 245	9,1	9 878	6,7	7 410	7,0	2 541	7,6	5 503	6,9	4 008	9,1	2 259	7,8	9 453	7,3	4 918	8,2
F	43 845	13,3	8 888	15,9	6 011	16,9	17 033	11,6	12 884	12,2	4 520	13,5	9 872	12,5	6 802	15,4	4 105	14,1	15 247	11,7	8 467	14,0
Unknown	3 184	1,0	602	1,1	274	0,8	1 382	0,9	1 586	1,5	297	0,9	329	0,4	537	1,2	229	0,8	1 129	0,9	454	0,8
Population Estimates (2020/2021)[53]	3 855 599		805 600		526 856		1 202 882		726 900		582 929		776 056		597 185		907 660		1 018 434		713 277	
TOTAL positives (227 762)	73 374		14 015		9 530		28 293		21 880		7 358		15 704		11 347		6 593		25 829		13 839	
TOTAL tests (PCR) 1 048 575	328 781		55 875		35 589		146 654		105 315		33 573		79 292		44 262		29 103		129 858		60 273	
PCR Positivity rate	22,3		25,1		26,8		19,3		20,8		21,9		19,8		25,6		22,7		19,9		23,0	
Contribution to positive cases	32,2		6,2		4,2		12,4		9,6		3,2		6,9		5,0		2,9		11,3		6,1	

n = positive cases % = positivity rate

Table 2: Characterisation of SARS-CoV-2 hotspots as per location, mean CT and variant distribution across epidemic waves in KZN Sub-districts, March 2020 – September 2021

	Wave 1			Post-wave 1		Wave 2		Post-wave 2	Wave 3
Health district (Sub-district)	Harry Gwala (Greater Kokstad)	Zululand (Abaqulusi)	Amajuba (Emadlangeni)	Umkhanyakude (Mtubatuba)	Ugu (Umzumbe; Umdoni)	Ugu (Umzumbe)	Uthukela (Alfred Duma);	Umzinyathi (Endumeni)	Amajuba (Newcastle; Emadlangeni; Dannhauser)
Mean CT value	26.6	26.4	24	24.8	28.3	27.8	27.9	30.7	25.6
Most frequently detected SARS-CoV-2 sequence	B.1.1.54 (23.7%) South African Lineage			B.1.1.448 (26.7%) South African identified lineage descendent of B.1.1.54		B.1.351 (100%) Variant of concern (Beta) first detected in South Africa		B.1.351 (78.2%) Variant of concern (Beta) first detected in South Africa	AY.45 (76.4%) Delta sub-lineage



*Description of Alert levels in Supplementary Data (Table D)

Figure 1: Timeline of KZN SARS-CoV-2 epidemic depicting case count contributions per healthcare district for first three epidemic waves.

