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Epidemiology of Potential SARS-CoV-2 Reinfection in a Pediatric Cohort in Kuwait

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-056371
Article Type:	Original research
Date Submitted by the Author:	23-Aug-2021
Complete List of Authors:	Alhaddad, Fatemah; Farwaniya Hospital, Department of Pediatrics Abdulkareem, Ali; Farwaniya Hospital, Department of Pediatrics Alsharrah, Danah; Farwaniya Hospital, Pediatrics Alkandari, Abdullah; Dasman Diabetes Institute, Bin-Hasan, Saadoun ; Farwaniya Hospital, Department of Pediatrics; Dasman Diabetes Institute Al-Ahmad, Mona; Kuwait University Faculty of Medicine, Department of Microbiology Al Hashemi, Hashem ; Farwaniya Hospital, Department of Pediatrics Alghounaim, Mohammed; Amiri Hospital, Pediatric Infectious Diseases Specialist and Medical Microbiologist
Keywords:	COVID-19, Epidemiology < INFECTIOUS DISEASES, PAEDIATRICS

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Epidemiology of Potential SARS-CoV-2 Reinfection in a Pediatric Cohort in Kuwait

Fatemah Alhaddad[1], MD, Ali Abdulkareem[1], MD, Danah Alsharrah[1], MD, Abdullah Alkandari[2], PhD, Saadoun Bin-Hasan[1,2], MD, Mona Al-Ahmad[3], MD, Hashem Al Hashemi[1], MD, Mohammad Alghounaim[4], MD

[1]Department of Pediatrics, Farwaniyah Hospital, Ministry of Health, Sabah Al-Nasser, Kuwait

[2] Department of Population Health, Dasman Diabetes Institute, Kuwait City, Kuwait

[3] Department of Microbiology, Faculty of Medicine, Kuwait University, Kuwait

[4]Department of Pediatrics, Amiri Hospital, Ministry of Health, Kuwait City, Kuwait

Keywords: Children, SARS-CoV-2, COVID-19, reinfection, epidemiology

Running title: SARS-CoV-2 Reinfection in Children.

Conflict of Interest Disclosures: The authors no conflicts of interest to disclose

Funding: None

Abbreviations:

COVID-19: Coronavirus disease 2019

SARS-CoV-2: Severe acute respiratory syndrome-related coronavirus 2

PCR-Q8: The Pediatric COVID Registry in Kuwait

WHO: World Health Organization.

PCR: polymerase chain reaction

IQR: interquartile range

Article summary: National-level study found that pediatric SARS-CoV-2 reinfection is uncommon, and estimated to be 1.02 (95% CI 0.71-1.45) infection per 100,000 person-days

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Corresponding author:

Dr. Mohammad Alghounaim

Department of Pediatrics

Amiri Hospital

900005 Ibn Misbah St. Sharq block 3

Kuwait City, Kuwait

Tel: +965-97333099

Fax: +965-22436149

E-mail: malghounaim@moh.gov.kw

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Contributors' Statement:

Drs Ali Abdulkareem, Danah Alsharrah, and **Fatemah Alhaddad** conceptualized and designed the study, curated the data, wrote the original draft of the paper, and revised the manuscript.

Dr Abdullah Alkandari conducted the formal analysis, and critically revised the manuscript

Dr Saadoun Bin-Hasan: conceptualized and designed the study, and critically revised the manuscript.

Drs Mona Al-Ahmad and **Hashem Al-Hashemi**: conceptualized the study, participated in data curation, and revised the manuscript

Mohammed Alghounaim: conceptualized and designed the study, supervised data collection, conducted initial analysis, and critically revised the manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Abstract

Objective: Subsequent protection from Severe acute respiratory syndrome-related coronavirus 2 (SARS-COV-2) infection in pediatrics is not well reported in the literature. We aimed to describe the clinical characteristics and dynamics of SARS-CoV-2 PCR repositivity in children.

Methods: This is a population-level retrospective cohort study included children 12 years and younger between February 28, 2020 and March 6, 2021. Patients were identified through multiple national-level electronic coronavirus disease 2019 (COVID-19) databases. SARS-CoV-2 reinfection was defined as having two or more positive SARS-CoV-2 PCR done on a respiratory sample, at least 45 days apart. Clinical data was obtained from the Pediatric COVID-19 Registry in Kuwait (PCR-Q8). Descriptive statistics and incidence-sensitivity analyses were performed.

Results: Thirty pediatric COVID-19 patients had SARS-CoV-2 reinfection at an incidence of 1.02 (95% CI 0.71-1.45) infection per 100,000 person-days and a median time to reinfection of 83 days (IQR 62-128.75). The incidence of reinfection decreased to 0.78 (95% CI 0.52-1.17) and 0.47 (95% CI 0.28-0.79) per person-days when the minimum interval between PCR repositivity was increased to 60 and 90 days, respectively. The mean age of reinfected subjects was 8.5 years (IQR 3.7-10.3) and majority (70%) were female. Most children (55.2%) had asymptomatic reinfection. Fever was the most common presentation in symptomatic patients. One immunocompromised experienced two reinfection episodes.

Conclusion: SARS-CoV-2 reinfection is uncommon in children. Previous confirmed COVID-19 in children seems to induce a protective immunity against future infections.

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Strength and limitations of the study

- Little is known about the risk of pediatric SARS-CoV-2 reinfection and children are more likely to be asymptomatic and therefore not all positive cases are detected.
- Estimating the duration of immunity after the primary infection is crucial in planning health care measures
- A population-level retrospective cohort study included children 12 years and younger was performed and included SARS-CoV-2 reinfection and descriptive statistics were preformed.

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Introduction:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) is a novel beta coronavirus that was first described in December 2019 and resulted in a pandemic of respiratory illness, coronavirus disease-2019 (COVID-19) [1]. By early May 2021, the pandemic has resulted in over 150 million cases worldwide and more than three million deaths reported by the World Health Organization (WHO)[2]. Despite the widespread of COVID-19, children compromised less than 15% of all reported cases [3-5]. Limited social interactions, and enhanced infection control measures such as school closure and online teaching, may have contributed to lower proportions of infected children [6]. In addition, children are more likely to have asymptomatic or mild infection compared to adults and, therefore, may not be tested [7].

Quantifying the duration of natural immunity after the primary SARS-CoV-2 infection has been crucial to address public health measures, to help predict the continuity of the pandemic, and to understand the effect of reinfection on disease severity. The first case of SARS-CoV-2 reinfection was reported in August 2020 [8]. Since then, interest in the exact risk and rate of reinfection has been increasing. Several reports estimated that reinfection occurs in less than 1% of previously infected individuals [9-11]. The duration between primary and secondary infection was reported to vary from 48 to 124 days in the first documented cases in literature [12, 13]. However, most of the reinfection studies focused mainly on the adult population. COVID-19 follows different disease dynamics in children, and studies addressing reinfection in this population are lacking.

The first pediatric case of COVID-19 in Kuwait was identified in February 2020 [7]. Since then, schools and daycare centers were closed, and classes were conducted virtually. In addition, commercial centers, gyms, and restaurants were open but with time restrictions and strict protective measures. Early in the pandemic, a national electronic COVID-19 testing database was created and included all SARS-CoV-2 test results of symptomatic individuals, contact tracing, and routine

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travel or hospitalization screening. Also, a national Pediatric COVID-19 Registry (PCR-Q8) was established for children aged 12 years and younger to better understand disease dynamics and update management protocols. This created the unique opportunity to investigate the possibility of reinfection with SARS-CoV-2, with an attempt to establish an average duration between the two positive results, potential factors linked to reinfection, and its clinical severity.

Methods:

A population-level retrospective cohort study was conducted in Kuwait between February 28th, 2020, and March 6th, 2021. The national COVID-19 test result database was used to identify children younger than 12 years and had two or more subsequent positive SARS-CoV-2 polymerase chain reaction (PCR) tests done on a respiratory sample, at least 45 days apart. Subjects who had two or more positive SARS-CoV-2 PCR but less than 45 days in-between, and patients who fulfilled the WHO definition of the multisystem inflammatory syndrome in children (MIS-C) were excluded [14].

To assure the inclusion of all SARS-CoV-2 infected children into this study, the national COVID-19 testing database was used for initial patient identification. This database includes the results for all individuals who had SARS-CoV-2 PCR done on a respiratory specimen nationwide. SARS-CoV-2 PCR is typically done to confirm the diagnosis of symptomatic individuals, detect secondary infections in contact tracing, and identify infected subjects prior to hospitalization or travel. During the study period, the SARS-CoV-2 antigen was not routinely performed on pediatric samples. Also, serum SARS-CoV-2 IgG or IgM tests were not routinely done to confirm current or prior infection. A secondary search query was done on the PCR-Q8 registry, which included patient information from the following sources: hospital records, institutional quarantine centers, patient transport units, as well as all laboratories that provide SARS-CoV-2 PCR.

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The PCR-Q8 registry retrospectively collected detailed individual-level demographic, laboratory and clinical characteristics of all children diagnosed with COVID-19 in Kuwait. The database was acquired to obtain demographic and clinical data. COVID-19 severity was categorized based on WHO disease classification [15]. Infected children with mild or asymptomatic COVID-19 may not require hospitalization hence, clinical data in the registry may be lacking. For those subjected, parents were contacted to complete missing disease-related data.

Descriptive analysis was performed to compare between primary and secondary infections. To calculate incidence and to account for delayed presentation of reinfection, calculated days at risk included the period from the day of first positive SARS-CoV-2 PCR starting February 28th, 2020 to the second positive test or March 6th, 2021, whichever is first. However, individuals with the first positive SARS-CoV-2 PCR starting January 1st, 2021, were not included in the calculation due to limited time for reinfection to occur. Clopper-Pearson test was used to calculate the 95% CI. Analysis was done using GraphPad Prism (v. 9.0). Also, sensitivity analysis assessing the clinical presentation and incidence of reinfection considering a minimum interval for PCR repositivity of 60 and 90 days.

Ethics:

The study was approved by the ethical board of the Ministry of Health, Kuwait (reference no. 1607/2020). Verbal consent was obtained from parents who agreed to participate to complete missing clinical data.

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Results

During the study period, there were 14,320 documented SARS-CoV-2 infections in children younger than 12 years in Kuwait, accounting for 2,954,372 person-days of follow-up. Among those, 421 children with repeat positive SARS-CoV-2 PCR were identified, of which, 30 patients had a repeat positive SARS-CoV-2 45 days or more after the first positive, 391 patients had their repeat test within 45 days and were excluded (figure 1). The incidence of reinfection was 1.02 (95% CI 0.71-1.45) infection per 100,000 previously infected person-days. The mean age of the reinfection cohort was 8.5 (IQR 3.7-10.3) years and majority (70%) were females (table 1). More than half (56.6%) of reinfection patients were not known to have any chronic comorbid conditions at the time of the first or second infection.

