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RESEARCH ARTICLE

Decrease in RT-PCR Ct values among SARS-CoV-2 positive samples during the emergence of B.1.617.2 (Delta) variant in Malaysia

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ABSTRACT

Reverse transcription-polymerase chain reaction (RT-PCR) cycle threshold (Ct) value in detecting the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection is inversely proportionate to the virus load in the patient's specimen. These values could be beneficial in the epidemic trajectory at the population level. The SARS-CoV-2 B.1.617.2 (Delta) variant which emerged in late 2020, caused an unprecedented exponential increase in SARS-CoV-2 infection cases worldwide. In Malaysia, the surge in coronavirus disease 2019 (COVID-19) cases and the inclining positivity rate contributed to the epidemic waves in late May 2021. Sudden surge in cases was suggested to be associated with increased transmission caused by the emergence of the B.1.617.2 variant. In the present study, Ct value distribution of the positive COVID-19 samples from 2020 and 2021 was tabulated against SARS-CoV-2 genomic variants determined from genomic sequencing. A significant decreasing pattern of median Ct values from overall 2020 and 2021 samples was evident ($p<0.01$). However, notable variability was observed in the Ct values between 2020 and 2021, which samples showing lower median Ct values in 2021. The percentages of SARS-CoV-2 genomic variants B.1.36 and B.1.524 were 31.6% and 68.4%, respectively, for samples obtained in October and December 2020. Whereas samples obtained in June and July 2021 were 100% of the B.1.617.2 variant. The population neutralizing antibody against SARS-CoV-2 during the initial peak of B.1.617.2 was low, however, increased during the B.1.617.2 wave. A decreasing trend in the Ct value distribution from samples tested in our laboratory correlated well with the increasing weekly COVID-19 cases reported by the Malaysia national data, which was subsequently attributed to the emergence of B.1.617.2 variant. This study proposes that analyzing Ct value distribution in screened SARS-CoV-2 samples could reveal population-level transmission dynamics and emerging variants. Coupled with genomic sequencing, it supports early control strategies against new SARS-CoV-2 strains.

Keywords: Coronavirus; COVID-19; variants; genome; transmission.

INTRODUCTION

The cycle threshold (Ct) value is the number of cycles required for the fluorescent signals to transcend the threshold of the background level implemented in the real-time reverse transcription-polymerase chain reaction (RT-PCR). Ct value is the surrogate endpoint or biomarker of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) virus burden, which is inversely correlated to the virus load (Rabaan *et al*., 2021). The interpretation from the Ct value is often semi-quantitatively represented in binary results, either negative or positive depending on the diagnostic test threshold specified by the different diagnostic reagent manufacturers, usually at a Ct value of ≤ 40. Conventionally, the Ct value is often excluded in the SARS-CoV-2 clinical test report requirement, which overlooked the potential importance of Ct values as a prognostic marker for infectiousness, notably during the inclining of epidemic growth (Hay *et al*., 2021). The skewness of Ct value varied throughout the pandemic waves between populations (Yin *et al*., 2021) and has proven beneficial to monitor viral fitness, influencing the epidemiologic phenomenon (Hay *et al*., 2021; Rodríguez-Grande *et al*., 2021; Yin *et al*., 2021). Therefore, observing the Ct value distribution at the population level would enable the estimation of the epidemic waves and is highly informative for monitoring the epidemiology and transmission dynamics of SARS-CoV-2 (Lin *et al*., 2022; Yin *et al*., 2021).

The rapid changes in SARS-CoV-2 infectiousness have been observed with the emergence of the B.1.617.2 (Delta) variant (Lopez-Bernal *et al*., 2021). The emergence of B.1.617.2 variant was first reported in India in late 2020. This SARS-CoV-2 variant carries multiple mutations in the N-terminal-domain (NTD), spike protein,

receptor-binding-domain (RBD), and furin-cleavage-site (Cherian *et al*., 2021; Planas *et al*., 2021). In less than five months following its emergence, B.1.617.2 became the dominant variant reported in India, showing high transmissibility (Lopez-Bernal *et al*., 2021). World Health Organization (WHO) has classified B.1.617.2 as a variant of concern (VOC) because the transmissibility of this variant was more than the existing VOC B.1.1.7 and has a higher affinity for binding to human angiotensin-converting enzyme 2 (ACE2) (Cherian *et al*., 2021; World Health Organization, 2023). In many countries, the B.1.617.2 infection has resulted in an exponential rise in COVID-19 cases, negatively impacting the global healthcare system (Twohig *et al*., 2022).

In Malaysia, epidemiological surveillance conducted by the Ministry of Health Malaysia revealed a surge in daily cases of COVID-19 and an inclining positivity rate starting from late May 2021 (MoH-Malaysia, 2022, 2023). Subsequently, B.1.617.2 variant was first detected in Malaysia in June 2021 from Sarawak, East Malaysia. Since then, this variant has been the predominant SARS-CoV-2 variant detected in Malaysia, with 99% of the genomic sequencing identifying the B.1.617.2 lineage (MoH-Malaysia, 2022). Additionally, from July 2021, B.1.617.2 variant has been reported from all states and territories across Malaysia due to community transmission or importation (MoH-Malaysia, 2022).