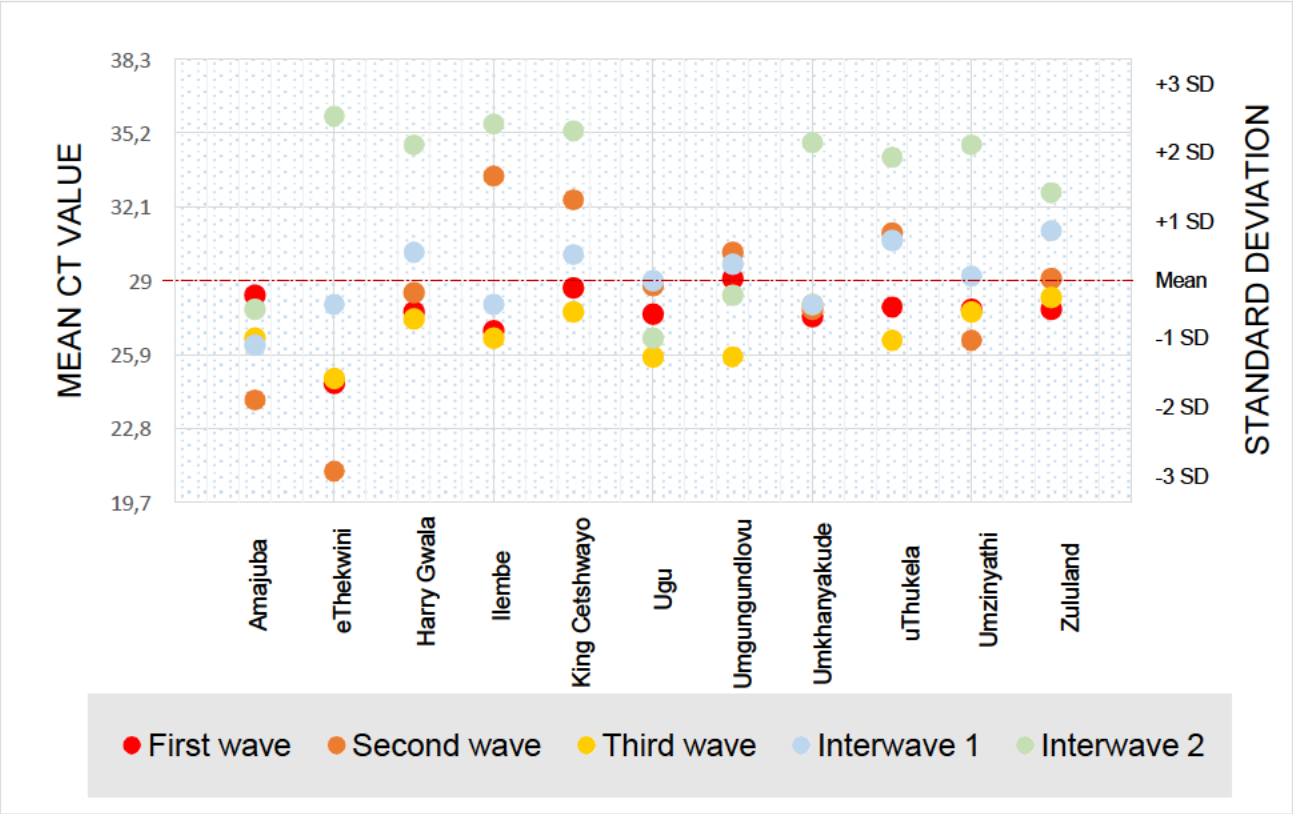


Figure 2: Mean cycle threshold value per epidemic wave and interwave period for KZN health districts

