

# SARS-CoV-2 (COVID-19) Virus Shedding Period among Bhutanese Population during Pandemic: A Retrospective Cohort Study from Bhutan

Kunznag Dorji<sup>1\*</sup>, Kelzang Choden<sup>2</sup>, Meejur Palden<sup>3</sup>, Sonam Wangchuk<sup>4</sup>, Tashi Dorji<sup>5</sup> and Sonam Gyeltshen<sup>1</sup>

<sup>1</sup>Department of Public Health, Royal Centre for Disease Control, Thimphu, Bhutan

<sup>2</sup>Department of Health, Mongar Eastern Regional Referral Hospital, Mongar, Bhutan

<sup>3</sup>Department of Public Health, Gelephu Central Regional Referral Hospital, Sarpang, Bhutan

<sup>4</sup>Department of Health, Phuentsholing General Hospital, Chukha, Bhutan

<sup>5</sup>Department of Medicine, Dewathang Military Hospital, Samdrupjongkhar, Bhutan

## Abstract

**Background:** Bhutan detected its first COVID-19 case on 5th March 2020 and implemented stringent public health measures. This study aims to describe the viral dynamics of SARS-CoV-2 and its clinical characteristics among the Bhutanese population during the pandemic.

**Methods:** This retrospective quantitative study included 500 COVID-19 laboratory-confirmed positives from five PCR testing labs from March 2020 to August 2021. The data were extracted from COVID-19 Integrated Influenza Surveillance System. Descriptive statistics and student t-tests were used to analyze the data in STATA version 13.1.

**Results:** The median detection period from the onset of symptoms to the first RT-PCR positive was 4 days (IQR, 2-12). The median period of virus shedding was 14 days (IQR, 8-27). The median period of virus shedding was longer in symptomatic cases (23 days) than in the asymptomatic cases (11 days). The median Ct value of the RT-PCR assay for 2020 was 31.95 (23.36-35.45), while for 2021 was 21.67 (16.17-29.15).

**Conclusion:** The findings suggest that SARS-CoV-2 can be detected for a long duration but the sensitivity of RT-PCR test is high during the early stage of infection.

**Keywords:** COVID-19, SARS-CoV-2, Viral shedding period, RT-PCR, Bhutan Coronavirus

**Abbreviations:** BMC: Boston Medical Center; HLH: Hemophagocytic Lymphohistiocytosis

## Introduction

The COVID-19 pandemic is a global outbreak of coronavirus; an infectious disease caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) viruses. This disease started to affect people in China in December 2019 and then spread quickly to other parts of the world. On 30 January 2020, WHO announced that this was a Public Health Emergency of International Concern (PHEIC), and on 11th March 2020, they declared it a pandemic. Since then, COVID-19 has become a global pandemic. Since then COVID-19 has affected more than 733 million people and caused more than 6.7 million deaths as of December 2022. The rapid spread and severity of COVID-19 have posed unprecedented challenges to the global health system, economy, and society [1].

SARS-CoV-2 is a member of the Coronaviridae family of enveloped RNA viruses. It is closely related to other bat coronaviruses, suggesting a zoonotic origin [2]. The virus infects human cells by binding to the Angiotensin-Converting Enzyme 2 (ACE2) receptor through its spike protein [3]. The clinical spectrum of COVID-19 varies from asymptomatic or mild respiratory symptoms to severe or fatal complications, such as ARDS, septic shock, and multi-organ failure [4]. The risk factors for severe COVID-19 include older age, comorbidities, male sex, and genetic susceptibility [5].

A key aspect of COVID-19 epidemiology and control is the understanding of viral dynamics and host response. Viral load kinetics and the duration of viral shedding are important indicators of disease severity, transmission potential, and treatment efficacy [6]. However, there is considerable variability in the reported viral shedding patterns and durations among different studies, depending on the sample type, collection method, detection assay, and patient characteristics [7]. Moreover, there is limited data on the molecular characterization of

SARS-CoV-2 strains circulating in different regions and populations, which may have implications for viral evolution, adaptation, and vaccine development [8].