Following the local shifting in epidemic trajectory, we suggested that the surge in COVID-19 cases was linked to the emergence of the B.1.617.2, which presumably possesses inherent higher transmissibility potential, hence, would present with a lower RT-PCR Ct values of the infected population (Liu & Rocklöv, 2021; Singanayagam *et al*., 2022; Tso *et al*., 2021). In the present study, we aimed to determine the distribution of the Ct value of positive SARS-CoV-2 specimen samples from the years 2020 and 2021 and link it to the virus genome sequence to identify the variants contributing to the surge in COVID-19 cases in Malaysia.

MATERIALS AND METHODS

Ethics approval and consent to participate

Ethical clearance has been obtained from Universiti Malaya Medical Centre (UMMC), MREC ID No.: 2021226-9886 for serology monitoring. Written informed consent was obtained from all volunteers for the blood sampling. The Ct value in the present study retrospectively analyzed the COVID-19 surveillance records from Tropical Infectious Diseases Research and Education Centre (TIDREC) online database and archived samples for the genomic sequencing. Data were anonymized before accessed and did not cause any harm to individuals or society.

Study design, SARS-CoV-2 RNA samples and cycle threshold (Ct) value collection

Tropical Infectious Diseases Research and Education Centre (TIDREC) at Universiti Malaya (UM) is a key contributor to Malaysia's National SARS-CoV-2 Genome Sequencing Consortium. The SARS-CoV-2 RNA samples utilized in this study were sourced from the COVID-19 Testing Laboratory at TIDREC, which complies with the MS ISO/IEC 17025:2017 standards. This COVID-19 screening is authorized by the Ministry of Health of Malaysia (MoH) and operates in collaboration with the Ministry of Science, Technology, and Innovation (MOSTI) and Ministry of Higher Education (MOHE) as part of the COVID-19 testing initiative. Samples from multiple healthcare facilities were sent to TIDREC for COVID-19 screening to enhance case identification and contact tracing capabilities during the pandemic.

SARS-CoV-2 RNA extraction and purification from nasopharyngeal and oropharyngeal swabs in viral transport media were performed using the MagMAX™ Viral RNA Isolation Kit (Thermo Fisher Scientific) according to the manufacturer's protocol. At the beginning of the pandemic, the laboratory primarily used both in-house and a commercial assay depending on the reagent availability which includes primer sets to detect the SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) gene. The in-house assay was based on the protocol by World Health Organisation (WHO) (Corman *et al*., 2020) and a non-structural protein 9 (Nsp9) as the confirmatory gene. The in-house real time reverse transcription-polymerase chain reaction (RT-PCR) assay was performed using SensiFAST™ Probe Hi-ROX™ One-Step Kit and SuperScript™ III Platinum™ One-Step qRT-PCR System. The commercial assay used was Allplex™ SARS-CoV-2 kit to detect RdRp, envelope (E), and nucleocapsid (N) genes for SARS-CoV-2 detection. The initial primer sets and commercial kit were evaluated, revealing a consistent and comparable Ct value with no significant differences. The real time RT-PCR was considered positive for SARS-CoV-2 if two gene targets were amplified with Cycle threshold (Ct) $\leq$ 40.

We compiled the SARS-CoV-2 Ct value based on the RdRp gene for standardization of Ct value distribution for the years 2020 and 2021 (N=854). This was done following the WHO protocol and utilizing commercial kit designed to detect the RdRp gene. The Ct values from positive SARS-CoV-2 samples were retrieved from the COVID-19 surveillance laboratory at TIDREC and the national weekly COVID-19 cases data from the MoH GitHub (MoH-Malaysia, 2023). For this study, we set the samples' cut-off Ct values of $\leq$ 30 to be further analyzed for the distribution trend and samples subjected to the virus genome sequencing. The cut-off Ct value is chosen in line with the finding that samples obtained throughout the study period did not produce culturable live virus in TIDREC when the Ct value was greater than 30. Therefore, analyzing samples of Ct value $\leq$ 30 compel to represent the infectious individuals to predict the infection trajectory.

Library preparation and sequencing of SARS-CoV-2

For the sequencing, the cDNA was synthesized using the SuperScript™ VILO™ cDNA synthesis kit (Invitrogen, Thermo Fisher Scientific). The SARS-CoV-2 full genome amplicon libraries were generated with Ion AmpliSeq™ SARS-CoV-2 Research Panel (Ion Torrent, Thermo Fisher Scientific) using the Ion AmpliSeq™ Library Kit Plus (Ion Torrent, Thermo Fisher Scientific) following protocol in Ion AmpliSeq™ SARS-CoV-2 Research Panel (Protocol publication number: MAN0019277). The purified amplified libraries were quantitated using the Qubit™ dsDNA HS Assay Kit (Invitrogen, Thermo Fisher Scientific). Sequencing template preparation was performed using Ion Chef System (Ion Torrent, Thermo Fisher Scientific). A flow of 550 was set for the sequencing run using Ion GeneStudio S5 System (Ion Torrent, Thermo Fisher Scientific).