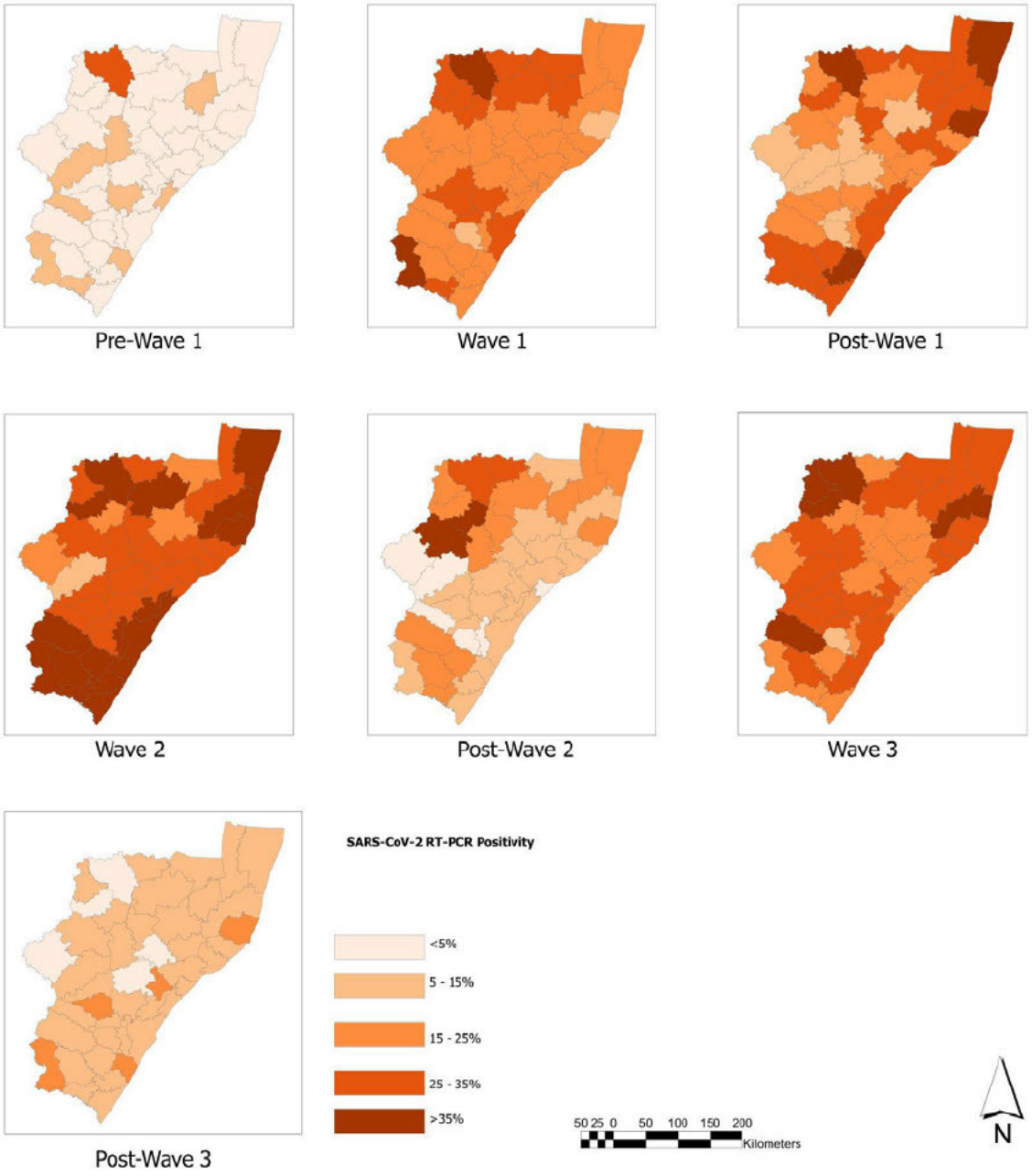


Figure 3: Heat maps depicting SARS-CoV-2 positivity rates per sub-district in KZN for time points within and between epidemic waves.