Bhutan detected its first COVID-19 case on 5th March 2020 and since then implemented stringent public health measures, including lockdowns, testing, tracing, isolation, and vaccination [9-13]. As of 26 December 2022, Bhutan has reported 62,538 confirmed cases of COVID-19, with 21 deaths [14,15]. Bhutan has adopted one of the most conservative protocols for COVID-19 diagnosis and management [16,17].

This study aims to describe SARS-CoV-2 viral detection results by RT-PCR across different time points throughout the disease course. It also seeks to understand the viral characteristics of SARS-CoV-2 and gain insights. This is the first paper to describe the virus characteristics of SARS-CoV-2 in the Bhutanese population during a pandemic.

## Materials and Methods

### Study design and setting, study participants, and sample size

A quantitative retrospective study was conducted to analyze the viral dynamics of SARS-CoV-2. We have adopted a total sampling strategy method to enroll 500 COVID-19 infected patients confirmed by RT-PCR

**\*Corresponding author:** Kunznag Dorji, Department of Public Health, Royal Centre for Disease Control, Thimphu, Bhutan; E-mail: kunzangdorji@health.gov.bt

**Received:** 31-Aug-2023, Manuscript No. JIDT-23-112587; **Editor assigned:** 04-Sep-2023, Pre QC No. JIDT-23-112587 (PQ); **Reviewed:** 19-Sep-2023, QC No. JIDT-23-112587; **Revised:** 26-Sep-2023, Manuscript No. JIDT-23-112587(R); **Published:** 04-Oct-2023, DOI:10.4173/2332-0877.23.S4.002.

**Citation:** Dorji K, Choden K, Palden M, Wangchuk S, Dorji T, et al. (2023) SARS-CoV-2 (COVID-19) Virus Shedding Period among Bhutanese Population during Pandemic: A Retrospective Cohort Study from Bhutan. J Infect Dis Ther S4:002.

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(250 from 2020 and 250 from 2021), isolated in four isolation facilities located in different regions of the country. There were four strategically identified COVID-19 Isolation Centres namely; Jigme Dorji Wangchuk National Referral Hospital (JDWRH), Thimphu, Royal Guest House, Mongar, Royal Institute for Governance and Strategic Studies (RIGSS) Hostel, Phuentsholing, and old Central Regional Referral Hospital, Gelephu. All the COVID-19-positive patients were isolated in these respective Isolation facilities and included in the study between 6th March, 2020-August, 2021.

All enrolled cases were diagnosed, isolated, and managed according to the National Preparedness and Response Plan for Outbreak of Novel Coronavirus (version 3) developed by the Ministry of Health, Bhutan. (Ministry of Health Bhutan, 2020)

**Inclusion criteria:** All COVID-19-positive cases confirmed by RT-PCR were included for analysis, irrespective of gender and age for the given timeline.

**Exclusion Criteria:** Clinically diagnosed or COVID-19 negative by RT-PCR were excluded from the study. We reviewed laboratory-confirmed COVID-19 symptomatic and asymptomatic cases, who eventually achieve the cessation of viral RNA shedding, defined as two consecutive negative SARS-CoV-2 PCR results on nasopharyngeal swabs collected at least 24 hours apart.

## Data collection

The data were collected as per the standard format developed in the electronic web-based system, which was managed by Royal Centre for Disease Control (RCDC) and National Surveillance Team, MoH. The representative data for two years were included (250 cases each in 2020 and 2021). The following information for 250 cases for the 2020 cohort was analyzed: age, gender, signs and symptoms, date of onset, date of first detection and date of discharge from the isolation ward, Cyclic threshold (Ct) for each periodic test to determine virus shedding period and viral load. Since no other variables were retrieved due to the system default, we analyzed only the Cyclic threshold (Ct) for each periodic test to determine the viral load of 250 cases for a cohort of 2021.