The median time between the two episodes of infection as evident by the sample collection date was 83 days (IQR 62-128.75 days) (figure 2). Majority of the patients had asymptomatic infection during the first and second episodes, 43.3% and 53.3% respectively (table 2). In symptomatic patients, fever, cough and shortness of breath were the most commonly reported symptoms. One (3.3%) patient had severe pneumonia during the first infection, whereas, four (13.3%) patients had severe pneumonia during second infection. Of those, three patients had mild or asymptomatic initial SARS-CoV-2 infection. One of the four patients was admitted for an acute exacerbation of asthma with three days history of fever. The median length of hospitalization was 9 days (IQR 5.75-13 days) and 6 days (IQR 3.75-6.75 days) for the first and second infection, respectively. None of the subjects received care in an intensive care unit.

One female patient previously diagnosed with hypereosinophilic syndrome on low-dose prednisone, had three episodes of SARS-CoV-2 infections. Her first infection was in July 2020 where she was admitted for 10 days as a case of severe pneumonia followed by two negative PCR tests done in August. Her second infection was in November 2020 when she was also admitted for

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severe pneumonia with prolonged hospital admission followed by a negative PCR test done in December. Interestingly, during her third infection in January 2021, she was asymptomatic and testing was done for screening before an outpatient appointment.

Around half (46.6%) of reinfections occurred more than 90 days after the initial infection. The incidence of reinfection decreased to 0.78 (95% CI 0.52-1.17) and 0.47 (95% CI 0.28-0.79) per infected person-days when the minimum acceptable duration between PCR repositivity was increased to 60 and 90 days, respectively. Also, there were more symptomatic children (64.3%) during the second infection using the 90-day definition when compared to the 45-day or 60-day minimum interval.

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Discussion

The exact risk and factors associated with SARS-CoV-2 reinfection in the pediatric population are still not well understood. We found that a second SARS-CoV-2 infection is uncommon in children with a rate of 1.02 reinfection per 100,000 previously infected person-days and a median time to reinfection of 83 days. The finding of this study is similar to previous reports which showed a reinfection incidence in adults of 0.09-0.13 per 10,000 person-day [9, 11]. Similarly, in a large Danish cohort, prior SARS-CoV-2 provided on average 80.5% protection against repeat infection. The estimated protection differed between different age groups ranging from 47.1% in individuals older than 65 years of age to 82.7% in younger subjects [16]. However, the epidemiology and disease dynamics of SARS-CoV-2 infection are different in children[17]. Therefore, using data generated from adult studies to infer on the pediatric population may not be accurate.

Magnitude and persistence of immunological responses conferred by SARS-CoV-2 infection can be variable based on age and medical comorbidities [18]. Hence, estimating the duration of protective immunity after acquiring SARS-CoV-2 infection has been the focus of several studies. In an adult study, looking at the neutralizing antibodies, the seropositivity was identified in all participants up to 53 days after infection [19]. However, the level of neutralizing antibodies varies greatly with disease severity and tend to wane over time [20, 21]. Few studies compared humoral responses between adults and children. Weisberg et. al. have shown that children exhibit lower antibody response compared to adults [22]. However, the incidence of reinfections observed in this study was similar to previously reported adult patients.

A standard definition of SARS-CoV-2 reinfection is lacking. Traditionally, the detection of viable virus by cell culture has been the standard to ascertain active infection [23]. However, this testing method lacks sensitivity and is time consuming [24]. In addition, due to the need of expertise

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and appropriate laboratory infrastructure, routine population-level testing using cell culture in not possible. For these reasons, the detection of genetically distinct SARS-CoV-2 in different infectious episodes or the use of the cycle threshold (Ct) value as a surrogate for viral load has been suggested [12, 13]. In Kuwait, genomic testing capacity is limited and retrieving patients' samples and laboratory data from several public and private laboratories was logistically challenging. In our study, a 45-day period between two-consecutive positive PCR test was selected based on the expected duration of molecular test positivity on a respiratory sample. Alsharrah et al. reported a median duration of PCR positivity among pediatric COVID-19 patients of 15 days, with maximum duration of 42 days [7].

To our knowledge, this is the first national-level cohort that estimates the risk of pediatric SARS-CoV-2 reinfection. Nevertheless, this study has some limitations. Due to limited genomic sequencing capacity, proving that PCR repositivity was caused by a genetically distinct virus was not possible. However, based on the expected duration of PCR-persistence in children mentioned above, one will not expect that PCR done on an upper respiratory specimen to remain positive beyond 45 days. Another limitation was the unavailability of some data recorded in the national pediatric COVID-19 registry. When data was missing, parents were contacted via the telephone, creating a recall bias. In addition, patients with mild upper respiratory tracts symptoms are underreported by parents. Therefore, mild cases are less likely to be tested and can be missed, and underestimate the reinfection rate. However, using the national level electronic record allowed the collection of data from variable sources and the detection of asymptomatically infected children. Despite these limitations, this study has shown that reinfection is generally uncommon. Further studies that correlate degree of humoral and cellular immunity, along with wide genomic sequencing surveillance with risk of reinfection are needed to better understand and quantify the risk of repeat SARS-CoV-2 infection.

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Acknowledgement:

We would like to thank Dr. Hessa Alkandari, chair of the pediatric council in the Ministry of Health, Kuwait, for her help in preparing this manuscript. We also thank the research group of the Pediatric COVID-19 Registry in Kuwait (PCR-Q8) for their contribution.

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Table 1. Demographic characteristics of the study population

Variable	Population (n=30)
Age [median, IQR]	8.6 years [3.7-10.3]
Male	9 (30%)
Comorbid conditions	
Asthma	2 (6.7%)
Diabetes	1 (3.3%)
Healthy	17 (56.7%)
Neurological disease	3 (10%)
Other	5 (16.7%)
Preterm	1 (3.3%)
Time between 2 episodes	
First positive PCR to reinfection [median, IQR]	83 days [62-128.75]
Symptom onset of first episode to reinfection [median, IQR]	83 days [62.5-130.5]

IQR, interquartile range.

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Table 2. Clinical characteristics of the study population based on different defined intervals of polymerase chain reaction (PCR) repositivity

Variable	First Infection			Second Infection		
	Minimum interval of PCR repositivity			Minimum interval of PCR repositivity		
	45 days (n=30)	60 days (n=23)	90 days (n=14)	45 days (n=29)*	60 days (n=23)	90 days (n=14)
Disease severity						
Asymptomatic	13 (43.3%)	12 (52.2%)	8 (57.1%)	16 (55.2%)	12 (52.2%)	5 (35.7%)
Mild illness	15 (33.3%)	9 (39.1%)	4 (28.6%)	9 (31%)	7 (30.4%)	5 (35.7%)
Mild pneumonia	1 (3.3%)	1 (4.3%)	1 (7.1%)	0	0	0
Severe pneumonia	1 (3.3%)	1 (4.3%)	1 (7.1%)	4 (13.8%)	4 (17.4%)	4 (28.6%)
Reason for testing						
Close contact testing	13 (43.3%)	12 (52.2%)	8 (57.1%)	6 (20.7%)	5 (21.7%)	4 (28.6%)
Hospitalization screening	5 (16.7%)	4 (17.4%)	3 (21.4%)	7 (24.1%)	6 (26.1%)	5 (35.7%)
Suspected COVID-19	12 (40%)	7 (30.4%)	3 (21.4%)	6 (20.7%)	5 (21.7%)	4 (28.6%)
Travel screening	1 (3.3%)	0	0	10 (34.5%)	7 (30.4%)	1 (7.1%)
Symptoms						
Abdominal pain	2 (4%)	1 (3.1%)	1 (5%)	0	0	0
cough	4 (8.2%)	4 (12.5%)	3 (15%)	8 (20.5%)	8 (21.6%)	7 (21.9%)
Diarrhea	2 (4%)	2 (6.3%)	2 (10%)	0	0	0
Fever	16 (32.6%)	11 (34.4%)	6 (30%)	12 (30.8%)	10 (27%)	8 (25%)
Headache	2 (4%)	2 (6.3%)	1 (5%)	1 (2.6%)	1 (2.7%)	1 (3.1%)
Loss of smell	4 (8.2%)	1 (3.1%)	0	1 (2.6%)	1 (2.7%)	1 (3.1%)
Loss of taste	4 (8.2%)	1 (3.1%)	0	1 (2.6%)	1 (2.7%)	1 (3.1%)
Myalgia	4 (8.2%)	3 (9.4%)	3 (15%)	1 (2.6%)	1 (2.7%)	1 (3.1%)
Rhinorrhea	4 (8.2%)	3 (9.4%)	1 (5%)	8 (20.5%)	8 (21.6%)	7 (21.9%)
Shortness of breath	3 (6.1%)	2 (6.3%)	2 (10%)	3 (7.7%)	3 (8.1%)	3 (9.4%)
Sore throat	3 (6.1%)	2 (6.3%)	1 (5%)	3 (7.7%)	3 (8.1%)	2 (6.3%)
Vomiting	1 (2%)	0	0	1 (2.6%)	1 (2.7%)	1 (3.1%)
Hospitalization	5 (16.7%)	4 (17.4%)	3 (21.4%)	6 (20.7%)	5 (21.7%)	5 (35.7%)
Length of stay in days [median, IQR]	9 [5.75-13]	9 [7.25-11]	8 [6.5-9]	6 [3-6]	6 [3-6]	6 [3-6]

IQR, interquartile range.

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*clinical data for one patient is missing

261

262

263

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Table 1. Demographic characteristics of the study population.

Table 2. Clinical characteristics of the study population based on different defined intervals of polymerase chain reaction (PCR) repositivity.

Figure 1: Flow diagram of the study population

Figure 2: Duration between first SARS-CoV-2 infection and subsequent infections. The shaded bars represent the duration between positive PCR results.