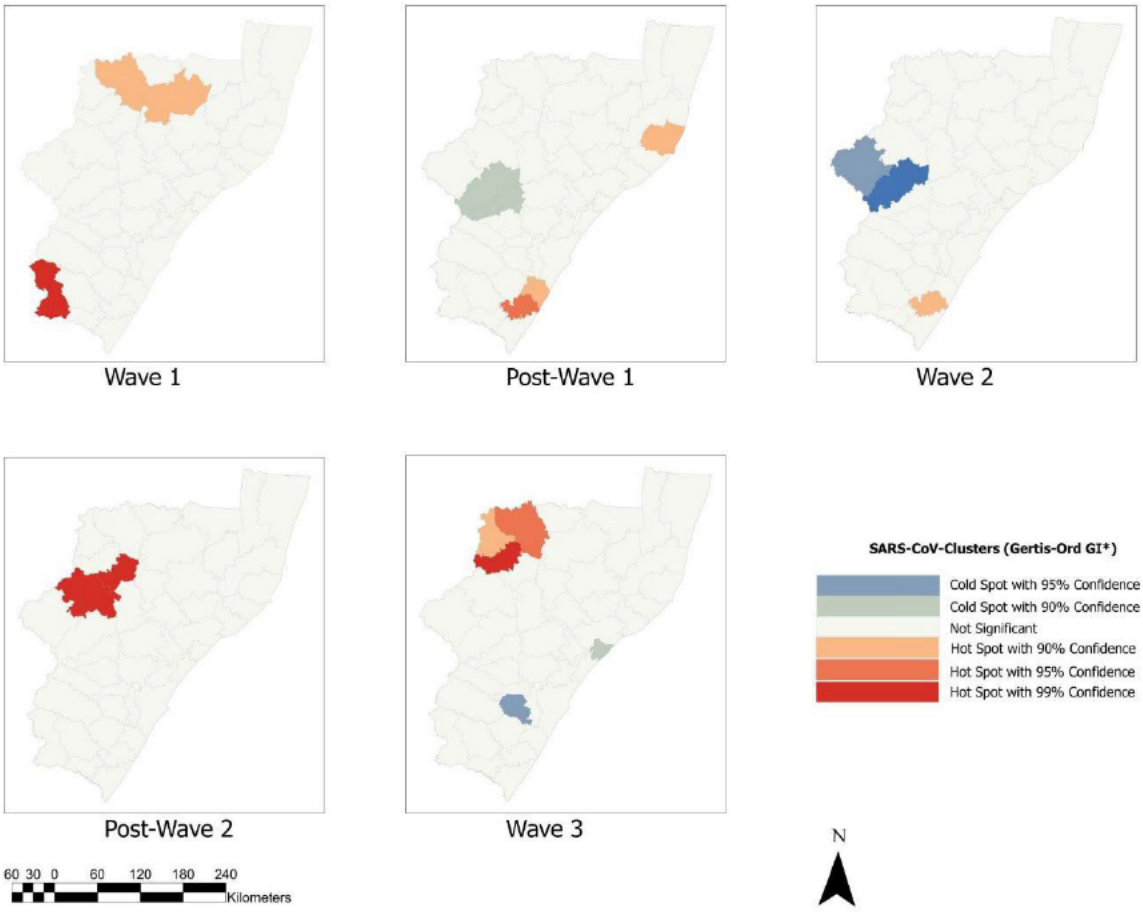


Figure 4: Maps depicting both SARS-CoV-2 hotspots and cold-spots in KZN sub-districts, March 2020 – September 2021

CHAPTER 4

DISCUSSION

4.1 Synthesis

The demographic data analysis revealed that the highest PCR positivity rate across all health districts occurred in the 19-59-year age category. This age category represents the economically active members of society and thus it is not an unexpected finding. The low positivity rate seen in the under 12-year age category correlates with global observations that children are more likely to have asymptomatic or mild infection that does not require presentation to healthcare facilities for testing (23). Closure of schools and day-cares early in the lockdown led to less frequent exposure and hence, possible lower infection rates in children. Excellent regeneration capacity of paediatric alveolar epithelium may contribute to early recovery from COVID-19(23), and therefore a low proportion of infected individuals present for testing in this age category. The test positivity rate of less than 1% in the over 80-year age category could be explained by a number of theories. First, the morbidity and mortality rate is higher in the elderly than in young people, with mortality rates ranging between 14 and 26% in the over 80-year age category (73). This disconnect between mortality rates and test positivity rates can be partly explained by the difficulty that this patient group has in accessing healthcare, and as such many do not present to healthcare and thus do not form part of the laboratory-confirmed cases. Another possible explanation is that a significant proportion of the individuals who tested in this age category were the "worried well", as a result of the early warning that the elderly was at risk for poorer outcomes (21). The large input of positive cases by eThekwini likely represents the easily accessible testing services present in the district, as well as the evolving testing strategy adopted by the province (34).

The KZN epidemic timeline indicates that eThekwini was the main contributor of positive cases both during and in between waves for the study period. In view of this study using the healthcare facility as a proxy for patient locater information, this likely reflects the larger number of hospitals and clinics located in the district. This would imply easier access to COVID testing services, thus allowing for greater detection of positive cases in eThekwini. The R_e increased above 1 during epidemic waves. This finding is expected, as this is indicative of a growing epidemic. The testing coverage was greater during wave periods and this is a reflection of the testing strategies employed by the province (53). The positivity rate followed a similar pattern, with higher positivity rates noted during peak periods. Of note, the positivity rate was greater in the second and third wave period which reflects the variants of concern which dominated these epidemic waves. These variants evolved towards increasing transmissibility, and thus more positive cases (10).

The mean CT analysis revealed lower CT values during epidemic waves than in between wave periods. CT values have an inverse relationship with viral load and lower CT values are also found to be associated with increased infectivity (51). Therefore, the trends of both the SARS-CoV-2 PCR CT values, as well as the positivity rate, indicate that the majority of the individuals tested during wave periods tested positive during times of higher viral loads. The CT trend noted in eThekwini had the greatest variability whilst both Ugu and Amajuba had limited variation. This is most probably a reflection of the varying testing coverage in the respective districts – eThekwini likely had higher testing coverage and so the CT trends noted in this district are an accurate reflection of changing CT values described for SARS-CoV-2 (51).

If we focus on eThekwini for the mean CT analysis, the mean CT was lower during epidemic waves than during the interwave periods. This is a well described phenomenon, recently infected individuals are known to have higher viral loads and therefore, greater infectiousness (51). This lower mean CT value, linked to an increase in infectious individuals, is indicative of an increased transmission potential. In SA, similar to the global trend, variants of concern dominated epidemic waves, with the Beta variant and the Delta variant dominating our second and third wave respectively. The Delta variant is known to be associated with more severe disease outcomes and lower PCR CT values (20). The mean CT value during Wave 2 was lower than during both the other epidemic waves in the study period. This finding differs from what other countries observed (20, 30) and this could be attributed to the stepwise SARS-

CoV-2 vaccine rollout strategy used in South Africa – with more individual having protection from vaccination by Wave 3. This perhaps blunted the mean CT value seen during Wave 3 to some extent. CT values were used to predict the onset of epidemic waves in provinces, as it was noticed that decreasing mean CT value coincided with increasing test positivity rates (74).