## Laboratory detection

Nasopharyngeal and throat swab samples were collected from all the symptomatic suspected cases, quarantine facilities, close contacts, and incoming travelers. The sample was collected and transported to PCR testing centers as per the National Preparedness and Response Plan for Outbreak of Novel Coronavirus (version 3) developed by the Ministry of Health, Bhutan [18]. The sample Viral RNA was extracted from 200 µl of nasopharyngeal swabs collected in VTM using QIAamp viral RNA mini kit (QIAGEN, Germany) and Quick-RNATM Viral Kit (Zymo Research, USA) following the manufacturer's instructions. Extracted Viral RNA was subjected to RT-PCR using Standard M nCOV Real-Time Detection Kit (SD Biosensor, Korea) targeting Envelop gene and ORF1ab gene. A total of 30µl reaction was prepared comprising 10 µl of eluted viral RNA, 14 µl of 2019-nCoV Reaction solution, 6 µl of RTase mix, 0.5 µl of ROX references and 0.5µl of Internal Control. The cycling condition consisted of 1 cycle for 15 minutes for 50°C, 3 minutes for 95°C followed by 5 cycles of pre-amplification at 95°C for 5 seconds, 60°C for 40 seconds followed by 40 cycles of 95°C at 5 seconds, 60°C for 40 seconds. Ct value <36 was considered positive. For each protocol Positive and negative controls were run in each test to validate the test result.

Patients were considered recovered under two circumstances; asymptomatic patients were tested after 7 days of isolation and considered recovered if they have two consecutive negative RT-PCR reports 24 hours apart and symptomatic patients were tested only after complete resolution of symptoms for at least three days and declared recovered if they have two RT-PCR reports negative within 24 hours apart. Recovered patients were further followed for the next 14 days after discharge from the isolation ward.

## Statistical analysis

All parametric variables were analyzed in the form of medians (Inter-Quartile Range, IQR) and mean using student t-test and ANOVA, while Pearson's Chi-square test was used to determine the significance level for nonparametric variables. All p values were reported as two-sided with a significance level of 0.05. All statistical tests were performed in STATA version 13.1.

## Results

### Demographic and clinical characteristics of COVID-19 patients

A total of 500 patients diagnosed with COVID-19 from March 2020 to August 2021 were included in this study, of which 296 were imported cases and 204 outbreak cases. However, we analyzed only 250 samples from 2020 for the most determinants of the dynamic profile, and the rest 250 samples from 2021 were exclusively used for Ct values comparison with the samples of 2020. No case was admitted to ICU. The median age was 28 years (IQR, 23-38; ranging from 0.2-91 years, of which 59.2% (296) were males. The most affected age group was 16-30 years (43.2%) followed by 31-45 years (27.4%)) (Table 1).

SN	Variables	(N=500)
1	Age	
	Median (IQR), y	28 (23-38)
	Mean	3080.00%
	Range	0.2-91
	Age group (years)	
	0-15	13.4% (67/500)
	16-30	43.2% (216/500)
2	Gender	
	Male	59.2% (296/500)
	Female	40.8% (204/500)
	greater than or equal to 65	3.6% (18/500)
3	SARS-CoV-2 RT-PCR assay (n=250)	
	Total tests	1764
	The median of Test/patient, IQR	6 (4-9) test/patient
	Range	2-21 test
	Nasal swab	100% (250/250)

4	Onset of symptoms to, median (IQR), d, (n=155)	
	First SARS-CoV-2 RT-PCR Assay	4 (2-12)
	Range	0-67
5	The median period of virus shedding	
	SARS-CoV-2 RT-PCR Assay (IQR), (n=250)	14 (8-27)
	Range	1-75
	Symptomatic SARS-CoV-2 (n=155)	23 (14-38)
	Range	1-77
	Asymptomatic SARS-CoV-2 (n=95)	10 (8-22)
	Range	4-49