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Figure 1: Flow diagram of the study population

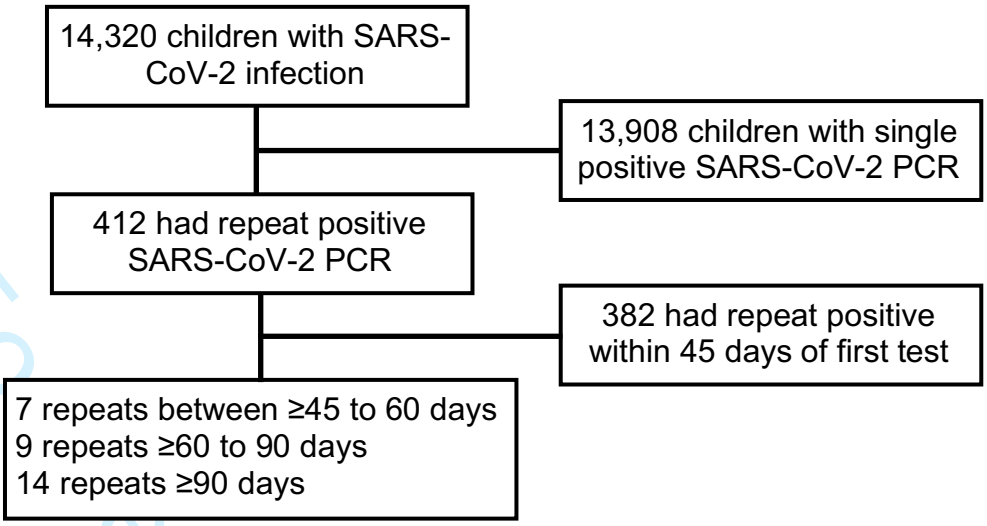

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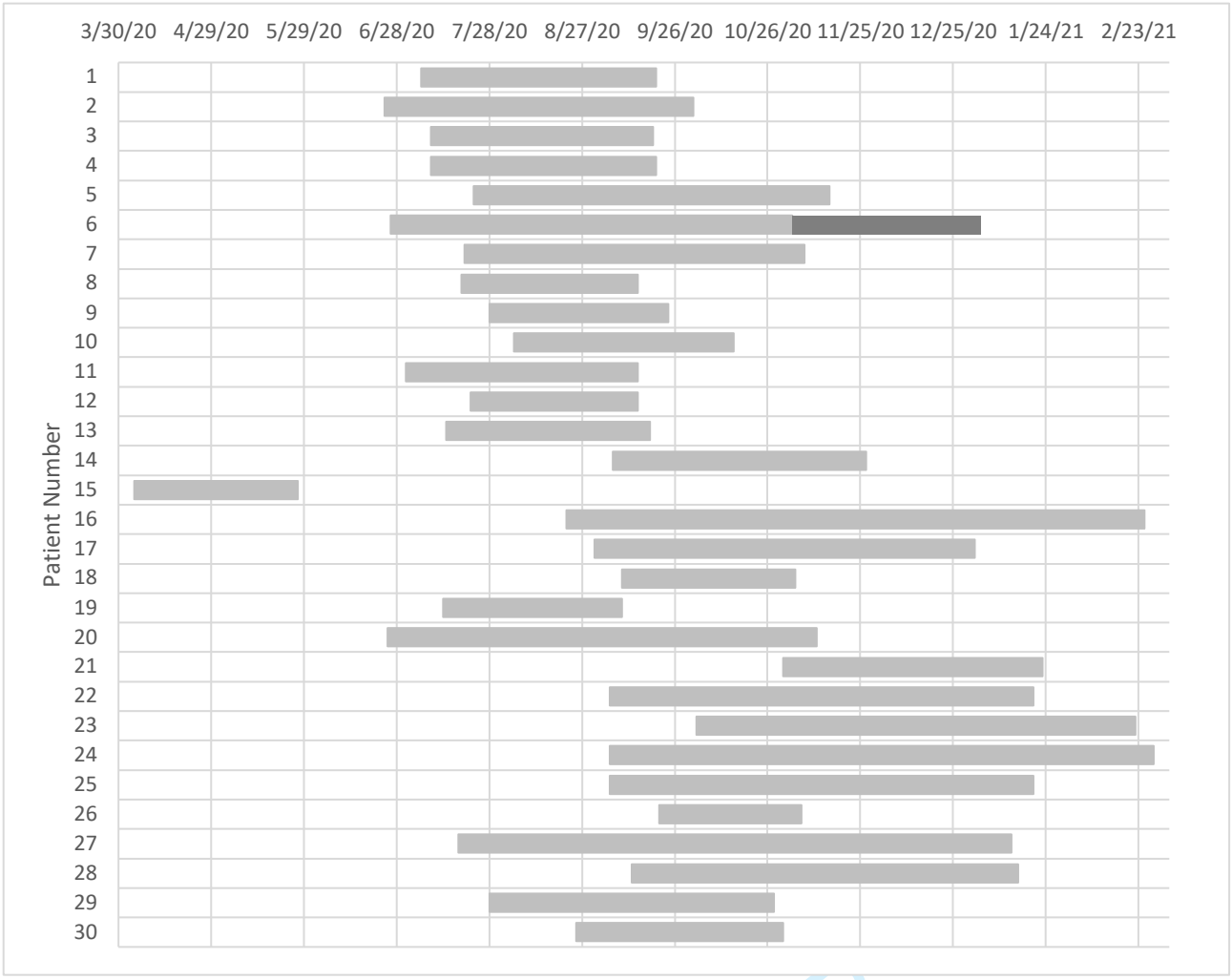
Figure 2: Duration between first SARS-CoV-2 infection and subsequent infections. The shaded bars represent the duration between positive PCR results.



*Patient 6 had two episodes of reinfection

BMJ Open

Incidence of SARS-CoV-2 Reinfection in a Pediatric Cohort in Kuwait

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-056371.R1
Article Type:	Original research
Date Submitted by the Author:	09-Feb-2022
Complete List of Authors:	Alhaddad, Fatemah; Farwaniya Hospital, Department of Pediatrics Abdulkareem, Ali; Farwaniya Hospital, Department of Pediatrics Alsharrah, Danah; Farwaniya Hospital, Pediatrics Alkandari, Abdullah; Dasman Diabetes Institute, Bin-Hasan, Saadoun ; Farwaniya Hospital, Department of Pediatrics; Dasman Diabetes Institute Al-Ahmad, Mona; Kuwait University Faculty of Medicine, Department of Microbiology Al Hashemi, Hashem ; Farwaniya Hospital, Department of Pediatrics Alghounaim, Mohammed; Amiri Hospital, Pediatric Infectious Diseases Specialist and Medical Microbiologist
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Infectious diseases, Paediatrics, Epidemiology
Keywords:	COVID-19, Epidemiology < INFECTIOUS DISEASES, PAEDIATRICS

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Incidence of SARS-CoV-2 Reinfection in a Pediatric Cohort in Kuwait

Fatemah Alhaddad[1], MD, Ali Abdulkareem[1], MD, Danah Alsharrah[1], MD, Abdullah Alkandari[2], PhD, Saadoun Bin-Hasan[1,2], MD, Mona Al-Ahmad[3], MD, Hashem Al Hashemi[1], MD, Mohammad Alghounaim[4], MD

[1]Department of Pediatrics, Farwaniyah Hospital, Ministry of Health, Sabah Al-Nasser, Kuwait

[2] Department of Population Health, Dasman Diabetes Institute, Kuwait City, Kuwait

[3] Department of Microbiology, Faculty of Medicine, Kuwait University, Kuwait

[4]Department of Pediatrics, Amiri Hospital, Ministry of Health, Kuwait City, Kuwait

Keywords: Children, SARS-CoV-2, COVID-19, reinfection, epidemiology

Running title: SARS-CoV-2 Reinfection in Children.

Conflict of Interest Disclosures: The authors no conflicts of interest to disclose

Funding: None

Abbreviations:

COVID-19: Coronavirus disease 2019

SARS-CoV-2: Severe acute respiratory syndrome-related coronavirus 2

PCR-Q8: The Pediatric COVID Registry in Kuwait

WHO: World Health Organization.

PCR: polymerase chain reaction

IQR: interquartile range

Article summary: National-level study found that pediatric SARS-CoV-2 reinfection is uncommon, and estimated to be 1.02 (95% CI 0.71-1.45) infection per 100,000 person-days

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Corresponding author:

Dr. Mohammad Alghounaim

Department of Pediatrics

Amiri Hospital

900005 Ibn Misbah St. Sharq block 3

Kuwait City, Kuwait

Tel: +965-97333099

Fax: +965-22436149

E-mail: malghounaim@moh.gov.kw

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Contributors' Statement:

Drs Ali Abdulkareem, Danah Alsharrah, and **Fatemah Alhaddad** conceptualized and designed the study, curated the data, wrote the original draft of the paper, and revised the manuscript.

Dr Abdullah Alkandari conducted the formal analysis, and critically revised the manuscript

Dr Saadoun Bin-Hasan: conceptualized and designed the study, and critically revised the manuscript.

Drs Mona Al-Ahmad and **Hashem Al-Hashemi**: conceptualized the study, participated in data curation, and revised the manuscript

Dr Mohammed Alghounaim: conceptualized and designed the study, supervised data collection, conducted initial analysis, and critically revised the manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Abstract

Objective: Subsequent protection from severe acute respiratory syndrome-related coronavirus 2 (SARS-COV-2) infection in pediatrics is not well reported in the literature. We aimed to describe the clinical characteristics and dynamics of SARS-CoV-2 PCR repositivity in children.

Methods: This is a population-level retrospective cohort study included children 12 years and younger between February 28, 2020 and March 6, 2021. Patients were identified through multiple national-level electronic coronavirus disease 2019 (COVID-19) databases. SARS-CoV-2 reinfection was defined as having two or more positive SARS-CoV-2 PCR done on a respiratory sample, at least 45 days apart. Clinical data was obtained from the Pediatric COVID-19 Registry in Kuwait (PCR-Q8). Descriptive statistics and incidence-sensitivity analyses were performed.

Results: Thirty pediatric COVID-19 patients had SARS-CoV-2 reinfection at an incidence of 1.02 (95% CI 0.71-1.45) infection per 100,000 person-days and a median time to reinfection of 83 days (IQR 62-128.75). The incidence of reinfection decreased to 0.78 (95% CI 0.52-1.17) and 0.47 (95% CI 0.28-0.79) per person-days when the minimum interval between PCR repositivity was increased to 60 and 90 days, respectively. The mean age of reinfected subjects was 8.5 years (IQR 3.7-10.3) and majority (70%) were female. Most children (55.2%) had asymptomatic reinfection. Fever was the most common presentation in symptomatic patients. One immunocompromised experienced two reinfection episodes.

Conclusion: SARS-CoV-2 reinfection is uncommon in children. Previous confirmed COVID-19 in children seems to induce a protective immunity against future infections.

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Data availability statement:

Data are available upon reasonable request

Strength and limitations of the study

- This study used a national-level electronic database that included all SARS-CoV-2 test results that allowed data collection from variable sources and the detection of asymptomatically infected children.
- The result highlights that SARS-CoV-2 reinfection is uncommon in children.
- The exact risk for SARS-CoV-2 reinfection is difficult to estimate as children are more likely to be asymptomatic, and therefore not all positive cases are detected.
- Patients' symptoms and severity in this retrospective analysis were dependent on the accuracy of reporting in medical notes and parental recall of symptoms.
- Due to limited genomic sequencing capacity, proving the direct causality between PCR repositivity and a genetically distinct virus was not possible.