Genome assembly and clade assignment

The generated reads were assembled using IRMAreport v1.3.0.2 as implemented in Torrent Suite 5.14.0 (Ion Torrent, Thermo Scientific). The SARS-CoV-2 lineage was assigned by the Phylogenetic Assignment of Named Global Outbreak LINeages (PANGOLIN) tool (Version 3.1.16, 2021-11-25, https://pangolin.cog-uk.io/).

Survey of SARS-CoV-2 antibodies

A cross-sectional survey was conducted to estimate the population antibody status, primarily IgG and neutralizing antibodies, from late May to July 2021, during the initial phase of the Delta variant peak in Malaysia to understand the temporal progression of sero-prevalence against SARS-CoV-2. Serum samples were collected from public residing in the Kuala Lumpur and Selangor area, specifically from healthy individuals aged 18 years old and above, regardless of their COVID-19 vaccination and infection statuses. Commercially available ELISA kits (EUROIMMUN Medizinische Diagnostika AG, Lübeck, Germany) were utilized to assess anti-S1-IgG levels and the percentage of inhibition against SARS-CoV-2. The assays were conducted following the manufacturer's instructions, with a

modification of the serum dilution factor from 1:101 to 1:10,100, to ensure a readable range of antibody titers estimated from the provided standards.

Data analysis

All data analysis and representation were performed in R statistical software version 1.3.1073. The distribution of Ct value from the SARS-CoV-2 screening and the serology distribution survey was plotted. Linear regression was used to observe the trend in Ct value variations from the overall study time and between 2020 and 2021. Analysis of Variance (ANOVA) using the F-test was used to compare the distribution mean of Ct value between 2020 and 2021.

RESULTS

Sample selection

This study involved a retrospective cross-sectional analysis of RdRp Ct values (Ct ≤ 30) using a final total of 441 respiratory specimen samples that tested positive for SARS-CoV-2 virus. The selection of samples with Ct values of ≤ 30 aligns with our observation that samples collected throughout the study period could not yield culturable live virus when the Ct value exceeded 30 in our laboratory. These 441 samples comprised 161 from the year 2020 and 280 from the year 2021. For genomic sequencing, we purposively selected 36 specimen samples from the TIDREC laboratory based on a range of Ct values: > 25 Ct value, 15 to 24 Ct value, and < 15 Ct value.

Decrease in RT-PCR Ct value in recent SARS-CoV-2 positive samples

We have observed an increasing trend in the monthly positivity rate of COVID-19 cases based on the samples received by our laboratory (Table 1). In 2020, the monthly positivity rate ranged from 0% to 13.56%, while in 2021, it ranged from 0% to 28.23%. The overall positivity rates for 2020 and 2021 were 2.16% and 15.23%, respectively. Notably, the samples from July 2021 exhibited the highest positivity rate in this study, reaching 28.23%.

Table 1. Summary of the samples received for SARS-CoV-2 detection from April 2020 to July 2021

Month	Number of Samples	Number of Positive	Positivity Rate
	2020		
April	4,605	46	0.10
May	4,127	34	0.82
June	627	0	0
July	73	0	0
August	74	0	0
September	1,205	0	0
October	2,836	65	2.29
November	932	22	2.36
December	1,276	173	13.56
Total	**15,755**	**340**	**2.16**
	2021		
January	175	0	0
February	4	0	0
March	18	2	11.11
April	34	0	0
May	429	41	9.56
June	1,793	211	11.77
July	921	260	28.23
Total	**3,374**	**514**	**15.23**

Figure 1 illustrates the distribution of Ct values among RT-PCR positive SARS-CoV-2 samples from April 2020 to July 2021. A statistically significant ($p < 0.01$) and consistent decline in the median Ct values for 2020 and 2021 was evident in Figure 2. A direct comparison between April 2020 and July 2021 demonstrated a reduction of three (3) Ct value units (a median of 26 Ct units in April 2020 and 23 Ct units in July 2021, based on the overall distribution). This decrease coincided with the increased positivity percentage of received samples, rising from 0.1% in April 2020 to 28.2% in July 2021 (as detailed in Table 1). This trend was visualized through the positivity rate and median Ct value regression lines, which demonstrated an inverse relationship.

Furthermore, the declining Ct values correlated with the escalation in weekly COVID-19 cases, as indicated in Figure 1(B). The decreasing median Ct value observed from 2020 to 2021 in samples obtained from the TIDREC laboratory closely mirrored the rise in weekly COVID-19 cases in Malaysia. This parallel relationship offered a valuable estimate of the epidemic curve.

Comparison of RT-PCR Ct values regression slope of SARS-CoV-2 samples from the years 2020 and 2021

Comparison between the Ct values of samples from 2020 and 2021 revealed significant differences in the slope for the respective years (Figure 2). Our observations indicate that in 2020, the gradient slope (m) of Ct values exhibited a mild decline (m = -0.13) from the median Ct value between April and December. Conversely, in 2021, the

