

1 **Age-related seroprevalence trajectories of seasonal coronaviruses in children**

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34 **Abstract**

35 Four seasonal coronaviruses, including HCoV-NL63 and HCoV-229E, HCoV-OC43 and
36 HCoV-HKU1 cause approximately 15–30% of common colds in adults. However, the
37 frequency and timing of early infection with four seasonal coronaviruses in the infant are still
38 not well studied. Here, we evaluated the serological response to four seasonal coronaviruses
39 in 1886 children under 18-year-old to construct the viral infection rates. The antibody levels
40 were also determined from the plasma samples of 485 pairs postpartum women and their
41 newborn babies. This passive immunity waned at one year after birth and the resurgence of
42 the IgGs were found thereafter with the increase of the age. Taken together, our results show
43 the age-related seroprevalence trajectories of seasonal coronaviruses in children and provide
44 useful information for deciding vaccine strategy for coronaviruses in the future.

45

46 **Main Text**

47 SARS-CoV-2 is now high prevalence worldwide and becomes persistence in the human
48 population. It is reasonable to expect that most people will be exposed to the virus for the first
49 time during their childhood. Understanding the development of acquired immunity against
50 the seasonal coronaviruses (HCoV-NL63 and HCoV-229E, HCoV-OC43 and HCoV-HKU1)
51 in young age group will thus give us a clue on the impact of SARS-CoV-2 to human in the
52 post COVID-19 era. These viruses have been circulating in human population for many years
53 and are accounted for approximately 15–30% of upper respiratory tract infection¹. Infection
54 of these viruses mainly cause self-limiting flu-like illnesses, but severe pediatric respiratory
55 infections are not rare²⁻⁴.

56

57 Children are not entirely immunological naïve when they are born⁵. IgG antibodies in the
58 neonates are transferred from their mother so as to provide a transient immune barrier against
59 the potential infection^{6,7}. This transferred immunity plays a protective role before the infants
60 establish their own specific adaptive immunity to the same pathogen. So far, there is paucity
61 of data to describe the transition period from transferred immunity to acquired immunity for
62 the seasonal coronavirus in children. Moreover, the accumulation of immune response to the
63 seasonal coronaviruses in children is also not yet well understood. Longitudinal study showed
64 that adults are repeatedly infected by the seasonal human coronaviruses for every 12 months⁸.
65 Although it was found that the induction of antibodies after each infection is short-lasting,
66 frequent reinfections lead to persistent levels of antibodies to the four seasonal coronaviruses
67 in most of the adults⁹. These pre-existing antibodies against seasonal coronaviruses were

68 recently found to be associated with the neutralizing antibody response against SARS-CoV-2
69 that may mitigate disease manifestations from SARS-CoV-2 infection¹⁰. In this study, we
70 determined the serological response against four seasonal coronaviruses in the plasma
71 samples of children and modelled the seroprevalence trajectories of the four virus subtypes
72 during the whole childhood period.

73

74 We tested the seroprevalence to the four seasonal coronaviruses by the Enzyme-linked
75 Immunosorbent Assay (ELISA) using the plasma samples collected from 1886 children
76 (Female: 43.9%) with age ranging from 0 (Neonates) to 18 years old in Guangzhou, China
77 between January and March in 2020. Among our cohort, 259 were under 6 months old, 161
78 were 6 months-1 year old, 278 were 1-2 years old, 603 were 3-6 years old, 466 were 7-12
79 years old, 119 were 13-18 years old (Supplementary Table 1). The spike (S) protein of
80 coronavirus, which plays an essential role in the receptor recognition and cell membrane
81 fusion process, is composed of two subunits, S1 and the stalk-like S2¹¹. Since there are 63–
82 98% of sequence similarity in the S2 among the seven human coronaviruses^{12,13}, we
83 specifically targeted to detect the level of IgG antibody to the S1 (HCoV-229E, HCoV-NL63,
84 HCoV-HKU1) or hemagglutinin-esterase (HE) (HCoV-OC43) of the viruses.