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See comments at reference list

The SARS-CoV-2 PCR positivity rates, like the mean CT findings, demonstrate a pattern that follows epidemic wave trends. The use of positivity rates over positive cases corrects for the notable differences in testing coverage seen across the province. Although, caution must be exercised when interpreting positivity data during periods of low testing coverage.

The pattern of test positivity could reflect the variable testing strategies used in the province. The epidemic trajectory in SA differed per province, Western Cape led the country into the first and second epidemic waves and Gauteng took over in subsequent waves (75). The widespread high positivity rates seen during Wave 2 are reflective of the pathogenic characteristics of the VOC, Beta, that dominated circulation during that time (20). The high positivity rates seen in eThekwini during all wave periods is telling of the urban nature of this district which implies greater access to healthcare and testing services. This sustained high positivity rate in eThekwini could also reflect the migratory behaviour of its population which results in greater exposure and resultant higher proportion testing positive. The pattern of decreasing positivity rates in the interwave periods could reflect the increasing population level immunity over time, either from vaccination or natural infection, that translated into less infections that presented for SARS-CoV-2 testing.

Analysis of the identified hotspots revealed that age, location and level of healthcare were not determinants of SARS-CoV-2 hotspots. The mean CT value seen in hotspots followed epidemic trends, i.e. lower CT values were seen during wave periods and higher CT values were seen in between waves. The differing CT values seen in hotspots from each wave could mirror the VOC that circulated during those time points (20). The predominant SARS-CoV-2 sequence per hotspot corresponded with variants highlighted in national genomic surveillance. The dominance of VOC B.1.351 (Beta) over two study time points underscores the increased transmissibility (10) and the prolonged viral shedding (20) associated with this variant. The dominant sequence identified in the hotspots found during wave 3 was AY.45 which is a Delta sub-lineage. This is consistent with national genomic surveillance which demonstrated that Delta was the predominant variant seen in SA's third epidemic wave (14).

The cluster analysis also identified cold-spots throughout the study period. Cold spots could represent areas that were spared from surrounding high SARS-CoV-2 positivity rates. Cold spots occurred in uThukela during Post-wave 1 and Wave 2. The testing strategy used during this earlier part of the pandemic was directed at high and medium priority populations(52), which included hospitalised patients, healthcare workers and people living in close proximity settings e.g. old age homes. uThukela district lacks a dedicated virology laboratory and as such testing capacity took longer to be established compared to other districts. The testing coverage for this district was analysed for the periods where cold-spots were identified. The testing coverage for uThukela for both these time points were below the provincial testing coverage of 333 tests per 100000 population and 2040 tests per 100000 population for Post-wave 1 and Wave 2 respectively. Perhaps the low testing rate resulted in a delay in aligning with recommended testing strategies which lead to the appearance of a "false" cold-spot in this district. The remaining cold-spots were identified in uMgungundlovu, during Post-wave 1 and Wave 3, and iLembe in Wave 3. The testing coverage was above the provincial rate for both districts for all time points where cold-spots were identified. Further analysis of patterns of testing during these time points revealed that the majority of tests performed during cold-spots were from Primary Healthcare facilities(see appendix 7).

Further investigation of how these health districts dealt with the SARS-CoV-2 pandemic may be warranted, in order to gain insight for future outbreak preparedness.

4.2 Recommendations for future research and conclusion

Further studies looking at subsequent epidemics waves for better comparison and to get an overall impression of the geographic distribution of the SARS-CoV-2 epidemic in KZN are needed. Another area for future research is the inclusion of genomic surveillance data, in order to study the correlation of transmission networks and hotspots. Near-real time hot spot mapping could be explored as a surveillance tool, which could act as an early warning indicator for resurgence.

A limitation of our study was the lack of clinical information for the patients who tested for SARS-CoV-2 i.e. symptomatic; severity of illness; outcomes etc. We were therefore unable to further describe the hotspots we identified according to varying clinical presentations. We also had limited access to testing strategies adopted at a provincial and district level and thus, we were unable to describe both the hotspots and cold-spots with relation to this.

