Case series

A SARS-CoV-2 variant of C-36 lineage with L452R mutation infects a family in Sri Lanka, but has limited spread – A Case Series

H Abeygoonawardena[1], H Jayasinghearachchi[2], T Senaratne[3], P Jayasekera[1], D Nakkawita[1], N Mubarak[1], U Kulasekera[1], P Kawyangana[1], D Govindapala[1], AD De Silva[1]

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Abstract

We report a family cluster of six members who tested positive for SARS-CoV-2 infection in April 2021 at University Hospital, Kotelawala Defence University, Sri Lanka. Samples from three members were subjected to Next Generation Sequencing (NGS) and all were identified as SARS-CoV-2 variant of pangolin lineage C.36, containing the L452R mutation which is associated with high infectivity. All patients recovered with mild symptoms and no complications. This is the first report identifying a new variant of the C.36 lineage containing the L452R mutation from Sri Lanka followed by detection of the same lineage from Northern Sri Lanka in June 2021 and no reported cases hereafter. At that time, it was relatively rare for a whole family to get infected, and the concern was that this strain would spread rapidly. Due to unknown circumstances it never spread to be detected by the national surveillance programme. Other variants, B.1.411 and B.1.1.7, more dominant and infectious, were already in circulation and presumably outcompeted this strain.

Keywords: *SARS-CoV-2, L452R, variants, Sri Lanka*

Introduction

During the COVID-19 pandemic in Sri Lanka, the third wave came eighteen months after the reporting of the first person infected by the SARS-CoV-2 virus.[1] During that time many countries were experiencing the third wave with over 180 million cases reported, and scarce resources to deal with such large numbers.[2] The first patient in Sri Lanka, a foreign national from China, was detected on the 27th of January 2020, and the first Sri Lankan patient was reported on the 10th of March 2020.[3] Since the detection of the first patient, Sri Lanka went on a strict and extensive lockdown for 10 days (on the 20th of March 2020), which limited the

[1] Faculty of Medicine, General Sir John Kotelawala Defence University, Rathmalana-10390, Sri Lanka
[2] KDU-CARE, General Sir John Kotelawala Defence University, Kadawala Estate, Rathmalana- 10390, Sri Lanka
[3]Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University, Werahera- 10290, Sri Lanka

Address for correspondence: Dr.A.D. De Silva, Faculty of Medicine, General Sir John Kotelawala Defence University, Rathmalana-10390, Sri Lanka; Telephone: +9477-8792186 email: dharshan_fom@kdu.ac.lk

https://orcid.org/0000-0001-5291-9543

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initial outbreak to certain areas of the Colombo Municipal Council (CMC) area. Although Sri Lanka successfully contained the epidemic until the end of September, with no locally detected cases from August to September 2020, a large outbreak emerged during early October, which rapidly spread island-wide.[1] Up to date, Sri Lanka has had three waves of COVID-19 and has been able to control the disease to a large extent due to a concerted vaccination campaign. In addition, the country has one of the lowest COVID-19 related death rates in the world.[4]

Multiple variants of SARS-CoV-2 have been documented globally during this pandemic and it is important to identify possible new variants that may be spreading within a community.[3,5] Sri Lanka had three waves with a total of 16,517 deaths, with the pandemic largely controlled by lockdowns, etc. With Sri Lanka's successful control of SARS-CoV-2 compared to other countries with much higher resources, it is important to monitor variants that come into circulation to be able to ramp up testing if a particularly infectious variant comes into the island.

The cases we present came up early in the third wave. Sri Lanka had fairly extensive testing and it was unusual to have a whole family (six members) infected within a few days and report to the University Hospital Kotelawala Defence University (UHKDU) at that time. It is important to study cases such as this which can serve as the "canary in the coal-mine" to warn of new variants that can spread rapidly.