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Introduction:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) is a novel beta coronavirus that was first described in December 2019 and resulted in a pandemic of respiratory illness, coronavirus disease-2019 (COVID-19) [1]. By early May 2021, the pandemic has resulted in over 150 million cases worldwide and more than three million deaths reported by the World Health Organization (WHO)[2]. Despite the widespread of COVID-19, children compromised less than 15% of all reported cases [3-5]. Limited social interactions, and enhanced infection control measures such as school closure and online teaching, may have contributed to lower proportions of infected children [6]. In addition, children are more likely to have asymptomatic or mild infection compared to adults and, therefore, may not be tested [7].

Quantifying the duration of natural immunity after the primary SARS-CoV-2 infection has been crucial to address public health measures, to help predict the continuity of the pandemic, and to understand the effect of reinfection on disease severity. The first case of SARS-CoV-2 reinfection was reported in August 2020 [8]. Since then, interest in the exact risk and rate of reinfection has been increasing. Several reports estimated that reinfection occurs in less than 1% of previously infected individuals [9-11]. The duration between primary and secondary infection was reported to vary from 48 to 124 days in the first documented cases in literature [12, 13]. However, most of the reinfection studies focused mainly on the adult population. COVID-19 follows different disease dynamics in children, and studies addressing reinfection in this population are lacking.

The first pediatric case of COVID-19 in Kuwait was identified in February 2020 [7]. Since then, schools and daycare centers were closed, and classes were conducted virtually. In addition, commercial centers, gyms, and restaurants were open but with time restrictions and strict protective measures. Early in the pandemic, a national electronic COVID-19 testing database was created and included all SARS-CoV-2 test results of symptomatic individuals, contact tracing, and routine

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travel or hospitalization screening. Also, a national Pediatric COVID-19 Registry (PCR-Q8) was established for children aged 12 years and younger to better understand disease dynamics and update management protocols. This created the unique opportunity to investigate the possibility of reinfection with SARS-CoV-2, with an attempt to establish an average duration between the two positive results, potential factors linked to reinfection, and its clinical severity.

Methods:

A population-level retrospective cohort study was conducted in Kuwait between February 28th, 2020, and March 6th, 2021. The national COVID-19 test result database was used to identify children younger than 12 years and had two or more subsequent positive SARS-CoV-2 polymerase chain reaction (PCR) tests done on a respiratory sample, at least 45 days apart. Subjects who had two or more positive SARS-CoV-2 PCR but less than 45 days in-between, and patients who fulfilled the WHO definition of the multisystem inflammatory syndrome in children (MIS-C) were excluded [14].

To assure the inclusion of all SARS-CoV-2 infected children into this study, the national COVID-19 testing database was used for initial patient identification. This database includes the results for all individuals who had SARS-CoV-2 PCR done on a respiratory specimen nationwide. SARS-CoV-2 PCR is typically done to confirm the diagnosis of symptomatic individuals, detect secondary infections in contact tracing, and identify infected subjects prior to hospitalization or travel. During the study period, the SARS-CoV-2 antigen was not routinely performed on pediatric samples. Also, serum SARS-CoV-2 IgG or IgM tests were not routinely done to confirm current or prior infection. A secondary search query was done on the PCR-Q8 registry, which included patient information from the following sources: hospital records, institutional quarantine centers, patient transport units, as well as all laboratories that provide SARS-CoV-2 PCR.

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The PCR-Q8 registry retrospectively collected detailed individual-level demographic, laboratory and clinical characteristics of all children diagnosed with COVID-19 in Kuwait. The database was acquired to obtain demographic and clinical data. COVID-19 severity was categorized based on WHO disease classification [15]. Infected children with mild or asymptomatic COVID-19 may not require hospitalization. Therefore, clinical data in the registry may be lacking. For those subjected, parents were contacted to complete missing disease-related data.

Descriptive analysis was performed to compare between primary and secondary infections. To calculate incidence and to account for delayed presentation of reinfection, calculated days at risk included the period from the day of first positive SARS-CoV-2 PCR starting February 28th, 2020 to the second positive test or March 6th, 2021, whichever is first. However, individuals with the first positive SARS-CoV-2 PCR starting January 1st, 2021, were not included in the calculation due to limited time for reinfection to occur. Clopper-Pearson test was used to calculate the 95% CI. Analysis was done using GraphPad Prism (v. 9.0). Due to the variability in established reinfection case definition, sensitivity analysis assessing the clinical presentation and incidence of reinfection considering a minimum interval for PCR repositivity of 60 and 90 days[16].

Patient and public involvement statement:

Patients were not included in the design, reporting or dissemination plans of our research. However, parents of children with PCR repositivity were contacted to complete missing data. This study was conducted to address epidemiological questions presented by the Pediatric COVID-19 Task Force (Ministry of Health, Kuwait). The initial concept and study plan was presented to the committee members, whose suggestions and comments were considered in the final study protocol

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Ethics:

The study was approved by the ethical board of the Ministry of Health, Kuwait (reference no. 1607/2020). Verbal consent was obtained from parents who agreed to participate to complete missing clinical data.

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Results

During the study period, there were 14,320 documented SARS-CoV-2 infections in children younger than 12 years in Kuwait, accounting for 2,954,372 person-days of follow-up. Among those, 421 children with repeat positive SARS-CoV-2 PCR were identified, of which, 30 patients had a repeat positive SARS-CoV-2 45 days or more after the first positive, 391 patients had their repeat test within 45 days and were excluded (figure 1). The incidence of reinfection was 1.02 (95% CI 0.71-1.45) infection per 100,000 previously infected person-days. The mean age of the reinfection cohort was 8.5 (IQR 3.7-10.3) years and majority (70%) were females (table 1). More than half (56.6%) of reinfection patients were not known to have any chronic comorbid conditions at the time of the first or second infection.

The median time between the two episodes of infection as evident by the sample collection date was 83 days (IQR 62-128.75 days) (figure 2). Majority of the patients had asymptomatic infection during the first and second episodes, 43.3% and 53.3% respectively (table 2). In symptomatic patients, fever, cough and shortness of breath were the most commonly reported symptoms. One (3.3%) patient had severe pneumonia during the first infection, whereas, four (13.3%) patients had severe pneumonia during second infection. Of those, three patients had mild or asymptomatic initial SARS-CoV-2 infection. One of the four patients was admitted for an acute exacerbation of asthma with three days history of fever. The median length of hospitalization was 9 days (IQR 5.75-13 days) and 6 days (IQR 3.75-6.75 days) for the first and second infection, respectively. None of the subjects received care in an intensive care unit.

One female patient previously diagnosed with hypereosinophilic syndrome on low-dose prednisone, had three episodes of SARS-CoV-2 infections. Her first infection was in July 2020 where she was admitted for 10 days as a case of severe pneumonia followed by two negative PCR tests done in August. Her second infection was in November 2020 when she was also admitted for

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severe pneumonia with prolonged hospital admission followed by a negative PCR test done in December. Interestingly, during her third infection in January 2021, she was asymptomatic and testing was done for screening before an outpatient appointment.

Around half (46.6%) of reinfections occurred more than 90 days after the initial infection. The incidence of reinfection decreased to 0.78 (95% CI 0.52-1.17) and 0.47 (95% CI 0.28-0.79) per infected person-days when the minimum acceptable duration between PCR repositivity was increased to 60 and 90 days, respectively. Also, there were more symptomatic children (64.3%) during the second infection using the 90-day definition when compared to the 45-day or 60-day minimum interval.

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Discussion

The exact risk and factors associated with SARS-CoV-2 reinfection in the pediatric population are still not well understood. We found that a second SARS-CoV-2 infection is uncommon in children with a rate of 1.02 reinfection per 100,000 previously infected person-days and a median time to reinfection of 83 days. The finding of this study is similar to previous reports which showed a reinfection incidence in adults of 0.9-1.3 per 100,000 person-day [9, 11]. Similarly, in a large Danish cohort, prior SARS-CoV-2 provided on average 80.5% protection against repeat infection. The estimated protection differed between different age groups ranging from 47.1% in individuals older than 65 years of age to 82.7% in younger subjects [17]. However, the epidemiology and disease dynamics of SARS-CoV-2 infection are different in children[18]. Therefore, using data generated from adult studies to infer on the pediatric population may not be accurate.

Magnitude and persistence of immunological responses conferred by SARS-CoV-2 infection can be variable based on age and medical comorbidities [19]. Hence, estimating the duration of protective immunity after acquiring SARS-CoV-2 infection has been the focus of several studies. In an adult study, looking at the neutralizing antibodies, the seropositivity was identified in all participants up to 53 days after infection [20]. However, the level of neutralizing antibodies varies greatly with disease severity and tend to wane over time [21, 22]. Few studies compared humoral responses between adults and children. Weisberg et. al. have shown that children exhibit lower antibody response compared to adults [23]. However, the incidence of reinfections observed in this study was similar to previously reported adult patients.

A standard definition of SARS-CoV-2 reinfection is lacking. Traditionally, the detection of viable virus by cell culture has been the standard to ascertain active infection [24]. However, this testing method lacks sensitivity and is time consuming [25]. In addition, due to the need of expertise

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and appropriate laboratory infrastructure, routine population-level testing using cell culture in not possible. For these reasons, the detection of genetically distinct SARS-CoV-2 in different infectious episodes or the use of the cycle threshold (Ct) value as a surrogate for viral load has been suggested [12, 13]. In Kuwait, genomic testing capacity is limited and retrieving patients' samples and laboratory data from several public and private laboratories was logistically challenging. In our study, a 45-day period between two-consecutive positive PCR test was selected based on the expected duration of molecular test positivity on a respiratory sample. Alsharrah et al. reported a median duration of PCR positivity among pediatric COVID-19 patients of 15 days, with maximum duration of 42 days [7]. However, we observed a decline in the rate of repositivity from 1.02 to 0.47 per 100,000 previously infected person-days when the definition of PCR-repositivity increased from 45 to 90 days. As almost half of cases are asymptomatic, this finding could be due to persistent detection of viral particles by PCR.

Asymptomatic SARs-CoV-2 infection is common in pediatrics. We found that around half of infections (43.3% in initial infection and 55.2% in reinfection) remained asymptomatic on follow-up. This finding is similar to other studies [26]. High proportion of silent infection may pose an important public health concern and limit the effectiveness of transmission mitigation efforts. Also, the possibility of reinfection within a relatively short period of time as observed in this study may be an overlooked source of community transmission. Real-world COVID-19 vaccine effectiveness data showed that reinfection is more common in unvaccinated adults[27]. These findings support the recommendation to offer vaccination to those previously infected.