Table 2. The SARS-CoV-2 lineages detected at the TIDREC laboratory in Malaysia

Year	Month	Reference code	Lineage
2020	October	TIDREC/CVD/20/12565	B.1.524
		TIDREC/CVD/20/11875	B.1.524
		TIDREC/CVD/20/11629	B.1.524
		TIDREC/CVD/20/11782	B.1.36
		TIDREC/CVD/20/12484	B.1.524
	December	TIDREC/CVD/20/15218	B.1.524
		TIDREC/CVD/20/15182	B.1.524
		TIDREC/CVD/20/15128	B.1.524
		TIDREC/CVD/20/15217	B.1.524
		TIDREC/CVD/20/15233	B.1.524
		TIDREC/CVD/20/14789	B.1.36
		TIDREC/CVD/20/15126	B.1.524
		TIDREC/CVD/20/15226	B.1.524
		TIDREC/CVD/20/14893	B.1.36
		TIDREC/CVD/20/14939	B.1.36
		TIDREC/CVD/20/14832	B.1.36
		TIDREC/CVD/20/15145	B.1.524
		TIDREC/CVD/20/14927	B.1.36
		TIDREC/CVD/20/15171	B.1.524
2021	June	TIDREC/CVD/21/2163	B.1.617.2
		TIDREC/CVD/21/2542	B.1.617.2
		TIDREC/CVD/21/2479	B.1.617.2
		TIDREC/CVD/21/2476	B.1.617.2
		TIDREC/CVD/21/2420	B.1.617.2
		TIDREC/CVD/21/2471	B.1.617.2
		TIDREC/CVD/21/2422	B.1.617.2
		TIDREC/CVD/21/2195	B.1.617.2
	July	TIDREC/CVD/21/3081	B.1.617.2
		TIDREC/CVD/21/3041	B.1.617.2
		TIDREC/CVD/21/3123	B.1.617.2
		TIDREC/CVD/21/3030	B.1.617.2
		TIDREC/CVD/21/3176	B.1.617.2
		TIDREC/CVD/21/3162	B.1.617.2
		TIDREC/CVD/21/3332	B.1.617.2
		TIDREC/CVD/21/3333	B.1.617.2
		TIDREC/CVD/21/3092	B.1.617.2