We report a family cluster of six members who tested positive for SARS-CoV-2 infection by both Rapid Antigen Test (RAT) and RT-PCR done from nasopharyngeal swab samples between the 22nd and 26th of April 2021 at UHKDU.

Methods

All six members (Patients P1-6) were tested by RAT & RT-PCR at the PCR laboratory of UHKDU. RNA samples from P3, P5 and P6 were recovered from the molecular diagnostic laboratory UHKDU and sent to Genelabs Medical (Pvt) Ltd, Sri Lanka for SARS-CoV-2 NGS sequencing. Sequencing (NGS) was carried out using the Ion Torrent platform and data analysis by torrent suite. Sequences were aligned using Geneious Prime software and a Phylogenetic tree was developed using Geneious Prime tree builder constructed using the Neighbour-joining method, Tamura-Nei genetic distance model. RNA of Patient 1 (P1), Patient 2 (P2) and Patient 4 (P4) were used as internal positive controls in the molecular laboratory of UHKDU. Sequencing analysis could therefore not be performed on the RNA samples from P1, P2 and P4.

Case Histories

Six members of one extended family were infected with the SARS-CoV-2 virus over a period of four days. Suspicion of the same strain of the virus was considered due to the short time frame of infection with the high number of cases (all exposed).

Patient 1 (P1) - Index case

The index case was a 36-year-old male from Colombo (Western Province) who was previously well without any comorbidities, and presented with fever (38.8 °C), arthralgia and myalgia since 8.00 pm on 21st April 2021. He had not received COVID-19 vaccine at that time (Figure 1).

He, with his wife (patient 5) and his 2-year-old daughter (patient 6) had visited his wife's parents in Galle (Southern Province) on 9th April 2021 in their private vehicle and stayed at their house for one week during the new year vacation. On the 15th of April, the index case, with his wife and daughter, visited his parents at Alawwa in the Kurunegala district (Northwestern Province) and stayed there for another 5 days until the 19th of April. On the 19th of April, the index case, with his wife, daughter, and mother (patient 3) travelled back to their house in Colombo.

On the 20th of April, the index case went again to Galle and on the following day came back to Colombo with his mother-in-law (patient 4). On the 21st of April he took her back to Alawwa in his private vehicle and returned to Colombo.

The index case tested positive for SARS-CoV-2 by RT-PCR and Rapid Antigen test (RAT) on 22nd April (day 2 of symptoms) and was admitted to University Hospital KDU (Table 1).

Patient 2 (P2)
P2 was the 75-year-old father of the index case (patient 1) residing in Alawwa, Kurunegala district. P2 was diagnosed with prostate cancer in 2009 and had undergone prostatectomy and orchidectomy in 2009 and 2018 respectively followed by cycles of chemotherapy. He had not been vaccinated against COVID-19. He developed fever and headache on 21st April (the same day as the index case) and tested positive on the following day with RAT and RT-PCR for SARS-CoV-2. He was admitted to University Hospital KDU with the index case.

Patient 3 (P3)
P3 was the 71-year-old mother of the index case residing in Alawwa, Kurunegala District. She was not vaccinated against COVID-19. She was a hypertensive patient on regular treatment and had developed sore throat without fever on 23rd April and tested positive with both RAT and PCR tests and was admitted to the same hospital.

Patient 4 (P4)
P4 was the 67-year-old mother-in-law of the index case, residing in Galle (Southern Province) and who travelled with the index case in the same vehicle on 21st April. She had diabetes mellitus, hypertension, ischemic heart disease and rheumatoid arthritis and was on multiple drugs. She received the first dose of the AstraZeneca (COVISHIELD) vaccine on 26th March 2021. On the 25th of April, she developed mild fever and sneezing and tested positive (RAT and RT-PCR for SARS-CoV-2) on the following day and was admitted to UHKDU.