To our knowledge, this is the first national-level cohort that estimates the risk of pediatric SARS-CoV-2 reinfection. Nevertheless, this study has some limitations. Due to limited genomic sequencing capacity, proving that PCR repositivity was caused by a genetically distinct virus was not possible. However, based on the expected duration of PCR-persistence in children mentioned

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above, one will not expect that PCR done on an upper respiratory specimen to remain positive beyond 45 days. Another limitation was the unavailability of some data recorded in the national pediatric COVID-19 registry. When data was missing, parents were contacted via the telephone, creating a recall bias. In addition, patients with mild upper respiratory tracts symptoms are underreported by parents. Therefore, mild cases are less likely to be tested and can be missed, and underestimate the reinfection rate. However, using the national level electronic record allowed the collection of data from variable sources and the detection of asymptomatically infected children. Despite these limitations, this study has shown that reinfection is generally uncommon. Further studies that correlate degree of humoral and cellular immunity, along with wide genomic sequencing surveillance with risk of reinfection are needed to better understand and quantify the risk of repeat SARS-CoV-2 infection.

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Acknowledgement:

We would like to thank Dr. Hessa Alkandari, chair of the pediatric council in the Ministry of Health, Kuwait, for her help in preparing this manuscript. We also thank the research group of the Pediatric COVID-19 Registry in Kuwait (PCR-Q8) for their contribution.

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Table 1. Demographic characteristics of the study population

Variable	Population (n=30)
Age [median, IQR]	8.6 years [3.7-10.3]
Male	9 (30%)
Comorbid conditions	
Asthma	2 (6.7%)
Diabetes	1 (3.3%)
Healthy	17 (56.7%)
Neurological disease	3 (10%)
Other	5 (16.7%)
Preterm	1 (3.3%)
Time between 2 episodes	
First positive PCR to reinfection [median, IQR]	83 days [62-128.75]
Symptom onset of first episode to reinfection [median, IQR]	83 days [62.5-130.5]

IQR, interquartile range.

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Table 2. Clinical characteristics of the study population based on different defined intervals of polymerase chain reaction (PCR) repositivity

Variable	First Infection			Second Infection		
	Minimum interval of PCR repositivity			Minimum interval of PCR repositivity		
	45 days (n=30)	60 days (n=23)	90 days (n=14)	45 days (n=29)*	60 days (n=23)	90 days (n=14)
Disease severity						
Asymptomatic	13 (43.3%)	12 (52.2%)	8 (57.1%)	16 (55.2%)	12 (52.2%)	5 (35.7%)
Mild illness	15 (33.3%)	9 (39.1%)	4 (28.6%)	9 (31%)	7 (30.4%)	5 (35.7%)
Mild pneumonia	1 (3.3%)	1 (4.3%)	1 (7.1%)	0	0	0
Severe pneumonia	1 (3.3%)	1 (4.3%)	1 (7.1%)	4 (13.8%)	4 (17.4%)	4 (28.6%)
Reason for testing						
Close contact testing	13 (43.3%)	12 (52.2%)	8 (57.1%)	6 (20.7%)	5 (21.7%)	4 (28.6%)
Hospitalization screening	5 (16.7%)	4 (17.4%)	3 (21.4%)	7 (24.1%)	6 (26.1%)	5 (35.7%)
Suspected COVID-19	12 (40%)	7 (30.4%)	3 (21.4%)	6 (20.7%)	5 (21.7%)	4 (28.6%)
Travel screening	1 (3.3%)	0	0	10 (34.5%)	7 (30.4%)	1 (7.1%)
Symptoms						
Abdominal pain	2 (4%)	1 (3.1%)	1 (5%)	0	0	0
cough	4 (8.2%)	4 (12.5%)	3 (15%)	8 (20.5%)	8 (21.6%)	7 (21.9%)
Diarrhea	2 (4%)	2 (6.3%)	2 (10%)	0	0	0
Fever	16 (32.6%)	11 (34.4%)	6 (30%)	12 (30.8%)	10 (27%)	8 (25%)
Headache	2 (4%)	2 (6.3%)	1 (5%)	1 (2.6%)	1 (2.7%)	1 (3.1%)
Loss of smell	4 (8.2%)	1 (3.1%)	0	1 (2.6%)	1 (2.7%)	1 (3.1%)
Loss of taste	4 (8.2%)	1 (3.1%)	0	1 (2.6%)	1 (2.7%)	1 (3.1%)
Myalgia	4 (8.2%)	3 (9.4%)	3 (15%)	1 (2.6%)	1 (2.7%)	1 (3.1%)
Rhinorrhea	4 (8.2%)	3 (9.4%)	1 (5%)	8 (20.5%)	8 (21.6%)	7 (21.9%)
Shortness of breath	3 (6.1%)	2 (6.3%)	2 (10%)	3 (7.7%)	3 (8.1%)	3 (9.4%)
Sore throat	3 (6.1%)	2 (6.3%)	1 (5%)	3 (7.7%)	3 (8.1%)	2 (6.3%)
Vomiting	1 (2%)	0	0	1 (2.6%)	1 (2.7%)	1 (3.1%)
Hospitalization	5 (16.7%)	4 (17.4%)	3 (21.4%)	6 (20.7%)	5 (21.7%)	5 (35.7%)
Length of stay in days [median, IQR]	9 [5.75-13]	9 [7.25-11]	8 [6.5-9]	6 [3-6]	6 [3-6]	6 [3-6]

IQR, interquartile range.

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*clinical data for one patient is missing

287

288

289

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Table 1. Demographic characteristics of the study population.

Table 2. Clinical characteristics of the study population based on different defined intervals of polymerase chain reaction (PCR) repositivity.

Figure 1: Flow diagram of the study population

Figure 2: Duration between first SARS-CoV-2 infection and subsequent infections. The shaded bars represent the duration between positive PCR results.

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387

388

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Figure 1: Flow diagram of the study population

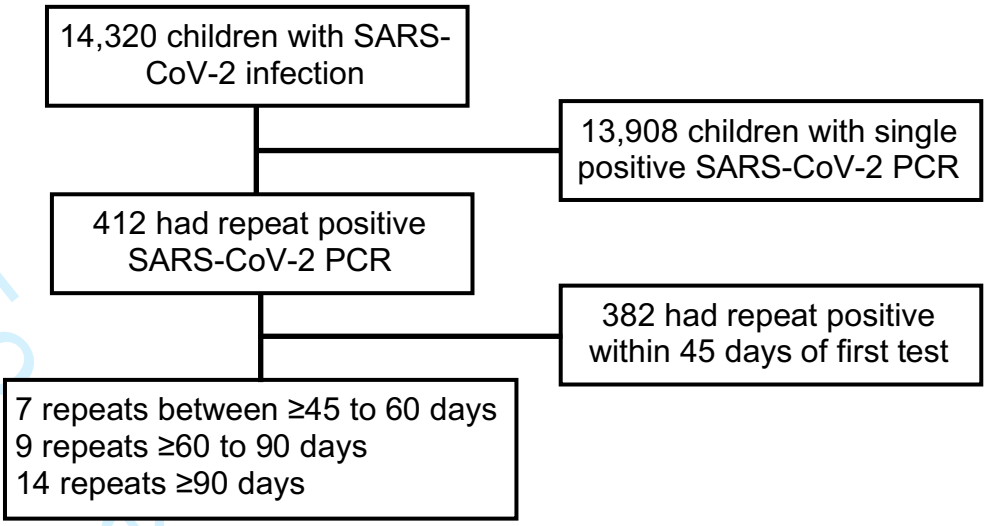

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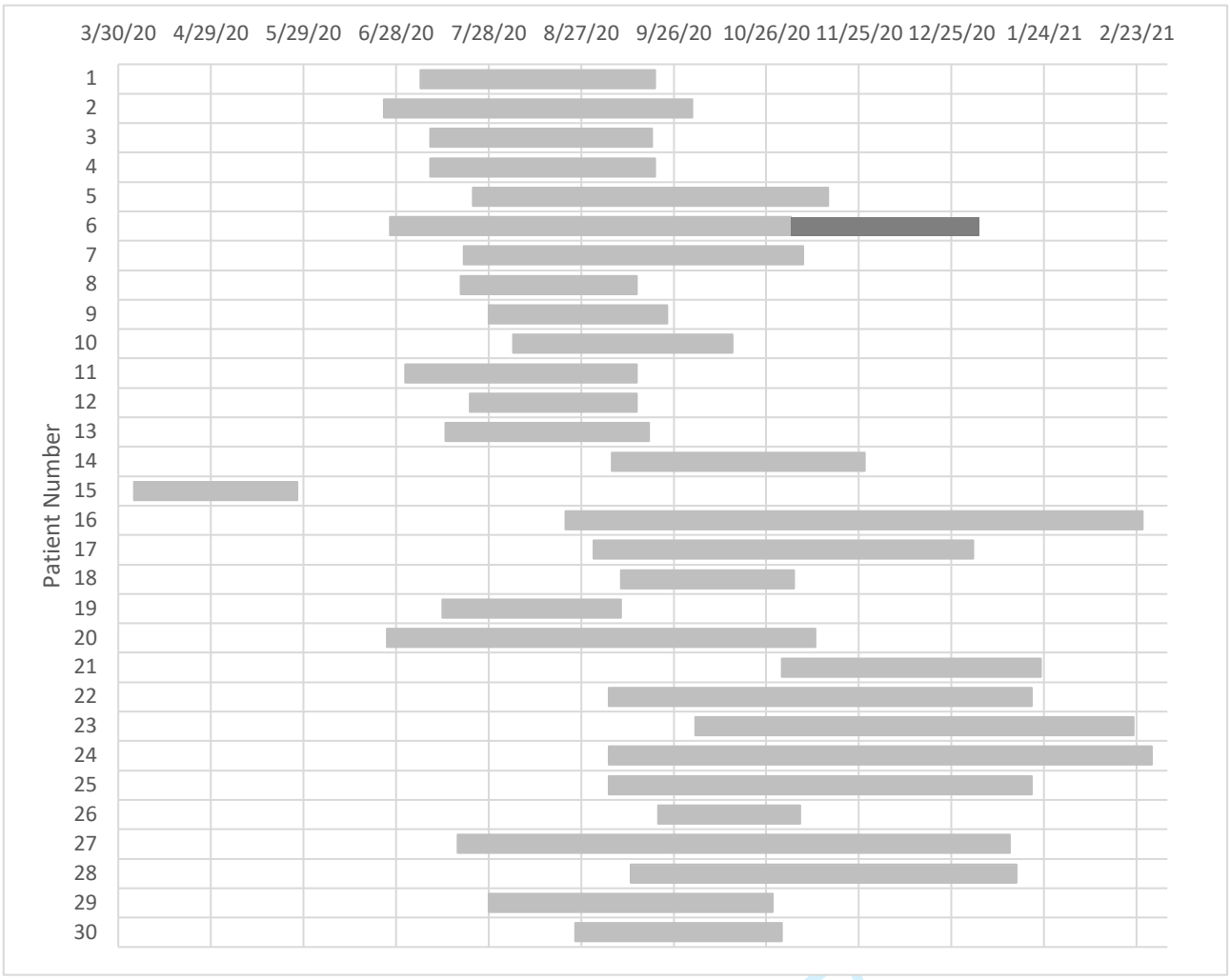
Figure 2: Duration between first SARS-CoV-2 infection and subsequent infections. The shaded bars represent the duration between positive PCR results.