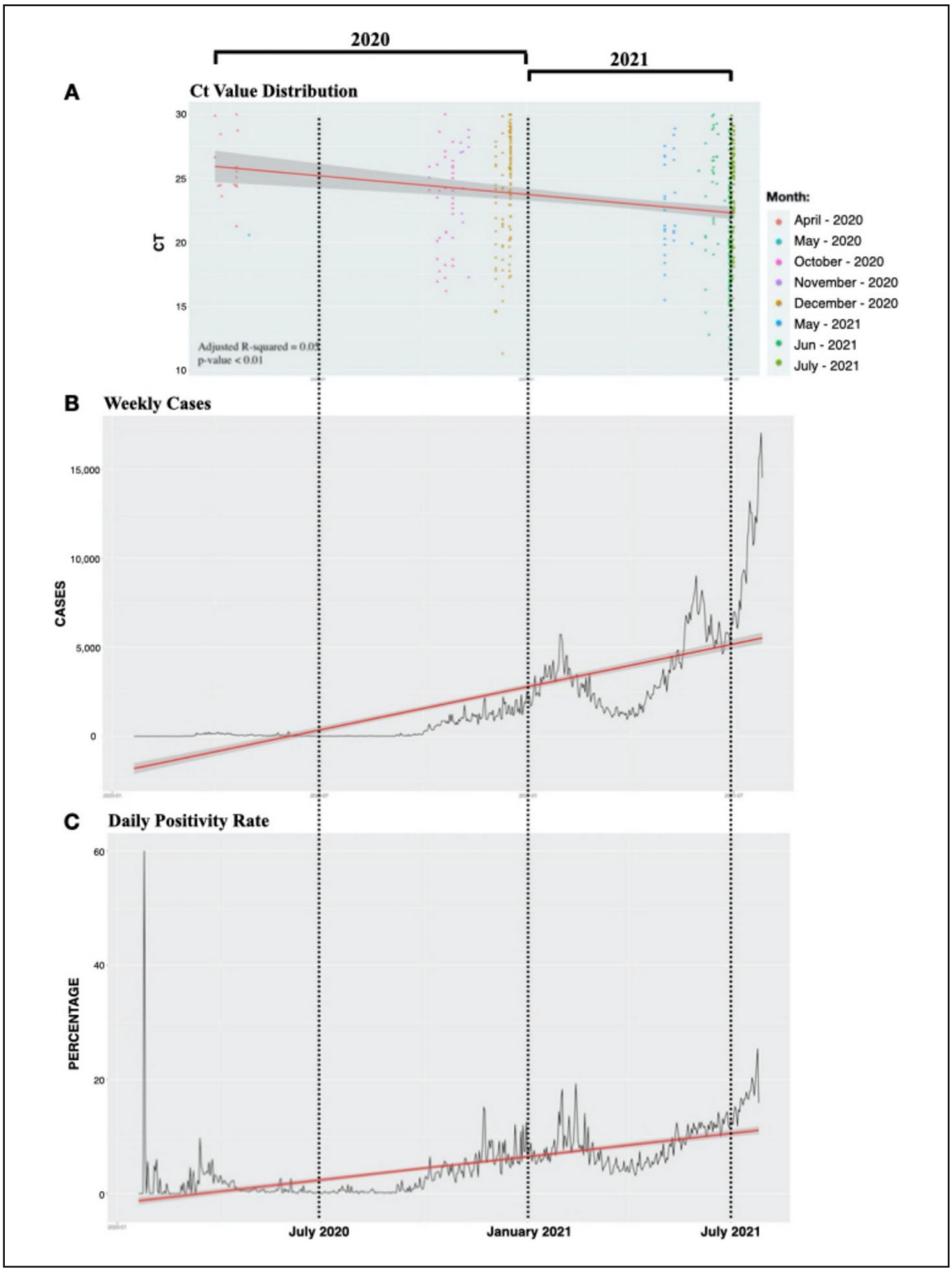

Figure 1. Overview of cross-sectional Ct value of SARS-CoV-2 samples against the SARS-CoV-2 epidemic trajectory in Malaysia. (A) Ct value distribution of positive SARS-CoV-2 samples from April 2020 until July 2021. The linear regression line from the mean Ct value shows the decreasing trend following later months. (B) Weekly SARS-CoV-2 confirmed cases in Malaysia from February 2020 until August 2021 with the linear regression line. (C) Daily SARS-CoV-2 positivity rate in Malaysia from February 2020 until August 2021 with the linear regression line.

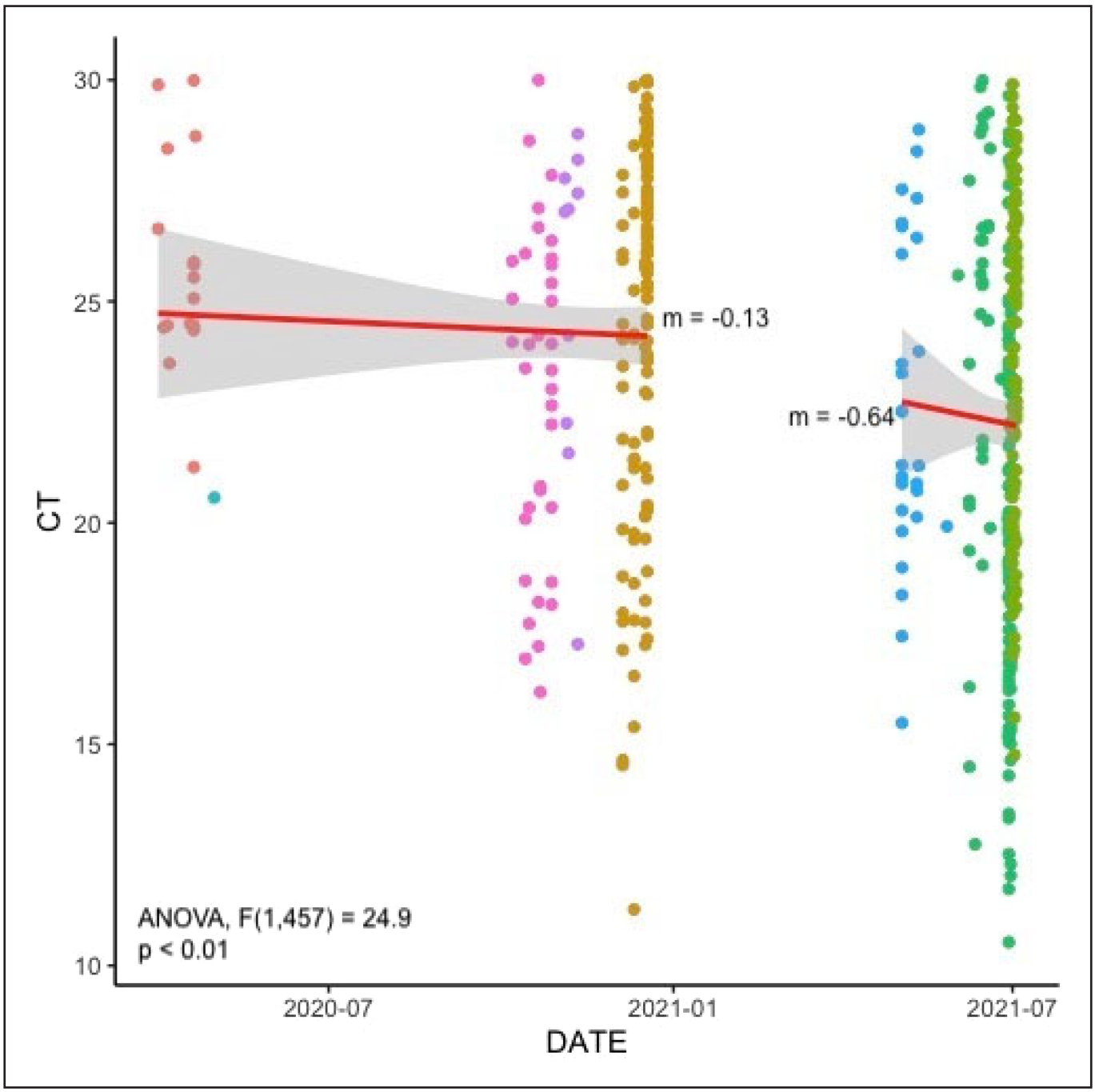

Figure 2. RT-PCR Ct value linear regression line of SARS-CoV-2 positive samples from the years 2020 and 2021. The mean distribution of Ct values from 2020 was higher than those from 2021, showed by the ANOVA in comparing the slope of the regression line. The comparison between 2020 and 2021 showed statistically significant differences in the slope from 2020 and 2021 ($p < 0.01$). The slope gradient (*m*) for 2020 and 2021 were -0.13 and -0.64, respectively.

gradient of the median Ct value slope from May to July displayed a more pronounced declining trend (m = -0.64), resulting in an overall lower median Ct value than that observed in 2020. This trend was visually evident in the scatter plot, where a noticeable decrease in the lowest Ct value units for each month was observed.