Patient 5 (P5)
P5, the 36-year-old wife of the index case was previously healthy without any comorbidities. She had received the first dose of the AstraZeneca (COVISHIELD) vaccine on 1st February. On 23rd April she was screened for SARS CoV-2 infection with RAT and RT-PCR and both tests were negative. She was asymptomatic throughout. However, on 26th April, she developed headache without fever and tested positive with RT-PCR and RAT.

Patient 6 (P6)
P6, the 2-year-old daughter of the index case, was previously healthy and remained asymptomatic throughout the period. She was screened for the infection on the 26th as her mother was positive, and was found to be positive on both tests. The father-in-law of the index case who lived in Galle was also tested on the 26th and was negative for SARS-CoV-2.

Results

Table 1: History and test results of the patients

Patient	Age (years)	Relationship	Symptoms	No. of days from initial case detection	Positive date	PCR CT values
P1	36	Index patient	fever (38.8 °C) with arthralgia and myalgia	--	22nd April 2021	E gene = 20.04, RdRP gene = 23.44 N gene = 18.11
P2	75	Father	headache and fever	--	22nd April 2021	E gene = 21.29 RdRP gene = 26.20 N gene =20.61
P3	71	Mother	Sore throat	02	23rd April 2021	E gene = 26.12 RdRP gene = 28.46 N gene = 25.07
P4	36	Wife	headache	05	26th April 2021	S gene = 19.64 E gene = 20.30
P5	02	Daughter	No symptoms	05	26th April 2021	S gene = 27.99 E gene = 28.99
P6	67	Mother-in-law	Cold, sneezing and fever	04	25th April 2021	S gene = 24.97 E gene = 25.79

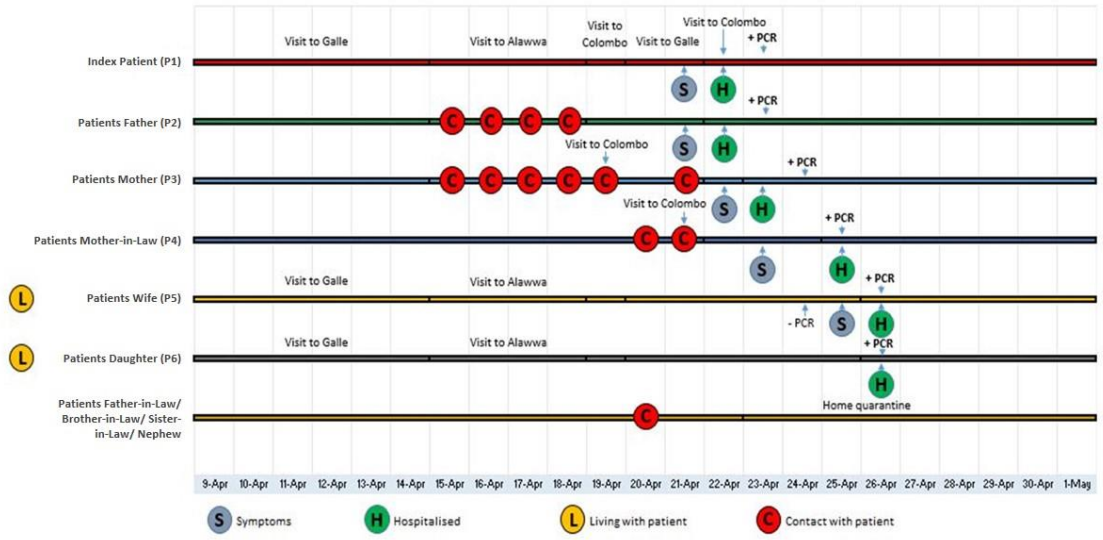

Figure 1: Timeline of the Index case

By considering the travel and contact history in this family cluster, we believe that the P1 and P2 were infected in Alawwa. Both had developed symptoms on the same day. P3 too might have been infected during the same period by P2 or from the index case (P1) while travelling in the car on 21st April. She presented with symptoms on 23rd April, 2 days after the onset of symptoms in P1 and P2. P4 who developed symptoms 4 days after P1 could have been infected by travelling in the same car from Galle to Colombo with P1 on 21st April (Figure 1). P5 and P6 may have acquired the infection from P1.