85

86 The IgG levels to the four seasonal coronaviruses were determined from each plasma sample.
87 The association between the IgG level and the age in each seasonal coronavirus was
88 constructed by generalized additive models (GAM) (Figure 1)¹⁴. The restricted cubic splines
89 (smooth curve) with five knots were used to visualize the association. We found that the
90 seroprevalences of the four seasonal coronaviruses showed a similar trajectory. Compared to
91 the entire childhood period, the levels of IgG in the neonates dropped significantly and
92 reached to the lowest level after the age of 1 year (1.25 years: HCoV-229E; 1 years: HCoV-
93 OC43; 1.08 years: HCoV-NL63; 1.08 years: HCoV-HKU1). The levels of IgG were then
94 increased and accumulated when the children became older in age. The IgG levels against
95 HCoV-OC43, HCoV-NL63 and HCoV-HKU1 were increased to the comparable levels of the
96 neonates at the age of 8, 9 and 6 years respectively. However, it was intriguing to find that
97 the IgG to the HCoV-229E was increased slower than the other seasonal coronaviruses and it
98 reached to the comparable level of the neonates at the age of 16 years. Thus, our results
99 implicate that the frequency of repeated infection of HCoV-229E was lower than that of the
100 other three subtypes¹⁵. The serological results of each coronavirus were further stratified into
101 two sex groups (male/female) and were further compared (Supplementary Figure 1).

102 Importantly, we found that the IgG waning of all four seasonal coronaviruses in male
103 neonates were much faster than that in female. The time required for dropping the IgG of
104 each coronavirus to their lowest level in male neonates were 1.89 (HCoV-229E: 0.75(M) vs
105 1.42(F), 1.89 (HCoV-OC43: 0.62(M) vs 1.17(F)), 1.72 (HCoV-NL63: 0.68(M) vs 1.17(F)),
106 1.75 (HCoV-HKU1: 0.67(M) vs 1.17(F)) folds faster than that of the female neonates.

107

108 The relatively high levels of IgG antibody to the four seasonal coronaviruses in the neonates
109 implicated a vertical transfer of the maternal immune response. It has been recently shown
110 that the passive immunity against SARS-CoV-2 of neonates was contributed by their
111 mothers⁶. We further collected plasma samples from 485 pairs of postpartum women and
112 their newborn baby for testing the levels of their IgG to the four seasonal coronaviruses using
113 similar serological assays. We found that the maternal IgG level was linearly associated with
114 their neonatal IgG levels in each seasonal coronavirus: HCoV-229E ($r=0.63$, 95% CI: 0.57-
115 0.68, $p<0.0001$), HCoV-OC43 ($r=0.65$, 95% CI: 0.60-0.70, $p<0.0001$), HCoV-NL63 ($r=0.69$,
116 95% CI: 0.64-0.74, $p<0.0001$), HCoV-HKU1 ($r=0.63$, 95% CI: 0.58-0.69, $p<0.0001$) (Figure
117 2). While comparing to the previous report that maternally derived antibodies against SARS-
118 CoV-2 could persist up to 6 months of age in their infant¹⁶, our results indicated that the
119 passive transferred immunity against the seasonal coronaviruses in neonates can maintain
120 longer time.

121

122 Prevalence of the seasonal coronaviruses in children is determined either by detecting the
123 specific nucleic acids from the respiratory specimen or through serology test. However, it is
124 difficult to define and collect true negative reference samples because the seasonal
125 coronaviruses are highly circulating in children. Previous studies adopted an approach in
126 which the cutoffs were determined from a small subset of reference samples who the children
127 were between 1-2 years old, and the tested samples were defined as positive if the results
128 were above the mean of the references^{17,18}. Here, we estimated the prevalence of the seasonal
129 coronaviruses by using the lowest level in the generalized additive models as our negative
130 reference (Supplementary Table 1). We assumed that children with IgG level above this point
131 indicate infection of the corresponding seasonal coronaviruses and thus defined it as
132 seropositive. 91.12%, 82.24%, 79.92% and 84.17% of sero-positivity to HCoV-229E, HCoV-
133 OC43, HCoV-NL63 and HCoV-HKU1 respectively were found in those under 6 months old.
134 In infants with the age between 6 months and 1-year-old, the seropositive rates dropped to
135 44.72% (HCoV-229E), 43.48% (HCoV-OC43), 45.96% (HCoV-NL63) and 45.96% (HCoV-

136 HKU1). The sero-positivity of each seasonal coronavirus increased with age and was over
137 64.51% of prevalence in the children at their pre-school age (3-6 years). The seroprevalences
138 for HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1 kept increasing and were
139 98.11%, 100%, 96.23% and 98.11%, respectively, at the age of 16-18 years.