*Patient 6 had two episodes of reinfection

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Dr. Danah Alsharrah
Department of Pediatrics,
Farwaniyah Hospital,
Ministry of Health, Sabah Al-Nasser, Kuwait

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Incidence of Potential SARS-CoV-2 Reinfection in a Pediatric Cohort in Kuwait

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page and line number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	P 1, L 1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	P 4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	P 6, L 105-123
Objectives	3	State specific objectives, including any prespecified hypotheses	P 7, L 132-134
Methods			
Study design	4	Present key elements of study design early in the paper	P 7, L 138
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Dates: P7, L139 Exp: P7, L 146 Data: P8, 157
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	P 7, L138 and P8, L 157 Exclusion: P 7, L142
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	P 7, L 146 Outcome: P7, L140
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	P8, L157
Bias	9	Describe any efforts to address potential sources of bias	Potential recall bias is mentioned in limitation
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	P 8, L165
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	NA
		(*b*) Describe any methods used to examine subgroups and interactions	P8, L 170
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	P8, L 170

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Results			**Page and line number**
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	P 9, L 183
		(b) Give reasons for non-participation at each stage	P 9, L187
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	P 9, L 193
		(b) Indicate number of participants with missing data for each variable of interest	Table 1- no missing data Table 2 – indicated by *
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	P9, L 184
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	P9, L183-190
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	P9, L187
		(*b*) Report category boundaries when continuous variables were categorized	done
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	P 10, L213
Discussion			
Key results	18	Summarise key results with reference to study objectives	P11, L 223
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	P12, L272
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	P11, 234-P12 L269
Generalisability	21	Discuss the generalisability (external validity) of the study results	P12, L 280
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	P1, L15

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely

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available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Incidence of SARS-CoV-2 Reinfection in a Pediatric Cohort in Kuwait

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-056371.R2
Article Type:	Original research
Date Submitted by the Author:	29-Mar-2022
Complete List of Authors:	Alhaddad, Fatemah; Farwaniya Hospital, Department of Pediatrics Abdulkareem, Ali; Farwaniya Hospital, Department of Pediatrics Alsharrah, Danah; Farwaniya Hospital, Pediatrics Alkandari, Abdullah; Dasman Diabetes Institute, Bin-Hasan, Saadoun ; Farwaniya Hospital, Department of Pediatrics; Dasman Diabetes Institute Al-Ahmad, Mona; Kuwait University Faculty of Medicine, Department of Microbiology Al Hashemi, Hashem ; Farwaniya Hospital, Department of Pediatrics Alghounaim, Mohammed; Amiri Hospital, Pediatric Infectious Diseases Specialist and Medical Microbiologist
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Infectious diseases, Paediatrics, Epidemiology
Keywords:	COVID-19, Epidemiology < INFECTIOUS DISEASES, PAEDIATRICS

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Incidence of SARS-CoV-2 Reinfection in a Pediatric Cohort in Kuwait

Fatemah Alhaddad[1], MD, Ali Abdulkareem[1], MD, Danah Alsharrah[1], MD, Abdullah Alkandari[2], PhD, Saadoun Bin-Hasan[1,2], MD, Mona Al-Ahmad[3], MD, Hashem Al Hashemi[1], MD, Mohammad Alghounaim[4], MD

[1]Department of Pediatrics, Farwaniyah Hospital, Ministry of Health, Sabah Al-Nasser, Kuwait

[2] Department of Population Health, Dasman Diabetes Institute, Kuwait City, Kuwait

[3] Department of Microbiology, Faculty of Medicine, Kuwait University, Kuwait

[4]Department of Pediatrics, Amiri Hospital, Ministry of Health, Kuwait City, Kuwait

Keywords: Children, SARS-CoV-2, COVID-19, reinfection, epidemiology

Running title: SARS-CoV-2 Reinfection in Children.

Conflict of Interest Disclosures: The authors no conflicts of interest to disclose

Funding: None

Abbreviations:

COVID-19: Coronavirus disease 2019

SARS-CoV-2: Severe acute respiratory syndrome-related coronavirus 2

PCR-Q8: The Pediatric COVID Registry in Kuwait

WHO: World Health Organization.

PCR: polymerase chain reaction

IQR: interquartile range

Article summary: National-level study found that pediatric SARS-CoV-2 reinfection is uncommon, and estimated to be 1.02 (95% CI 0.71-1.45) infection per 100,000 person-days

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Corresponding author:

Dr. Mohammad Alghounaim

Department of Pediatrics

Amiri Hospital

900005 Ibn Misbah St. Sharq block 3

Kuwait City, Kuwait

Tel: +965-97333099

Fax: +965-22436149

E-mail: malghounaim@moh.gov.kw

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Contributors' Statement:

Drs Ali Abdulkareem, Danah Alsharrah, and **Fatemah Alhaddad** conceptualized and designed the study, curated the data, wrote the original draft of the paper, and revised the manuscript.

Dr Abdullah Alkandari conducted the formal analysis, and critically revised the manuscript

Dr Saadoun Bin-Hasan: conceptualized and designed the study, and critically revised the manuscript.

Drs Mona Al-Ahmad and **Hashem Al-Hashemi**: conceptualized the study, participated in data curation, and revised the manuscript

Dr Mohammed Alghounaim: conceptualized and designed the study, supervised data collection, conducted initial analysis, and critically revised the manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Abstract

Objective: Subsequent protection from severe acute respiratory syndrome-related coronavirus 2 (SARS-COV-2) infection in pediatrics is not well reported in the literature. We aimed to describe the clinical characteristics and dynamics of SARS-CoV-2 PCR repositivity in children.

Design: This is a population-level retrospective cohort study

Setting: Patients were identified through multiple national-level electronic coronavirus disease 2019 (COVID-19) databases that covers all primary, secondary and tertiary centers in Kuwait.

Participants: The study included children 12 years and younger between February 28, 2020 and March 6, 2021. SARS-CoV-2 reinfection was defined as having two or more positive SARS-CoV-2 PCR done on a respiratory sample, at least 45 days apart. Clinical data was obtained from the Pediatric COVID-19 Registry in Kuwait (PCR-Q8).

Primary and secondary outcome measures: The primary measure is to estimate SARS-CoV-2 PCR repositivity rate. The secondary objective was to establish average duration between first and subsequent SARS-CoV-2 infection. Descriptive statistics was used to present clinical data for each infection episode. Also, incidence-sensitivity analysis was performed to evaluate 60- and 90-day PCR repositivity intervals.

Results: Thirty pediatric COVID-19 patients had SARS-CoV-2 reinfection at an incidence of 1.02 (95% CI 0.71-1.45) infection per 100,000 person-days and a median time to reinfection of 83 days (IQR 62-128.75). The incidence of reinfection decreased to 0.78 (95% CI 0.52-1.17) and 0.47 (95% CI 0.28-0.79) per person-days when the minimum interval between PCR repositivity was increased to 60 and 90 days, respectively. The mean age of reinfected subjects was 8.5 years (IQR 3.7-10.3) and the majority (70%) were females. Most children (55.2%) had asymptomatic reinfection. Fever was the most common presentation in symptomatic patients. One immunocompromised experienced two reinfection episodes.

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Conclusion: SARS-CoV-2 reinfection is uncommon in children. Previous confirmed COVID-19 in children seems to result in a milder reinfection.

Data availability statement:

No additional data available.

Strength and limitations of the study

- This study used a national-level electronic database that included all SARS-CoV-2 test results that allowed data collection from variable sources and the detection of asymptomatically infected children.
- The exact risk for SARS-CoV-2 reinfection is difficult to estimate as children are more likely to be asymptomatic, therefore not all positive cases are detected.
- Factors contributing to reinfection such as the circulating variants, and the degree of community SARS- CoV-2 transmission were not addressed in this study.
- Patients' symptoms and severity in this retrospective analysis were dependent on the accuracy of reporting in medical notes and parental recall of symptoms.
- Due to limited genomic sequencing capacity, proving the direct causality between PCR repositivity and a genetically distinct virus was not possible.

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Introduction:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) is a novel beta coronavirus that was first described in December 2019 and resulted in a pandemic of respiratory illness, coronavirus disease-2019 (COVID-19) [1]. By early May 2021, the pandemic has resulted in over 150 million cases worldwide and more than three million deaths reported by the World Health Organization (WHO)[2]. Despite the widespread of COVID-19, children compromised less than 15% of all reported cases [3-5]. Limited social interactions, and enhanced infection control measures such as school closure and online teaching, may have contributed to lower proportions of infected children [6]. In addition, children are more likely to have asymptomatic or mild infection compared to adults and, therefore, may not be tested [7].

Quantifying the duration of natural immunity after the primary SARS-CoV-2 infection has been crucial to address public health measures, to help predict the continuity of the pandemic, and to understand the effect of reinfection on disease severity. The first case of SARS-CoV-2 reinfection was reported in August 2020 [8]. Since then, interest in the exact risk and rate of reinfection has been increasing. Several reports estimated that reinfection occurs in less than 1% of previously infected individuals [9-11]. The duration between primary and secondary infection was reported to vary from 48 to 124 days in the first documented cases in literature [12, 13]. However, most of the reinfection studies focused mainly on the adult population. COVID-19 follows different disease dynamics in children, and studies addressing reinfection in this population are lacking/or limited.

The first pediatric case of COVID-19 in Kuwait was identified in February 2020 [7]. Since then, schools and daycare centers were closed, and classes were conducted virtually. In addition, commercial centers, gyms, and restaurants were open but with time restrictions and strict protective measures. Early in the pandemic, a national electronic COVID-19 testing database was created and

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included all SARS-CoV-2 test results of symptomatic individuals, contact tracing, and routine travel or hospitalization screening. Also, a national Pediatric COVID-19 Registry (PCR-Q8) was established for children aged 12 years and younger to better understand disease dynamics and update management protocols. This created the unique opportunity to investigate the possibility of reinfection with SARS-CoV-2, with an attempt to establish an average duration between the two positive results, potential factors linked to reinfection, and its clinical severity.

Methods:

A population-level retrospective cohort study was conducted in Kuwait between February 28th, 2020, and March 6th, 2021. The national COVID-19 test result database was used to identify children younger than 12 years and had two or more subsequent positive SARS-CoV-2 polymerase chain reaction (PCR) tests done on a respiratory sample, at least 45 days apart. Subjects who had two or more positive SARS-CoV-2 PCR but less than 45 days in-between, and patients who fulfilled the WHO definition of the multisystem inflammatory syndrome in children (MIS-C) were excluded [14].