In conclusion, the SARS-CoV-2 pandemic has been unprecedented in many aspects and therefore, lessons must be learnt for better preparedness and to avoid devastating losses. This study was undertaken to fill a gap in understanding the geographic distribution of SARS-CoV-2 in KZN and to use geospatial analysis to identify hotspots throughout the first three epidemic waves. Although no predictors of SARS-CoV-2 hotspots were identified, our study was able to demonstrate that genomic surveillance and CT values in hotspots followed trends noted in national surveillance.

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Scott LE, Hsiao NY, Dor G, Hans L, Marokane P, da Silva MP, Preiser W, Vreede H, Tsoka J, Mlisana K, Stevens WS. How South Africa Used National Cycle Threshold (Ct) Values to Continuously Monitor SARS-CoV-2

APPENDICES

APPENDIX 1:

Gatekeeper's letter of approval

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NATIONAL HEALTH LABORATORY SERVICE

17 February 2021

Dear Sir *I* Madam

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his letie- serves kl confirm lllat penmi:ssion has. been g;ra nled to conduct fne study tifl'ed: 'Genomic and geographic dis bUJtion of SARS--CoV 2:in KZN' I.O be conducted in the Department of Viro'logy, atiO'nal !bUea11h Laboratory Servioe -Academic Carnplll'tx, KZN_

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APPENDIX 2:

Ethic committee approval letter

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05 March 2021

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APPENDIX 3:

Categorization of Healthcare facilities in Kwa-Zulu Natal according to level of care

KZN Health District	Facility level of care	Number of facilities
Amajuba	Tertiary	0
	Regional	0
	District	6
	Primary healthcare	23
eThekwini	Tertiary	1
	Regional	5
	District	18
	Primary healthcare	103
Harry Gwala	Tertiary	0
	Regional	0
	District	6
	Primary healthcare	39
Ilembe	Tertiary	0
	Regional	1
	District	5
	Primary healthcare	35
King Cetshwayo	Tertiary	0
	Regional	3
	District	8
	Primary healthcare	64
Ugu	Tertiary	0
	Regional	1
	District	5
	Primary healthcare	52
Umgungundlovu	Tertiary	1
	Regional	3
	District	6

	Primary healthcare	62
Umkhanyakude	Tertiary	0
	Regional	1
	District	5
	Primary healthcare	58
Umzinyathi	Tertiary	1
	Regional	0
	District	3
	Primary healthcare	45
uThukela	Tertiary	0
	Regional	1
	District	3
	Primary healthcare	42
Zululand	Tertiary	0
	Regional	0
	District	8
	Primary healthcare	80

APPENDIX 4:

Results from the Spatial Autocorrelation (Moran's I statistic) for SARS-CoV-2 test positivity in KZN Sub-districts across all study time periods

Period	Moran's I	Expected Values	Variance	Z-Score	p-Value
Pre-Wave 1[α]	-0.149	-0.023	0.010	-1.26	0.208
Wave 1	0.369	-0.023	0.015	3.17	0.0015
Post-Wave 1	0.479	-0.023	0.016	4.04	<0.0001
Wave 2	0.414	-0.023	0.025	3.58	0.0003
Post-Wave 2	0.48	-0.023	0.016	4.04	<0.0001
Wave 3	0.323	-0.023	0.015	2.82	0.005
Post-Wave 3[α]	0.087	-0.023	0.015	0.91	0.36

[α]No statistically significant clustering noted

APPENDIX 5:

Categorization of Testing populations according to priority level(53).

High priority populations
1 Inpatients-general wards and ICU [a]
2 Hospital pre-admission testing for subset of clinically relevant conditions that pose a risk to patients (e.g. cancer patients) or staff (e.g. ENT surgery; bronchoscopy etc) [a]
3 Symptomatic hospital staff [a]
4 Hospital staff working with high risk patients (e.g. immunosuppressed patients such as oncology, transplantation patients)
5 Hospital staff regardless of symptoms
Medium priority populations
6 Care home staff & residents
7 Isolation facilities at entry [a]
8 Symptomatic essential service personnel
9 Symptomatic high-risk occupation personnel (lab staff, miners, prison wardens, etc.)
10 Individuals exposed in outbreaks
Low priority populations
11 Individuals attending primary care facilities [b]
12 Contacts of known positives [b]
13 Repatriation testing
14 Community active case finding [b]

[a] Populations prioritised for point-of-care testing

[b] Pooled testing could be considered for these populations given likely low prevalence

APPENDIX 6:

Summary of South African Alert levels (Taken from the South African Government COVID-19 information portal, Accessed on: https://www.gov.za/covid-19/about/about-alert-system)

Alert level	Measures
5 (drastic measures to contain spread)	Only essential services permitted; retail permitted for essential items; limited movement of non-essential personnel; No inter-provincial travel; No public gatherings permitted. Limited public transport services;
4 (extreme precautions to limit community transmission)	Limited number of additional sectors (high economic value; low risk of transmission) allowed to resume activity; limited sale of alcohol allowed and delivery of food items allowed; limited movement of non-essential personnel; Curfew: 7pm – 5am; No inter-provincial travel; No public gatherings permitted. Limited public transport services;
3 (restrictions to address a high risk of transmission)	Wider range of sectors permitted to open (low-moderate risk of transmission); clothing and hardware stores open; encourage limited interaction with others; Curfew: 7pm – 5am; No inter-provincial travel; No public gatherings permitted. Limited public transport services;
2 (restrictions to prevent a resurgence of the virus)	Most sectors permitted to open; All retail permitted; encourage limited interaction with others; Curfew: 7pm – 5am; Limit inter-provincial travel from provinces on higher alert levels; No public gatherings permitted. Limited public transport services and domestic air travel;
1 (Most normal activity can resume; some precautions remain)	All sectors permitted to open; All retail permitted; Restaurants permitted with social distancing measures; Curfew lifted; Inter-provincial movement allowed; International travel allowed with restrictions; No public gatherings permitted.

APPENDIX 7:

Reference map for Kwa-Zulu Natal districts with assigned sub-districts.

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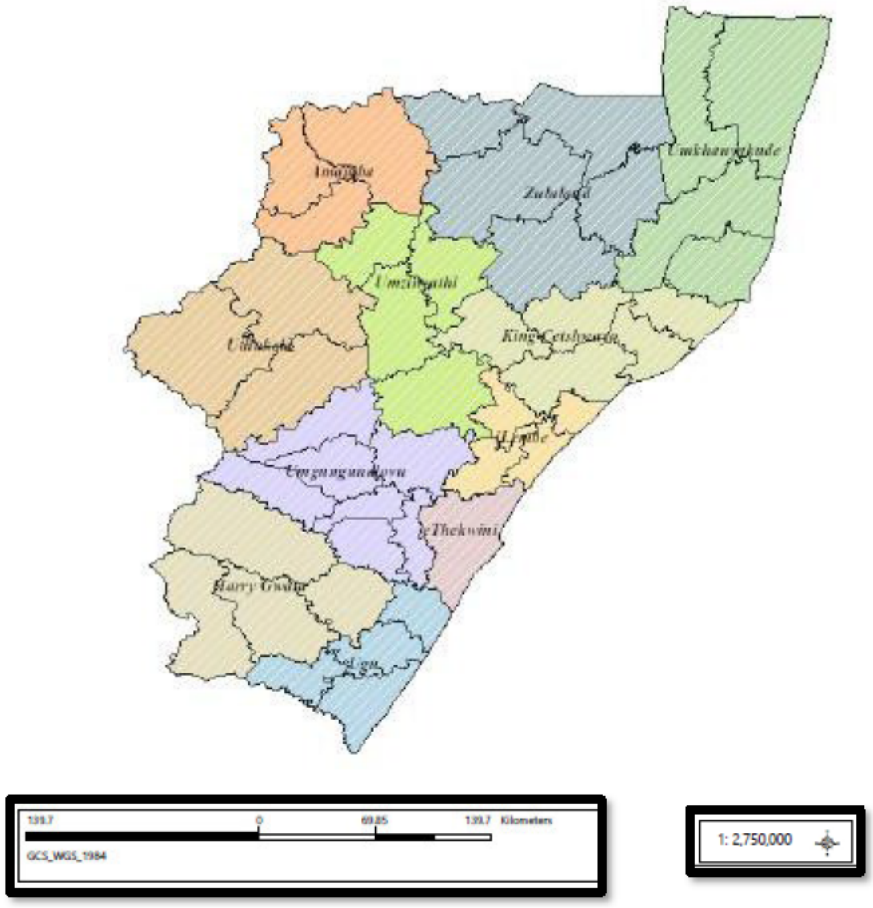
Names on figure is not clear- add text boxes for names You can increase the map size a bit more if needed



APPENDIX 8:

Pie charts depicting requests for SARS-CoV-2 PCR by facility level of care during the identified cold-spots.