140

141 Our study described the transition from passive to acquired immunity for seasonal
142 coronaviruses in children. The established approach here provides a view to identify the
143 waning period of immunity against coronavirus after born, that will be useful to apply on
144 SARS-CoV-2. The best timing to receive COVID-19 vaccine is still under debated. Though
145 US CDC suggested that COVID-19 vaccination is recommended for children aged 6 months
146 or older, it is mainly based on the safety concern rather than aiming for better protection.
147 Defining the waning period in SARS-CoV-2 using our approach will provide scientific
148 evidence to determine the vaccination window for children in post-COVID era. There were
149 some limitations in our study. Firstly, the trajectories were illustrated using cross-sessional
150 samples from population age groups, not in the longitudinal cohort. Secondly, the
151 seroprevalences from our cohort were determined by ELISA only. The neutralizing effect to
152 the seasonal coronaviruses was not evaluated. Thirdly, although the children were recruited
153 from the non-respiratory ward or routine body check center, we did not collect their clinical
154 background for analysis in this study.

155

156 In conclusion, we described that IgG antibody against four seasonal coronaviruses could be
157 transferred from mother to their infant in a large-scale cohort. Importantly, we reported this
158 transferred immunity waned for one year after born and children could acquire immunity
159 against four seasonal coronaviruses with the increase of the age. Overall, these results
160 provide a comprehensive analysis of the antibody dynamic in the early life of the children.

161

162 **Methods**

163 **Sample Collection.** Pediatric patients in non-respiratory diseases wards and children aged
164 under 18 and without signs of influenza-like illness were recruited in our study. All plasma
165 samples were obtained from the EDTA anti-coagulated peripheral blood samples in the
166 Guangdong Women and Children Hospital, Guangzhou, China. Peripheral whole blood
167 samples were centrifuged at 3000 x g for 10 minutes at room temperature for plasma
168 collection. All plasma samples were kept in -80°C until used. Moreover, 500 plasma samples
169 from postpartum women were collected between January and March 2020, with paired

170 plasma samples collected from their newborn babies. All study procedures were performed
171 after informed consent. The study was approved by the Human Research Ethics Committee at
172 the Guangdong Women and Children Hospital (Approval number: 202101231).

173

174 **ELISA.** The S1 subunits of spike protein (His tag) of HCoV-229E, HCoV-HKU1, and
175 HCoV-NL63 and the hemagglutinin esterase protein (His Tag) of HCoV-OC43 were
176 purchased from Sino Biological (China). The experiments were carried out according to our
177 previous study¹⁷.

178

179 **Modelling.** Generalized additive models (GAM) was fitted to investigate the association
180 between age and the ELISA results. The restricted cubic splines (smooth curve) with five
181 knots were used to construct the model¹⁴. Of note, percentile places knots at five spaced
182 percentiles of the explanatory variable, which are the 5th, 27.5th, 50th, 72.5th and 95th
183 percentile. R version 4.0.4 was used for the analysis.

184

185 **Statistical Analysis.** Significance between two groups was determined by the Mann-Whitney
186 test, with a *p*-value smaller than 0.05 being considered statistically significant. Correlation
187 between plasma samples were evaluated by using Pearson's correlation coefficients.

188

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194

195 **Author contributions**

196 H.L., N.C.W. and C.K.P.M. conceived the research idea and designed the study. Y.L., Y.S.
197 C.L., Y.D., B.L. and X.M coordinated and carried out cohort recruitment. H.L., S.Z., K.K.,
198 C.K.P.M. and H.M.T., analyzed the data. Y.L., H.L., C.C., W.L, Q.W.T, R.T.Y.S., Y.L, Z.D.,
199 J.Z., D.Z. and J.F. performed the experiments. H.L., R.B., H.M.T., and C.K.P.M. wrote the
200 manuscript.