**Table 1:** Demographic characteristics and dynamics of SARS CoV-2 in Bhutan, 2020-2021 (n=500).

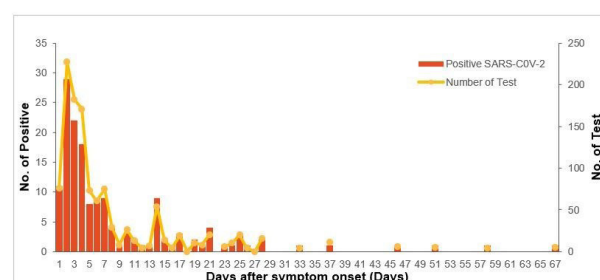
Around 64% (160/250) were imported cases (COVID-19 imported cases refer to individuals who have been infected with the SARS-CoV-2 virus in a different country or region and have traveled to Bhutan while infected). Most were symptomatic (62.0%). The most commonly presented symptoms were fever (47.7%) followed by headache (39.1%), cough (38.1%), and sore throat (31.0%), while loss of appetite (3.9%) and shortness of breath (4.5) were least presented among COVID-19 patients in 2020 cohort (Table 2). The median incubation period was 5 days (IQR: 3-11) (Table 2).

SN	Variables	Proportion (N=250)
1	Case type	
	Imported	64.0% (160/250)
	Local transmission	36.0% (90/250)
2	Symptomatic	62.0% (155/250)
	Fever	47.7% (74/155)
	Cough	38.1% (59/155)
	Shortness of breath	4.5% (7/155)
	Sore throat	31.0% (48/155)
	Headache	39.4% (61/155)
	Diarrhea	6.5% (10/155)
	Myalgia	15.5% (24/155)
	Runny Nose	21.9% (34/155)
	Loss of Smell	23.2% (36/155)
3	Incubation period	
	Median (IQR), d	5 (3-11)
	Range	1-99
4	Asymptomatic	38% (95/250)

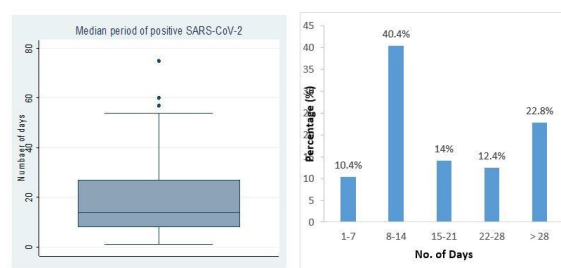
**Table 2:** Clinical characteristics and manifestation amongst COVID-19 positives.

## Dynamic profile of SARS-CoV-2 infection primary outcome

Table 1 shows the total number of SARS-CoV-2 RT-PCR assays conducted was 1764 among 250 COVID-19 cases (2020), which was an average of 6 tests per patient. The median detection period from onset of symptoms to the first RT-PCR positive was 4 days (IQR, 2-12; range, 0-67) among symptomatic cases (Figure 1). The median period of virus shedding (first positive till the detection of two consecutive negatives) of SARS-CoV-2 was 14 days (IQR, 8-27; range, 1-75, N=250). The median period of virus shedding period was longer in symptomatic cases (23 days, IQR: 14-38) than the asymptomatic cases (11 days, IQR: 8-22), which was statistically significant (p=0.0001) as shown in Table 1. Duration of virus shedding was observed maximum between 8-14 days (40.4%), however, around 22.8% of them were found still positive after 28 days (Figure 2).



**Figure 1:** Number of Positives SARS-CoV-2 detected after symptom onset (N=155). Note: (■): Positive SARS-CoV-2, (—): Number of Test.



**Figure 2:** Duration of viral RNA shedding period by days and median of viral shedding period.

## The influence of demographic factors on the temporal variation of SARS-CoV-2

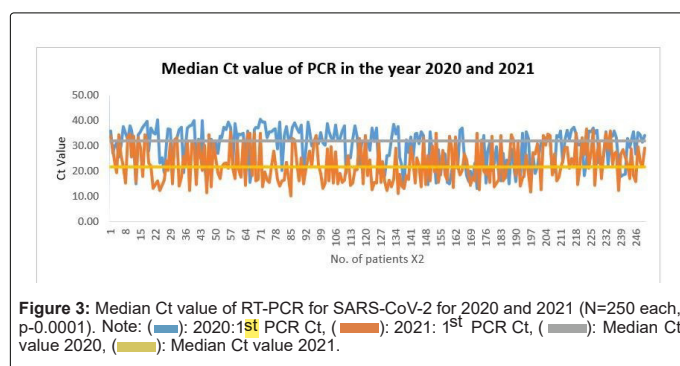
We investigated the impact of age and gender on the dynamic profile of SARS-CoV-2 by RT-PCR assay. The mean virus-shedding period was slightly longer in the 16-30 years age group (19.3 days, p=0.936) than in the other age groups (Table 3). Females had a shorter mean period of virus shedding than the male patients (17.6 days vs. 19.5 days, p=0.2728).