For instance, the lowest Ct value among samples taken in April 2020 was 21.26, while in July 2021 was 14.76. The lowest Ct value unit 10.53 was obtained in June 2021. The simple linear regression lines representing both years' median Ct value units can illustrate the disparities in SARS-CoV-2 infectiousness within the population, possibly indicating the emergence of more infectious variants.

Variants of SARS-CoV-2 and the emergence of B.1.617.2 (Delta)

We conducted sequencing of randomly selected positive SARS-CoV-2 samples from October 2020, December 2020, June 2021, and July 2021 (Table 2). This sequencing revealed the presence of three SARS-CoV-2 variant lineages, including one variant of concern (VOC): B.1.524, B.1.36, and B.1.617.2. The pattern of these three variant lineages closely resembled that of the median Ct value distribution shown in Figure 2, occurring before the subsequent rise in the positive rate.

Figure 3 provides an overview of the SARS-CoV-2 B.1.36 and B.1.524 variants detected among COVID-19 positive samples in 2020, with occurrence rates of 31.6% and 68.4%, respectively. In June 2021, the B.1.617.2 variant emerged in positive samples submitted to the TIDREC laboratory. Notably, the B.1.617.2 variant was detected in 100% of our RT-PCR positive SARS-CoV-2 samples, indicating its dominance over the existing B.1.36 and B.1.524 variants.

Sero-prevalence of IgG and neutralising antibodies against SARS-CoV-2

Serum samples were obtained from a total of 215 volunteers in a cross-sectional sampling. Notably, 85.1% (n=183/215) of these volunteers had completed their vaccinations of at least two doses, while the remaining 14.9% (n=32/215) had contracted SARS-CoV-2 within six weeks before collecting serum samples. Figure 4 illustrates the distribution pattern of IgG and neutralizing antibodies (Nab) among volunteers spanning late May through July 2021. This pattern represents the protection status within the population, which can act as a limiting factor for breakthrough infections during the emergence of the Delta variant.

In May 2021, IgG titers were notably high because most volunteers had been fully vaccinated within two months. However, a decline in these titers can be observed in subsequent months, stabilizing from June to July 2021. Specifically, the median IgG titer was 1412.5 IU/mL in May 2021, 18.6 IU/mL in June, and 215.3 IU/mL in July. During the initial peak of the Delta variant in June 2021, neutralizing antibodies exhibited a median inhibition rate of 36% against the SARS-CoV-2 wildtype compared to the period before the peak, which registered at 98% (Figure 4B).

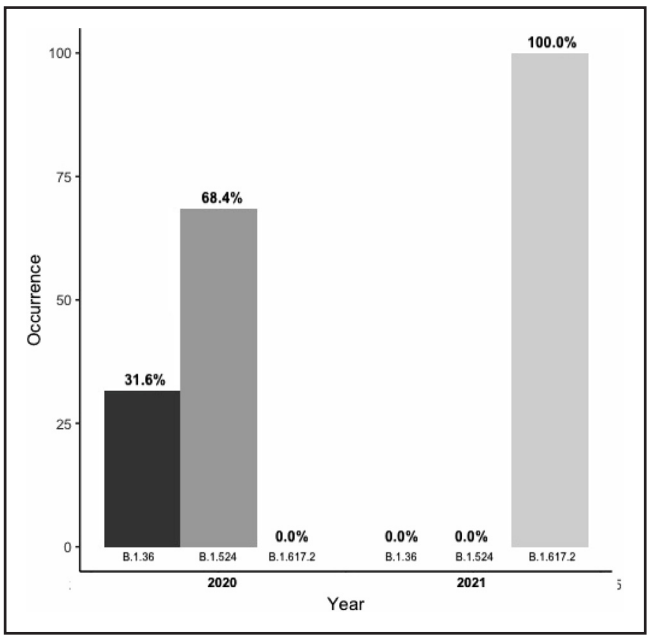

Figure 3. Percentage of variants detected from the randomly selected positive SARS-CoV-2 samples from the years 2020 and 2021 (n=36). Variant B.1.524 was more predominant (13 out of 19 positive SARS-CoV-2 samples) than B.1.36 (6 out of 19 positive SARS-CoV-2 samples) in 2020. After the B.1.617.2 SARS-CoV-2 variant emerged in 2021, B.1.617.2 was the only variant detected (17 out of 17 positive SARS-CoV-2 samples).