201

202 **Competing Interests**

203 The authors declare no competing interests.

204 **Reference**

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249

250 **Supplementary table 1. Prevalence of the seasonal coronaviruses in children**

		Participants	Number of Positive (%)			
			229E-S1	NL63-S1	OC43-HE	HKU1-S1
Male	<0.5	155	141 (90.97)	123 (79.35)	122 (78.71)	130 (83.87)
	0.5-1	99	37 (37.37)	41 (41.41)	39 (39.39)	47 (47.47)
	1-2	151	64 (42.38)	65 (43.05)	69 (45.70)	69 (45.70)
	3-6	332	212 (63.86)	273 (82.23)	289 (87.05)	286 (86.14)
	7-12	266	214 (80.45)	252 (94.74)	264 (99.25)	256 (96.24)
	13-15	32	32 (100.00)	31 (96.88)	31 (96.88)	32 (100.00)
	16-18	23	23 (100.00)	23 (100.00)	23 (100.00)	23 (100.00)
Female	<0.5	104	95 (91.35)	84 (80.77)	91 (87.50)	88 (84.62)
	0.5-1	62	35 (56.45)	33 (53.23)	31 (50.00)	27 (43.55)
	1-2	127	51 (40.16)	61 (48.03)	64 (50.39)	63 (49.61)
	3-6	271	177 (65.31)	221 (81.55)	237 (87.45)	241 (88.93)
	7-12	200	167 (83.50)	188 (94.00)	197 (98.50)	191 (95.50)
	13-15	34	33 (97.06)	33 (97.06)	31 (91.18)	34 (100.00)
	16-18	30	29 (96.67)	28 (93.33)	30 (100.00)	29 (96.67)
Overall	<0.5	259	236 (91.12)	207 (79.92)	213 (82.24)	218 (84.17)
	0.5-1	161	72 (44.72)	74 (45.96)	70 (43.48)	74 (45.96)
	1-2	278	115 (41.36)	126 (45.32)	133 (47.84)	132 (47.48)
	3-6	603	389 (64.51)	494 (81.92)	526 (88.89)	527 (87.40)
	7-12	466	381 (81.76)	440 (94.42)	461 (98.93)	447 (95.92)
	13-15	66	65 (98.48)	64 (96.97)	62 (93.94)	66 (100.00)
	16-18	53	52 (98.11)	51 (96.23)	53 (100.00)	52 (98.11)

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257 **Figure legends**

258

259 **Figure 1. Seroprevalence trajectory of the four seasonal coronaviruses in children.** The
260 plasma samples were collected from 1886 children who aged from 0 (neonates) to 18 years
261 old. Each sample was tested by ELISA against either S1 (HCoV-229E, HCoV-NL63 or
262 HCoV-HKU1) or hemagglutinin-esterase (HCoV-OC43) protein. Generalized additive
263 models (GAM) was used to model the association between the serological data and the age.
264 The black lines showed the fitted values and gray areas showed the 95% confidence intervals.
265 Each sample was tested in duplicate, and the results were represented by the mean of the two
266 values.

267

268 **Figure 2. Correlation between the maternal and neonatal IgG levels of the four seasonal**
269 **coronaviruses.** 485 paired of maternal and neonatal plasma samples were collected and
270 tested by ELISA. Antibody levels against A) HCoV-229E-S1, B) HCoV-NL63-S1, C)
271 HCoV-OC43-HE, and D) HCoV-HKU1-S1 were determined and the correlations between
272 the paired samples in the four seasonal coronavirus groups were shown. The black lines
273 showed the fitted values and gray areas showed the 95% confidence intervals. The r
274 represented the correlation coefficient.

275

276 **Figure S1. Antibody levels against the four seasonal coronaviruses in different genders.**
277 The 1886 plasma samples which were collected from children were further stratified into
278 female (n=828 samples) and male (n=1058 samples) for analysis. Each sample was tested by
279 ELISA against either S1 (A: HCoV-229E, C: HCoV-NL63 or D: HCoV-HKU1) or
280 hemagglutinin-esterase (B: HCoV-OC43) protein. Generalized additive models (GAM) was
281 used to model the association between the serological data and the age. The black lines
282 showed the fitted values and gray areas showed the 95% confidence intervals. Each sample
283 was tested in duplicate, and the results were represented by the mean of the two values.

284