Variables	N=250, mean (days)	p-value
<b>Mean period virus shedding by gender</b>		
Female		
Male	17.6 (6-60 days)	0.2728
	19.5 (1-75 days)	

<b>Mean period of detection after symptom onset</b>		
Imported		
Local transmission	12.1 days	
	4.7 days	0.0001
<b>The median period of Virus shedding, IQR, d</b>		
Symptomatic SARS-CoV-2 (n=155)	23 (14-38)	0.0001
Range	1-77	
Asymptomatic SARS-CoV-2 (n=95)	10 (8-22)	
Range	4-49	
Median Ct value of First RT-PCR assay for;	24.38 (18.40-31.83)	P=0.0001
Local cases (n=90)	13.08- 38.29	
Range	33.47 (29.47-36.34)	
Imported cases (n=160)	14.94-40.44	
Range		P=0.0001
Median Ct value of, median (IQR)	31.95 (23.36-35.45)	
First RT-PCR (n=250)	13.09-40.44	
Range	35.0 (32.90-36.63)	
Last RT-PCR (n=250)	18.0- 41.71	P=0.0001
Range		
Median Ct value of RT-PCR;	31.95 (23.36-35.45)	
2020: Median (IQR)	12.8-40.0	
Range	21.67 (16.17-29.15)	P=0.0001
2021: Median (IQR)	10-36.7	
Range	-	

**Table 3:** Clinical characteristics and viral dynamic.

The mean period of SARS-CoV-2 detection after symptom onset was shorter than the local community cases (4.7 days) than imported cases (12.1 days), which was statistically significant ( $p=0.0007$ ). The median Ct value of the first RT-PCR assay was compared between the cohorts of two years. The median Ct value of the first RT-PCR assay of 2020 was 31.95 (23.36-35.45), and for the year 2021 was 21.67 (16.17-29.15),  $p=0.0001$  (Table 3 and Figure 3).



## Discussion

We conducted a cohort study of 500 cases of laboratory-confirmed COVID-19 infection in Bhutan. This is the first paper to describe the viral dynamic of SARS-CoV-2 in the Bhutanese population during a pandemic. Our main findings are: The median detection period from

the date of onset to the first RT-PCR positive was 4 days (IQR, 2-12). The median period of virus shedding SARS-CoV-2 was 14 days (IQR, 8-27). The median period of virus shedding was longer in symptomatic cases (23 days) than in the asymptomatic cases (11 days). The median Ct value of the RT-PCR assay for 2020 was 31.95 (23.36-35.45), while for 2021 was 21.67 (16.17-29.15). The most affected age group was young adults (median age: 28 years; IQR: 23-38 years), and males were more affected than females (59.2% vs. 40.8%). Fever (47.7%) was the most common symptom among other clinical features.

Our results indicated that the SARS-CoV-2 mean viral shedding duration in the upper respiratory tract was 14 days which is a little shorter than the previous studies which has 17 days [19,20]. However, the studies found that there is no live virus isolated from the culture after 9 days of symptom onset [20]. These findings indicate that most of the cases were detected positive within 2-12 days and the virus keeps shedding on average 8-27 days. The key determinants that affect the detection and viral shedding periods as stated in previous studies depend on multiple factors such as the SARS-CoV-2 variant, and host factors, such as patient age and sex, and immune status, influence shedding dynamics [21].

However, we found a significant difference in the viral shedding duration between symptomatic and asymptomatic cases, which is contrary to some previous studies [22,23]. However, Cevik's 2020 study states that though the viral load among symptomatic and asymptomatic are the same, the viral clearance is faster among asymptomatic [19]. This could be due to different testing protocols, inclusion criteria, or viral variants circulating among different populations. Further studies are needed to confirm this finding and explore its implications for transmission dynamics and infection control measures.