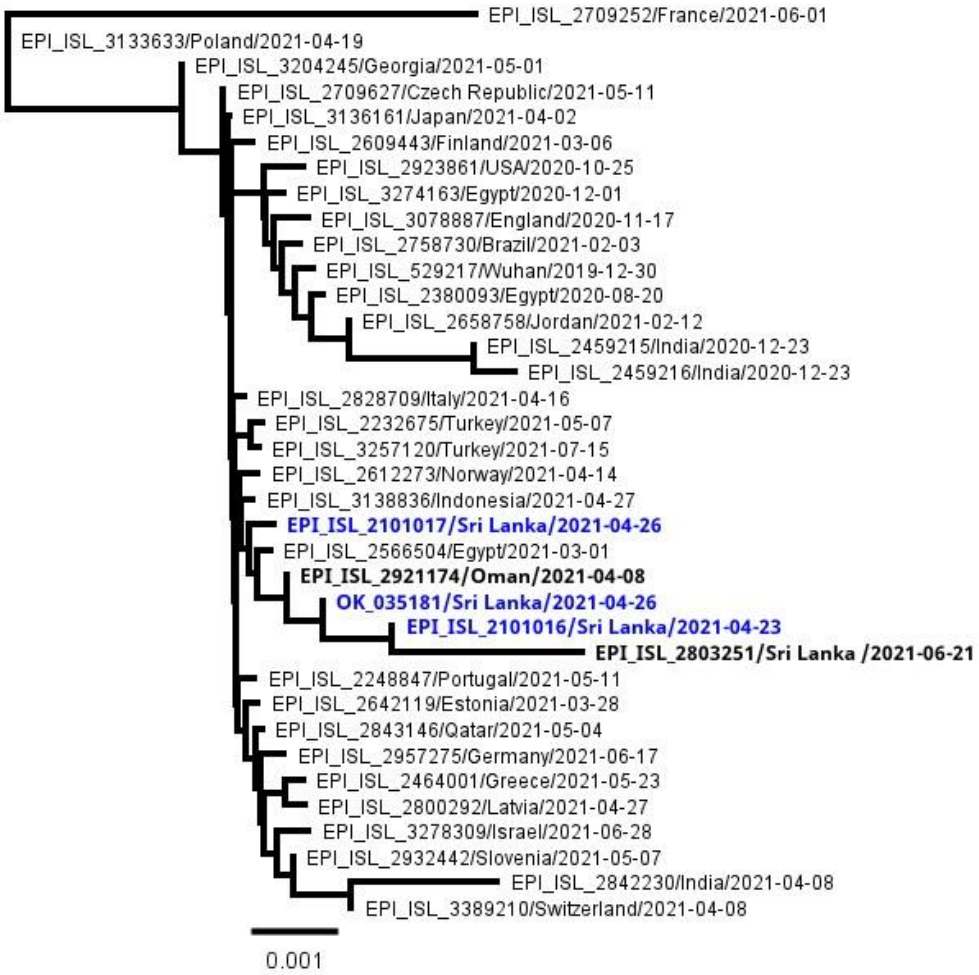

Figure 2: Phylogenetic tree

The sequences were submitted to the GISAID database, a European database enabling rapid and open access to epidemic and pandemic virus data, and to GenBank.