DISCUSSION

Laboratory diagnosis and confirmation of SARS-CoV-2 virus predominantly relied on detecting viral RNA in nasopharyngeal and oropharyngeal swab samples. The RT-PCR Ct value cut-off point is commonly used to determine whether a sample should be classified as positive. The RT-PCR Ct value demonstrates an inverse relationship with viral load (Rabaan *et al*., 2021). While Ct values are often considered unnecessary in SARS-CoV-2 clinical test reports, particularly during surges in epidemic waves (Hay *et al*., 2021), they can serve as indicators for the virus responsible for population-level epidemic waves (Mishra *et al*., 2022). Ct values obtained from a single population group can be correlated with the community-level epidemic trajectory (Hay *et al*., 2021). In Malaysia, the surge in weekly COVID-19 cases began in May 2021. It became intriguing to retrospectively analyze the trend in SARS-CoV-2 RT-PCR Ct value distribution in samples obtained from screening and associate this data with genomic information from the sequencing of selected genomic RNA samples. We postulated that Ct values of samples obtained through surveillance screening could offer insights into the local SARS-CoV-2 epidemiology.

From the study, we observed a shift in the median Ct value distribution pattern from positive RT-PCR SARS-CoV-2 specimen samples from April 2020 until July 2021. From 2020 to 2021, the overall median Ct value pattern demonstrates that the distribution slope in 2021 was statistically significantly lower than in 2020. Accordingly, we observed a decrease of three (3) median Ct value units in July 2021 compared to April 2020. The drop in the Ct value units corresponded to the increase in the COVID-19 positivity rate in this study, paralleling the surge of weekly cases in Malaysia. The

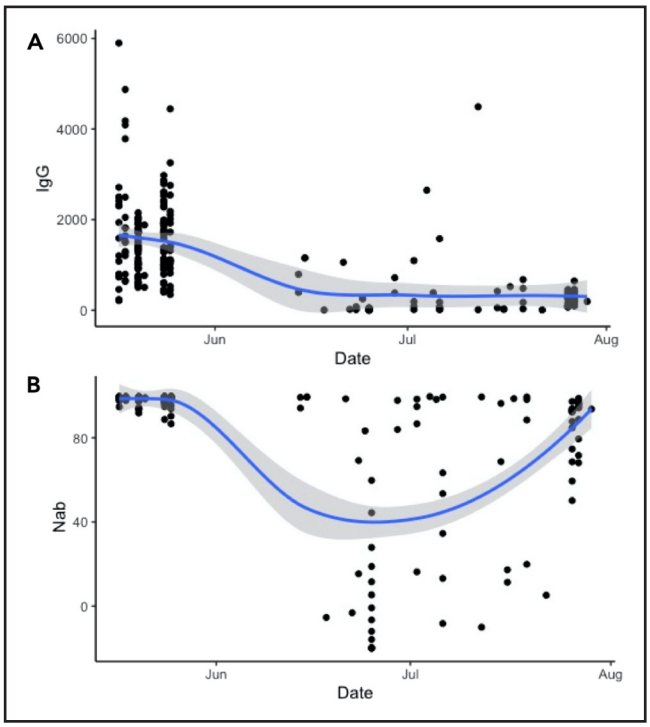

Figure 4. Sero-prevalence of IgG and neutralizing antibodies (NAb) collected from volunteers in late May until July 2021, during the initial peak of B.1.617.2 SARS-CoV-2 variant.

lower Ct value from population surveillance drives higher SARS-CoV-2 transmission since the Ct value is inversely proportional to the viral load of SARS-CoV-2 (Liu & Rocklöv, 2021; Singanayagam *et al*., 2022; Tso *et al*., 2021). It was estimated that a decrease of one (1) Ct value unit is comparable to an increment of two-fold in SARS-CoV-2 viral loads, which increased the infection probability, hence, would contribute to the epidemic growth (Levine-Tiefenbrun *et al*., 2021). From our study, the lowest Ct value unit of 10.53 was observed in June 2021, which coincided with more than 8,000 weekly cases (more than 200 cases per million people) just before the exponential increase in reported cases in July 2021 which reached more than 15,000 weekly cases (more than 600 cases per million people). The decreasing trend of the median Ct value distribution observed from our laboratory was comparable to that of the national data of increasing cases and positivity rate, suggesting that Ct value-based surveillance from a single sentinel laboratory could reflect the national trajectory of the SARS-CoV-2 epidemic waves. Hence, the use of RT-PCR Ct value can be integrated into policymaking for SARS-CoV-2 surveillance for monitoring the epidemic and to estimate the dynamics of spreading at the population level.

Our study's genomic sequencing of SARS-CoV-2 isolates revealed the detection of three variants–B.1.36, B.1.524, and VOC B.1.617.2–from the selected samples obtained from our laboratory between 2020 and 2021. Notably, the B.1.617.2 variant emerged in samples from 2021, aligning with the concurrent decline in Ct value distribution. This suggests a plausible link between the variant's infectivity and the rise in cases. This observation is consistent with earlier reports indicating that individuals with the B.1.617.2 variant carry a viral load 1,260 times higher than that of the original SARS-CoV-2 wildtype strain (phylogenetic clades 19A and 19B). Moreover, these individuals exhibit a shortened incubation period, lasting only four days compared to the usual six (Li *et al*., 2022). Furthermore, the B.1.617.2 variant has demonstrated the capability to cause reinfection in individuals previously exposed to different variants (Planas *et al*., 2021), as well as breakthrough infections among those vaccinated (Planas *et al*., 2021; Pouwels *et al*., 2021). Consequently, the population of susceptible hosts extends beyond the immunized population. Notably, no variations in the B.1.617.2 variant's viral load were observed in these subpopulations post-infection, facilitating the sustained transmission of the variants.