Our study finding showed that the mean virus concentration in 2021 was more compared to the year 2020 suggesting a possible increase in viral load or transmissibility over time [24]. This could be related to the emergence of new variants of SARS-CoV-2 (Delta variant) that have been detected in Bhutan since late 2020 [25,13,14].

Our study showed that SARS-CoV-2 affected mostly young adult men of the Bhutanese people, which is similar to previous other studies [25-28]. In Bhutan most young adults and teenagers returned from abroad who traveled for education and work in 2021. The cases were also added by expatriates of workers who were all men [29-31]. (Women are less likely to be infected by bacterial, viral, and fungal than men, due to their more innate and adaptive immune responses, moreover, Bhutanese women are indoor type culture, they prefer to stay at home) [32].

This study showed that fever, followed by cough was the most common clinical symptom of SARS-CoV-2 in Bhutan, which is consistent with several other studies in China and Pakistan [3,33,34]. However, other features like abdominal cramps and diarrhea, breathing difficulty, and loss of appetite were also observed though in less proportion. There was a high proportion of asymptomatic cases (40%), which is similar to the global trend of the COVID-19 burden [20,33-35]. This highlights the need for active surveillance and testing of travelers and contacts to prevent undetected transmission.

## Conclusion

Our results suggest that the SARS-CoV-2 virus can be detected for longer duration and sensitivity of the RT-PCR test is very high during the early stage of infection. Further, the study reveals the molecular and clinical features of SARS-CoV-2 infection in Bhutan, showing a similar detection period and viral shedding duration as other countries,



but a significant difference between symptomatic and asymptomatic cases. The study also suggests a possible increase in viral load or transmissibility over time and a higher prevalence of COVID-19 among young adults and males.

## Limitations

Our study has some limitations. First, we relied on self-reported data on symptoms onset and travel history, which may be subject to recall bias or incomplete information. Second, we did not collect data on comorbidities, clinical outcomes, or treatment options for our cases, which may affect their viral shedding duration and disease severity. Third, we did not perform any statistical adjustments for potential confounders, such as age, sex, or viral variant, which may influence our results.

## Recommendations

To improve the timeliness and accuracy of COVID-19 diagnosis, testing should be performed as soon as possible after the onset of symptoms or exposure to a confirmed case.

To reduce the risk of transmission and infection, isolation and quarantine measures should be implemented for at least 14 days for both symptomatic and asymptomatic cases, and longer if symptoms persist or worsen.

To understand the epidemiology and monitor the evolution and spread of SARS-CoV-2 in Bhutan, genomic sequencing and phylogenetic analysis should be conducted regularly and integrated with epidemiological and clinical data.

Our study provides novel insights into the molecular and clinical characteristics of SARS-CoV-2 infection in Bhutan. Our results have important implications for public health policy and practice, as well as for future research. We hope that our study will contribute to the global efforts to combat the COVID-19 pandemic

## Acknowledgment

We are grateful to the Ministry of Health, Bhutan for organizing the meeting to write the research proposal. We are grateful to Dr. Sonam Wangchuk, Head of the Royal Centre for Disease Control, for motivating and inspiring us to write this research article. We would like to convey our gratitude to all health workers, especially those involved in COVID-19 testing RT-PCR laboratories.

## Author contribution

All authors have equally made substantial contributions in writing this manuscript as follows; KD-Conception and design of the study, acquisition of data, data analysis and/or interpretation, drafting of manuscript and/or critical revision. SG-conception and design of the study, acquisition of data. MP, KC, TD, SW-acquisition of data. All authors reviewed for the final approval of the version to be submitted.

## Ethical Consideration

The study proposal was submitted to the research ethics board of Health (REBH) in Bhutan for administrative clearance and ethical review. The study was approved by REBH: REBH/Approval/2022/026, with administrative clearance: MoH/PPD/ADM.CL/9/2022/029. The study used routine surveillance data, we have maintained and protected patient privacy information.

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