The Accession numbers of the GISAID database are as follows:
P3: **EPI_ISL_2101016** (GISAID)
P5: **EPI_ISL_2101017** (GISAID) **MZ801830** (GenBank)
P6: **OK035181** (GenBank)

Discussion

As of 25th May 2022, WHO reported a total of 523,786,368 COVID-19 confirmed cases in the world with 6,279,667 deaths. There have been 663,823 infections and 16,517 coronavirus-related deaths reported in Sri Lanka since the pandemic began up to 31st May 2022. Most of the infected patients in Sri Lanka had mild disease and recovered as in most other countries.[1] Sri Lanka administered at least 40,319,161 doses of COVID vaccines as of 31st October 2022, and successfully protected the vulnerable population with 85.4% vaccine coverage by full vaccination with the last dose of the primary series and 47.6% with booster dose (up to 31st October 2022).[3,2]

The SARS CoV-2 variant found by our team was found to be in the pangolin lineage C.36, an alias of B.1.1.1.36, found mainly in Egypt and in Oman according to our phylogenetic tree. [6] There is a possibility that the strain was introduced to Sri Lanka from Oman as the data found in the GISAID database has the closest link following phylogenetic analysis (Figure 2). Oman has been a popular destination for employment of Sri Lankans at various levels. Although there is no travel history to Oman related to this family, the possibility that this strain came from Oman is high. All six members of the family had mild illnesses and none of them became oxygen-dependent or developed complications during the 14 days hospital stay.

We report rapid transmission of SARS-CoV-2 infection among six members of a family, with three members confirmed to have this new variant at the beginning of the third wave in Sri Lanka. Since all members of the family had SARS-CoV-2 within days of each person being infected, with minimal contact with outside parties, we presume all six were infected with the same variant. To the best of our knowledge, this is the first time a new variant of the C.36 lineage containing the L452R mutation was identified in Sri Lanka up to that time. The rapid spread of this virus variant within this family cluster suggests high infectivity of this variant. The presence of L452R spike protein mutation in the variant explains the highly infectious nature and as reported previously, is due to the ability of the spike protein mutation to evade human immune responses which enhances infectivity, fusogenicity and viral replication.[7] However, despite the infectious nature of the virus, all six members had mild illness without any complications, despite some members having several co-morbidities, including old age and lack of vaccine immunity. We assumed this SARS-CoV-2 infection could have been transmitted during their travel in a relatively small vehicle (passenger travel space ~3.0 m^3) with confined space as described in Figure 1. It is possible that the index patient's father/brother/sister-in-law did not get the infection because they did not travel together with the index patient. The air conditioner and the airflow circulation within the confined limits of the car may have been the reason for the fast spread of the disease among these members. As previously shown, the possibility of SARS-CoV-2 transmission may increase significantly inside a vehicle.[8,9] The index patient did not use public transport due to COVID-19 and there were strict regulations for wearing face masks and hand rubbing in public places. Transmission was therefore limited to the family, as no masks were used at home or while travelling in their car.

As mentioned above, six family members were infected within days and there was concern that this variant would spread rapidly. However, due to unknown circumstances, it did not spread to levels detectable by the National Surveillance programme, at a time when the dominant strain B.1.1.7 (alpha) was circulating in the country. It is surprising that a new variant was found to spread rapidly among family members and was more infectious, as all the family members became positive for antigen and by PCR. It was also surprising this strain was not reported further except in the single case in northern Sri Lanka as shown in the GISAID database. This variant was the closest match with the Oman strain reported on April 8th, 2021 (Figure 2) as it is possible that the new strain came from Sri Lankans returning from Oman. Although this variant is known to be highly infectious, it did not spread. This is possibly because the index patient lives in a rural area in Sri Lanka, which is not heavily congested such as towns with multi-storied houses.