Genomic studies of SARS-CoV-2 detected in Malaysia have revealed the presence of various strains of SARS-CoV-2 variants. Initial COVID-19 outbreaks were linked to dominant B.6 lineages, and the first three pandemic SARS-CoV-2 waves in Malaysia were primarily attributed to the B.6(O) and B.1.524(G) lineages (Chong *et al*., 2020; Tan *et al*., 2021). These outbreaks were mainly driven by importation and subsequent human-to-human transmission (Tan *et al*., 2021). However, as of now, Malaysia has not experienced sustained transmission of a single SARS-CoV-2 strain (Tan *et al*., 2021). The introduction of B.1.617.2 variant into Malaysia in 2021 likely played a pivotal role in the significant surge in weekly cases and the higher positivity rate. Rapidly overshadowing the B.1.36 and B.1.524 variants, the B.1.617.2 variant constituted 100% of the variants detected in our genomic sequencing by May 2021. It's important to note that the B.1.617.2 variant has been widely reported globally and is linked to importation from high-risk regions and community or clustered transmission (Lopez-Bernal *et al*., 2021). The transmissibility of this variant of concern (VOC) surpasses that of its predecessors (Pyke *et al*., 2021).

While the emergence of the highly transmissible B.1.617.2 SARS-CoV-2 variant coincided with a rise in reported COVID-19 cases in Malaysia, underscoring its heightened infectivity, additional factors might have influenced this emergence. An essential consideration is the failure to promptly recognize the urgency for immediate control measures and the subsequent delay in implementing actions to contain the spread of SARS-CoV-2. Notably, impactful decisions such as the substantial reduction of population movement control restrictions (Quilty *et al*., 2021), the abbreviated quarantine period for travellers from high-risk regions (Quilty *et al*., 2021), the shortened isolation duration for SARS-CoV-2 positive individuals (Quilty *et al*., 2021), and permitting large public indoor gatherings to occur (Barker, 2020; Chong *et al*., 2020), all transpired when there was still an absence of conclusive evidence regarding the transmissibility and infectivity of these emerging variants. These decisions could have substantially contributed to the surge in cases. Conversely, the limited tools at the disposal of health policy decision-makers could have driven choices that, unfortunately, fuelled the surge of COVID-19. These decisions led to elevated rates of hospitalization and mortality.

Furthermore, our serology survey showed that during the initial peak of the Delta variant emergence, the prevalence of neutralizing antibodies from the volunteers was notably low. However, at later phases, the increase in the neutralizing antibody inhibition percentage became evident in July 2021. This rise might be attributed to the proactive vaccination initiatives undertaken by the Malaysian government and/or heightened exposure to COVID-19. In hindsight, continuous virus detection and serology data monitoring are essential for swift actions to curb viral transmission.

The distribution of RT-PCR Ct values among positive COVID-19 samples could be a valuable tool for monitoring and predicting the infectivity and transmissibility of an emerging SARS-CoV-2 variant (Roquebert *et al*., 2021; So *et al*., 2021). In addition to considering factors like vaccination status, population susceptibility, and demographic characteristics, it is imperative to include the RT-PCR Ct value of all positive samples and continually track it throughout the various epidemic waves. This underscores the need for further exploration of these factors, which could yield insights into the trajectory of COVID-19 epidemic waves in Malaysia. However, it's important to note that the findings of this study were influenced by the emergence of the B.1.617.2 (Delta) variant within the study period. Future population studies must assess ongoing waves, including variants like Omicron.

CONCLUSION

Here, we described the information on the Ct value distribution specific to Malaysia, which provides different transmission behaviour changes for the B.1.617.2 (Delta) variant. A statistically significant decreasing pattern of the mean SARS-CoV-2 RT PCR Ct value distribution for positive COVID-19 samples obtained in 2020 and 2021 was observed. We identified the VOC B.1.617.2 in 100% of our genomic sequenced samples in June and July 2021, potentially outcompeting the preceding B.1.36 and B.1.524 variants. The emergence of the B.1.617.2 variant starting in late May 2021 from the national database coincided with the exponential increase in the COVID-19 weekly cases in Malaysia. Overall, our findings suggest that the RT-PCR Ct value could be useful for monitoring the emergence and transmissibility of SARS-CoV-2 variants which could reflect changes in the viral load trajectories at the population level.

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Conflict of Interest Statement

The authors declare that they have no competing interests in the publication of this manuscript.

List of Abbreviation

Ct: Cycle threshold; RT-PCR: reverse transcription-polymerase chain reaction; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; RBD: receptor-binding domain; WHO: World Health Organization; VOC: variant of concern; ACE2: angiotensin-converting enzyme 2; COVID-19: coronavirus diseases 2019; RdRp gene: RNA-dependent RNA polymerase gene; Nsp9 gene: nonstructural protein 9 gene; E gene: envelope gene; N gene: nucleocapsid gene

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