To assure the inclusion of all SARS-CoV-2 infected children into this study, the national COVID-19 testing database was used for initial patient identification. This database includes the results for all individuals who had SARS-CoV-2 PCR done on a respiratory specimen nationwide. SARS-CoV-2 PCR is typically done to confirm the diagnosis of symptomatic individuals, detect secondary infections in contact tracing, and identify infected subjects prior to hospitalization or travel. During the study period, the SARS-CoV-2 antigen was not routinely performed on pediatric samples. Also, serum SARS-CoV-2 IgG or IgM tests were not routinely done to confirm current or prior infection. A secondary search query was done on the PCR-Q8 registry, which included patient

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information from the following sources: hospital records, institutional quarantine centers, patient transport units, as well as all laboratories that provide SARS-CoV-2 PCR.

The PCR-Q8 registry retrospectively collected detailed individual-level demographic, laboratory and clinical characteristics of all children diagnosed with COVID-19 in Kuwait. The database was acquired to obtain demographic and clinical data. COVID-19 severity was categorized based on WHO disease classification [15]. Infected children with mild or asymptomatic COVID-19 may not require hospitalization. Therefore, clinical data in the registry may be lacking/or limited. For those subjected, parents were contacted to complete missing disease-related data.

Descriptive analysis was performed to compare between primary and secondary infections. To calculate incidence and to account for delayed presentation of reinfection, calculated days at risk included the period from the day of first positive SARS-CoV-2 PCR starting February 28th, 2020 to the second positive test or March 6th, 2021, whichever is first. However, individuals with the first positive SARS-CoV-2 PCR starting January 1st, 2021, were not included in the calculation due to limited time for reinfection to occur. Clopper-Pearson test was used to calculate the 95% CI. Analysis was done using GraphPad Prism (v. 9.0). Due to the variability in established reinfection case definition, sensitivity analysis assessing the clinical presentation and incidence of reinfection considering a minimum interval for PCR repositivity of 60 and 90 days[16].

Patient and public involvement statement:

Patients were not included in the design, reporting, or dissemination plans of our research. However, parents of children with PCR repositivity were contacted to complete missing data. This study was conducted to address epidemiological questions presented by the Pediatric COVID-19 Task Force (Ministry of Health, Kuwait). The initial concept and study plan was presented to the committee members, whose suggestions and comments were considered in the final study protocol

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Ethics:

The study was approved by the ethical board of the Ministry of Health, Kuwait (reference no. 1607/2020). Verbal consent was obtained from parents who agreed to participate to complete missing clinical data.

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Results

During the study period, there were 14,320 documented SARS-CoV-2 infections in children younger than 12 years in Kuwait, accounting for 2,954,372 person-days of follow-up. Among those, 421 children with repeat positive SARS-CoV-2 PCR were identified, of which, 30 patients had a repeat positive SARS-CoV-2 45 days or more after the first positive, 391 patients had their repeat test within 45 days and were excluded (figure 1). The incidence of reinfection was 1.02 (95% CI 0.71-1.45) infection per 100,000 previously infected person-days. The mean age of the reinfection cohort was 8.5 (IQR 3.7-10.3) years and majority (70%) were females (table 1). More than half (56.6%) of reinfection patients were not known to have any chronic comorbid conditions at the time of the first or second infection.

The median time between the two episodes of infection as evident by the sample collection date was 83 days (IQR 62-128.75 days) (figure 2). Majority of the patients had asymptomatic infection during the first and second episodes, 43.3% and 53.3% respectively (table 2). In symptomatic patients, fever, cough and shortness of breath were the most commonly reported symptoms. One (3.3%) patient had severe pneumonia during the first infection, whereas, four (13.3%) patients had severe pneumonia during second infection. Of those, three patients had mild or asymptomatic initial SARS-CoV-2 infection. One of the four patients was admitted for an acute exacerbation of asthma with three days history of fever. The median length of hospitalization was 9 days (IQR 5.75-13 days) and 6 days (IQR 3.75-6.75 days) for the first and second infection, respectively. None of the subjects received care in an intensive care unit.

One female patient previously diagnosed with hypereosinophilic syndrome on low-dose prednisone, had three episodes of SARS-CoV-2 infections. Her first infection was in July 2020 where she was admitted for 10 days as a case of severe pneumonia followed by two negative PCR tests done in August. Her second infection was in November 2020 when she was also admitted for

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severe pneumonia with prolonged hospital admission followed by a negative PCR test done in December. Interestingly, during her third infection in January 2021, she was asymptomatic and testing was done for screening before an outpatient appointment.

Around half (46.6%) of reinfections occurred more than 90 days after the initial infection. The incidence of reinfection decreased to 0.78 (95% CI 0.52-1.17) and 0.47 (95% CI 0.28-0.79) per infected person-days when the minimum acceptable duration between PCR repositivity was increased to 60 and 90 days, respectively. Also, there were more symptomatic children (64.3%) during the second infection using the 90-day definition when compared to the 45-day or 60-day minimum interval.

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Discussion

The exact risk and factors associated with SARS-CoV-2 reinfection in the pediatric population are still not well understood. We found that a second SARS-CoV-2 infection is uncommon in children with a rate of 1.02 reinfection per 100,000 previously infected person-days and a median time to reinfection of 83 days. The finding of this study is similar to previous reports, which showed a reinfection incidence in adults of 0.9-1.3 per 100,000 person-day [9, 11]. Similarly, in a large Danish cohort, prior SARS-CoV-2 provided on average 80.5% protection against repeat infection. The estimated protection differed between different age groups ranging from 47.1% in individuals older than 65 years of age to 82.7% in younger subjects [17]. However, the epidemiology and disease dynamics of SARS-CoV-2 infection are different in children[18]. Therefore, using data generated from adult studies to infer on the pediatric population may not be accurate.

Magnitude and persistence of immunological responses conferred by SARS-CoV-2 infection can be variable based on age and medical comorbidities [19]. Hence, estimating the duration of protective immunity after acquiring SARS-CoV-2 infection has been the focus of several studies. In an adult study, looking at the neutralizing antibodies, the seropositivity was identified in all participants up to 53 days after infection [20]. However, the level of neutralizing antibodies varies greatly with disease severity and tend to wane over time [21, 22]. Few studies compared humoral responses between adults and children. Weisberg et. al. have shown that children exhibit lower antibody response compared to adults [23]. However, the incidence of reinfections observed in this study was similar to previously reported adult patients.

A standard definition of SARS-CoV-2 reinfection is lacking. Traditionally, the detection of viable virus by cell culture has been the standard to ascertain active infection [24]. However, this testing method lacks sensitivity and is time consuming [25]. In addition, due to the need of expertise

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and appropriate laboratory infrastructure, routine population-level testing using cell culture in not possible. For these reasons, the detection of genetically distinct SARS-CoV-2 in different infectious episodes or the use of the cycle threshold (Ct) value as a surrogate for viral load has been suggested [12, 13]. In Kuwait, genomic testing capacity is limited and retrieving patients' samples and laboratory data from several public and private laboratories was logistically challenging. In our study, a 45-day period between two-consecutive positive PCR test was selected based on established definition in previously published studies and the expected duration of molecular test positivity on a respiratory sample [26-29]. Alsharrah et al. reported a median duration of PCR positivity among pediatric COVID-19 patients of 15 days, with maximum duration of 42 days [7]. However, we observed a decline in the rate of repositivity from 1.02 to 0.47 per 100,000 previously infected person-days when the definition of PCR-repositivity increased from 45 to 90 days. As almost half of cases are asymptomatic, this finding could be due to persistent detection of viral particles by PCR.

Asymptomatic SARS-CoV-2 infection is common in pediatrics. We found that around half of infections (43.3% in initial infection and 55.2% in reinfection) remained asymptomatic on follow-up. This finding is similar to other studies [30]. High proportion of silent infection may pose an important public health concern and limit the effectiveness of transmission mitigation efforts. Also, the possibility of reinfection within a relatively short period of time as observed in this study may be an overlooked source of community transmission. Further epidemiological studies are needed to assess the risk of transmission in patients with PCR repositivity within 90 days. Real-world COVID-19 vaccine effectiveness data showed that reinfection is more common in unvaccinated adults[31]. These findings support the recommendation to offer vaccination to those previously infected.

To our knowledge, this is the first national-level cohort that estimates the risk of pediatric SARS-CoV-2 reinfection. Nevertheless, this study has some limitations. Due to limited genomic

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sequencing capacity, proving that PCR repositivity was caused by a genetically distinct virus was not possible. However, based on the expected duration of PCR-persistence in children mentioned above, one will not expect that PCR done on an upper respiratory specimen to remain positive beyond 45 days. Also, the effect of different SARS-CoV-2 variants on the risk of reinfection was difficult to assess. Another limitation was the unavailability of some data recorded in the national pediatric COVID-19 registry. When data was missing, parents were contacted via the telephone, creating a recall bias. In addition, patients with mild upper respiratory tracts symptoms are underreported by parents. Therefore, mild cases are less likely to be tested and can be missed, and underestimate the reinfection rate. However, using the national level electronic record allowed the collection of data from variable sources and the detection of asymptomatically infected children. Lastly, the exact incidence of reinfection is dependent on several factors that may be difficult to control in cohort studies and may affect data generalizability. These factors include rate SARS-CoV-2 transmission in the community, and preexisting population immunity. However, the effect of existing immunity is limited in our cohort as it included pediatric patients that were followed from the start of the pandemic. Despite these limitations, this study has shown that reinfection is generally uncommon. Further studies that correlate degree of humoral and cellular immunity, along with wide genomic sequencing surveillance with risk of reinfection are needed to better understand and quantify the risk of repeat SARS-CoV-2 infection.

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Acknowledgement:

We would like to thank Dr. Hessa Alkandari, chair of the pediatric council in the Ministry of Health, Kuwait, for her help in preparing this manuscript. We also thank the research group of the Pediatric COVID-19 Registry in Kuwait (PCR-Q8) for their contribution.

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Table 1. Demographic characteristics of the study population

Variable	Population (n=30)
Age [median, IQR]	8.6 years [3.7-10.3]
Male	9 (30%)
Comorbid conditions	
Asthma	2 (6.7%)
Diabetes	1 (3.3%)
Healthy	17 (56.7%)
Neurological disease	3 (10%)
Other	5 (16.7%)
Preterm	1 (3.3%)
Time between 2 episodes	
First positive PCR to reinfection [median, IQR]	83 days [62-128.75]
Symptom onset of first episode to reinfection [median, IQR]	83 days [62.5-130.5]

IQR, interquartile range.