The SARS-CoV-2 variant found by our team has been reported from the United Kingdom (17.0%), United States of America (14.0%), Germany (12.0%) and Denmark (7.0%).[6] As previously mentioned, it is possible that the strain was introduced to Sri Lanka from Oman (Figure 2) as the data found in the GISAID database showed the closest matching strain was

from Oman. The significance of the mutation L452R was studied by several research groups. Liu, et al., 2020, showed in a study co-incubating pseudotyped virus with SARS-CoV-2 spike proteins and monoclonal antibodies, that viruses with S:L452R mutations escaped neutralisation by monoclonal antibodies SARS2-01, SARS-02, and SARS2-32 and some convalescent sera. Additionally, a study found an increase in infectivity as measured by soluble mACE2.[10] Another study[11] showed these spike protein mutations may increase binding affinity with ACE2 receptors. It was further revealed that L452R mutation showed several mechanisms to evade the host immune response.[12,13]

During the detection of COVID-19 among the extended family in April 2021, only clusters of infected people were identified in the country with community transmission. The SARS- CoV-2 variants identified before and during this period in Sri Lanka were mainly B.1 and B.4 lineages with the D614G mutation.[1] According to our analysis, this reported strain belongs to C.36 lineage and is reported to have the D614G mutation as well as the L452R mutation.

As the global SARS-CoV-2 pandemic expanded, genomic epidemiology and whole genome sequencing and analysis of spike protein mutations using sequence and structural approaches were undertaken to identify possible new variants and to gauge the fitness of current circulating strains.

The emergence of new variants in Sri Lanka through the accumulation of convergent mutations during the COVID-19 third wave needs further investigation to analyse the impact on public health and its possibility of becoming a variance of concern (VOC). A study done in Sri Lanka in March 2020 indicated that the SARS-CoV-2 sequences from Sri Lanka have the highest genomic similarity to sequences isolated from Italy, Germany and England.[14] The variant B1.427/B1.429 containing the L452R emerged in May 2020 and from September 2020 to January 2021 increased 0% to >50% of sequenced cases in California, USA.[15] The mutation L452R in the spike protein was first reported in USA, March 2020 and also reported in Denmark during the same period.[7] The SARS-CoV-2 variant with this mutation was originally known as Variant:20C/S:452R and phylogenetically belongs to the CLADE 20C.[7,16] The L452R variant was named as a variance of concern (VOC) due to the presence of key mutations in the spike protein and is among the top ten mutations listed by the Centers for Diseases Control and Prevention.

At the time of presentation of the patients in this case study, the delta variant (B.1.617.2) was emerging and reached its peak level where L452R was a common mutation, which may have outcompeted all other “weaker” variants. Due to this common mutation, we assume this new variant would become a variant of concern. This is the first reported case, identified as a new variant of the C.36 lineage containing the L452R mutation from Sri Lanka followed by detection of the same lineage from northern Sri Lanka in June 2021. No other cases have been reported thereafter (up to 10th November 2022 according to GISAID database).

The emergence of such variants through the accumulation of convergent mutations or from travellers coming into the country requires a system of monitoring. Such monitoring of the spread of SARS-CoV-2 variants of concern and the new Sri Lankan variants is required to determine the impact on public health in Sri Lanka. SARS-CoV-2 is relatively under control now in Sri Lanka, but continued surveillance is important.

Conclusion

Two important points can be highlighted from this study. Even though the COVID-19 situation is currently under control in Sri Lanka, new cases that continue to come up should be monitored and the virus sequenced, since these types of cases, as highlighted in this study, can serve as the "canary in the coal-mine" to warn epidemiologists of highly infectious mutants that may spread rapidly throughout the population. The second point is the possible rapid transmission of the virus during travel inside the closed vehicle with air-conditioning, as is common in tropical countries. More studies should be done such as by Park, et al., (2022) who studied transmission in buses as well as cars, with suggestions for improvement in technology to mitigate such spread of viruses.[17]

Declarations

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Ethics statement: All the participants in this family cluster gave their consent to conduct this research study.
Authors' contributions: Concepts & Design: ADS, HA, HJ; Clinical management: PJ, DG; Experiments/Testing: TS, DN, NM, UK, PK; Data acquisition & analysis: HA, HJ; Manuscript preparation: HA, HJ, TS, DN, PK, ADS.

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