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Table 2. Clinical characteristics of the study population based on different defined intervals of polymerase chain reaction (PCR) repositivity

Variable	First Infection			Second Infection		
	Minimum interval of PCR repositivity			Minimum interval of PCR repositivity		
	45 days (n=30)	60 days (n=23)	90 days (n=14)	45 days (n=29)*	60 days (n=23)	90 days (n=14)
Disease severity						
Asymptomatic	13 (43.3%)	12 (52.2%)	8 (57.1%)	16 (55.2%)	12 (52.2%)	5 (35.7%)
Mild illness	15 (33.3%)	9 (39.1%)	4 (28.6%)	9 (31%)	7 (30.4%)	5 (35.7%)
Mild pneumonia	1 (3.3%)	1 (4.3%)	1 (7.1%)	0	0	0
Severe pneumonia	1 (3.3%)	1 (4.3%)	1 (7.1%)	4 (13.8%)	4 (17.4%)	4 (28.6%)
Reason for testing						
Close contact testing	13 (43.3%)	12 (52.2%)	8 (57.1%)	6 (20.7%)	5 (21.7%)	4 (28.6%)
Hospitalization screening	5 (16.7%)	4 (17.4%)	3 (21.4%)	7 (24.1%)	6 (26.1%)	5 (35.7%)
Suspected COVID-19	12 (40%)	7 (30.4%)	3 (21.4%)	6 (20.7%)	5 (21.7%)	4 (28.6%)
Travel screening	1 (3.3%)	0	0	10 (34.5%)	7 (30.4%)	1 (7.1%)
Symptoms						
Abdominal pain	2 (4%)	1 (3.1%)	1 (5%)	0	0	0
cough	4 (8.2%)	4 (12.5%)	3 (15%)	8 (20.5%)	8 (21.6%)	7 (21.9%)
Diarrhea	2 (4%)	2 (6.3%)	2 (10%)	0	0	0
Fever	16 (32.6%)	11 (34.4%)	6 (30%)	12 (30.8%)	10 (27%)	8 (25%)
Headache	2 (4%)	2 (6.3%)	1 (5%)	1 (2.6%)	1 (2.7%)	1 (3.1%)
Loss of smell	4 (8.2%)	1 (3.1%)	0	1 (2.6%)	1 (2.7%)	1 (3.1%)
Loss of taste	4 (8.2%)	1 (3.1%)	0	1 (2.6%)	1 (2.7%)	1 (3.1%)
Myalgia	4 (8.2%)	3 (9.4%)	3 (15%)	1 (2.6%)	1 (2.7%)	1 (3.1%)
Rhinorrhea	4 (8.2%)	3 (9.4%)	1 (5%)	8 (20.5%)	8 (21.6%)	7 (21.9%)
Shortness of breath	3 (6.1%)	2 (6.3%)	2 (10%)	3 (7.7%)	3 (8.1%)	3 (9.4%)
Sore throat	3 (6.1%)	2 (6.3%)	1 (5%)	3 (7.7%)	3 (8.1%)	2 (6.3%)
Vomiting	1 (2%)	0	0	1 (2.6%)	1 (2.7%)	1 (3.1%)
Hospitalization	5 (16.7%)	4 (17.4%)	3 (21.4%)	6 (20.7%)	5 (21.7%)	5 (35.7%)
Length of stay in days [median, IQR]	9 [5.75-13]	9 [7.25-11]	8 [6.5-9]	6 [3-6]	6 [3-6]	6 [3-6]

IQR, interquartile range.

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*clinical data for one patient is missing

299

300

301

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Table 1. Demographic characteristics of the study population.

Table 2. Clinical characteristics of the study population based on different defined intervals of polymerase chain reaction (PCR) repositivity.

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Figure 1: Flow diagram of the study population

Figure 2: Duration between first SARS-CoV-2 infection and subsequent infections. The shaded bars represent the duration between positive PCR results.

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Figure 1: Flow diagram of the study population

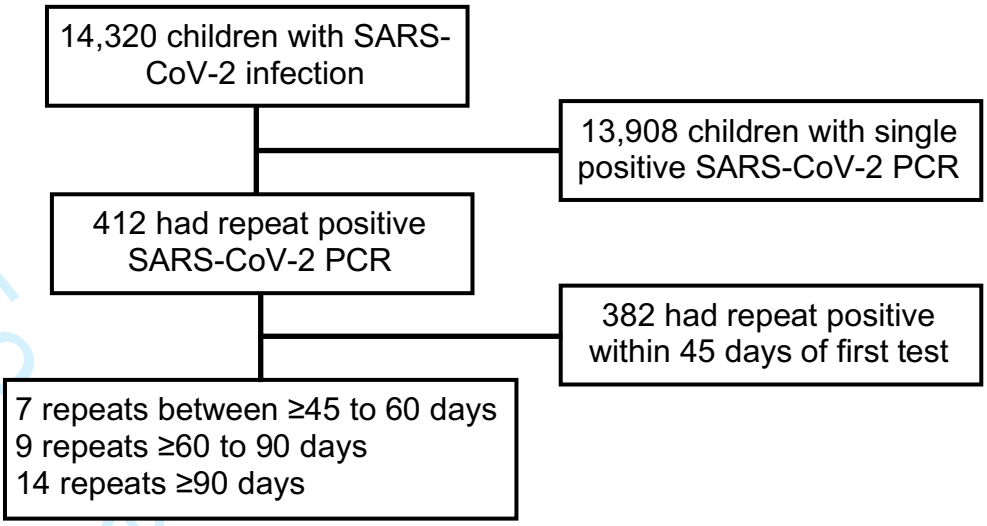

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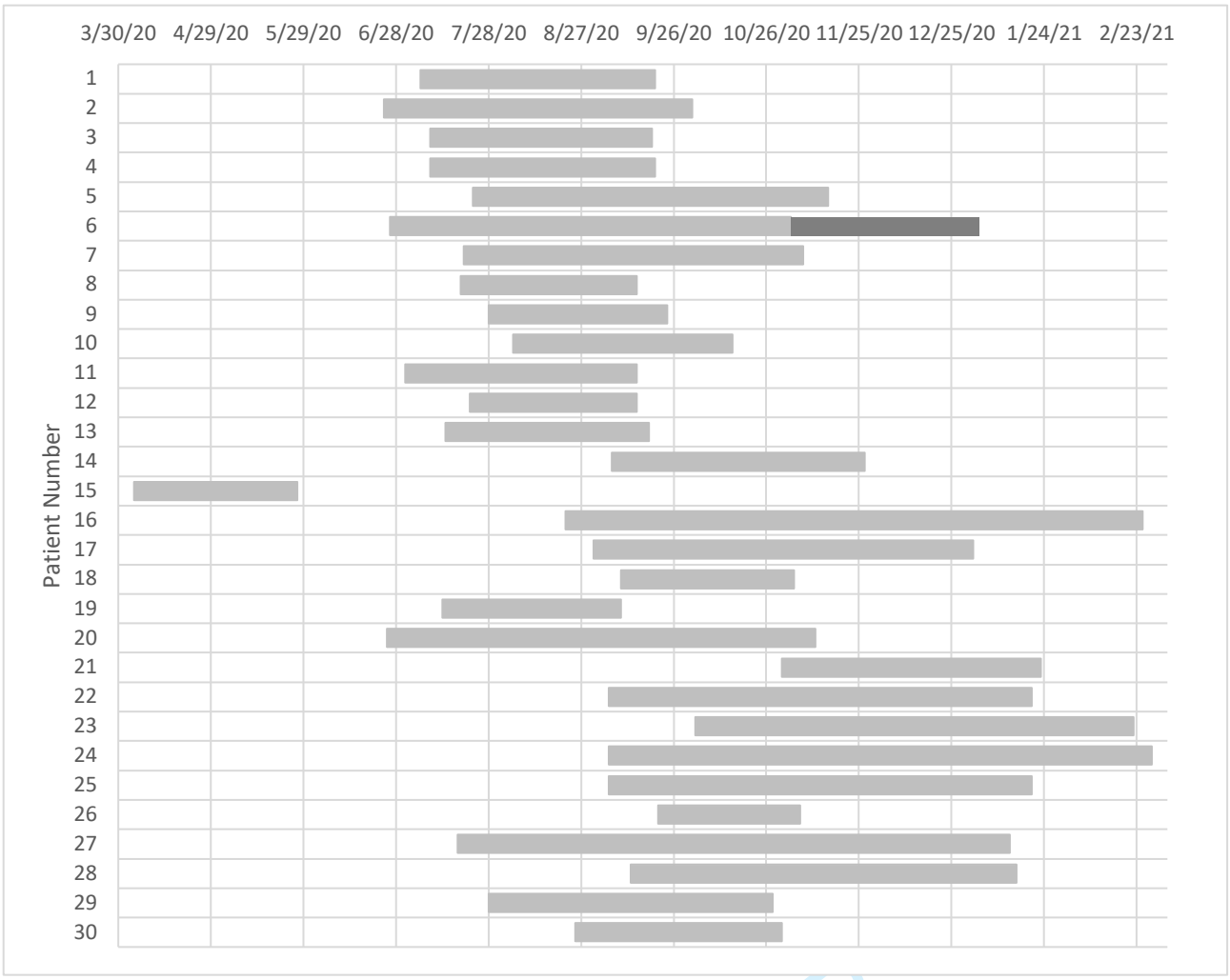
Figure 2: Duration between first SARS-CoV-2 infection and subsequent infections. The shaded bars represent the duration between positive PCR results.



*Patient 6 had two episodes of reinfection

Incidence of Potential SARS-CoV-2 Reinfection in a Pediatric Cohort in Kuwait

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page and line number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	P 1, L 1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	P 4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	P 6, L 105-123
Objectives	3	State specific objectives, including any prespecified hypotheses	P 7, L 132-134
Methods			
Study design	4	Present key elements of study design early in the paper	P 7, L 138
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Dates: P7, L139 Exp: P7, L 146 Data: P8, 157
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	P 7, L138 and P8, L 157 Exclusion: P 7, L142
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	P 7, L 146 Outcome: P7, L140
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	P8, L157
Bias	9	Describe any efforts to address potential sources of bias	Potential recall bias is mentioned in limitation
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	P 8, L165
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	NA
		(*b*) Describe any methods used to examine subgroups and interactions	P8, L 170
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	P8, L 170

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Results			**Page and line number**
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	P 9, L 183
		(b) Give reasons for non-participation at each stage	P 9, L187
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	P 9, L 193
		(b) Indicate number of participants with missing data for each variable of interest	Table 1- no missing data Table 2 – indicated by *
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	P9, L 184
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	P9, L183-190
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	P9, L187
		(*b*) Report category boundaries when continuous variables were categorized	done
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	P 10, L213
Discussion			
Key results	18	Summarise key results with reference to study objectives	P11, L 223
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	P12, L272
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	P11, 234-P12 L269
Generalisability	21	Discuss the generalisability (external validity) of the study results	P12, L 280
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	P1, L15

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely

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available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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