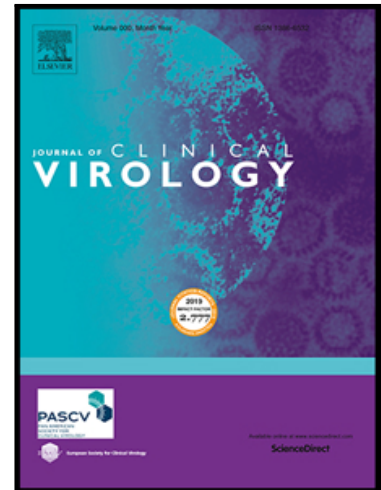


Journal Pre-proof

RSV reemergence in Argentina since the SARS-CoV-2 pandemic

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1 **Title: RSV reemergence in Argentina since the SARS-CoV-2 pandemic**

2

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16

17 **Competing interests**

18 The authors declare no competing interests.

19

20

21 **Word count: 1255**

Highlights:

- In ARG, during the 2020-lockdown due to COVID-19 pandemic, no RSV-cases were found
- RSV reemerged in ARG in 2021 with a delayed outbreak and fewer cases than 2018-2019
- Both subgroups A and B co-circulated during the three outbreaks analyzed
- The lineages detected in 2021 were the same as those that circulated in 2018-2019
- Phylogenetic analyses showed that 2021 lineages were new viral introductions to ARG

Abstract:

Introduction: The community mitigation measures taken because of the COVID-19 pandemic had side effects on the circulation of the most frequent respiratory viruses during 2020. In the case of respiratory syncytial virus (RSV), an important paediatric pathogen, a decrease in the number of cases and delayed outbreaks was previously described.

Aim and Methods: The genetic characteristics of the RSV circulating strains in paediatric patients in Buenos Aires, Argentina before and during the COVID-19 pandemic were studied. RSV (+) samples taken from hospitalised patients with respiratory tract infections (2018- 2021) were analysed through G gene sequencing and evolutionary analyses.

Results: No RSV hospitalised paediatric patients were registered in Buenos Aires during 2020; however, RSV reemerged in 2021 with a lower number of cases and a delayed outbreak, peaking in July-August. A total of 147 G gene sequences were analysed. RSV-B (N=85) predominated during 2018 and 2021 whereas in 2019 RSV-A were more prevalent (N=62). All RSV-A sequences were ON1-like strains, and all RSV-B were BA-like. Phylogenetic analyses showed that the same genetic lineages circulated before and after

48 2020, but RSVs from 2021 corresponded to new viral introductions rather than cryptic
49 circulation of the previous genetic clusters in Buenos Aires during 2020.

50 Conclusions: Following the reopening of borders, the reemergence of RSV in Argentina
51 brought new viral introductions from other countries. Therefore, it is important to continue a
52 deep global molecular surveillance to characterise RSV strains in post-pandemic circulation
53 with an impact in future vaccine implementation.

54 **Keywords:** Respiratory Syncytial Virus, COVID-19 pandemic, Molecular epidemiology,
55 genetic lineages

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58 Introduction:

59 Human respiratory syncytial virus (RSV) is one of the most common viral pathogens causing
60 acute lower respiratory tract infections (ALRTI) in paediatric patients (1). In Argentina, RSV
61 has contributed to approximately 80% of paediatric viral ALRTI yearly (2).

62 RSV has been classified into two antigenic subgroups A and B. Within each subgroup there
63 are numerous genotypes, which have historically been classified based on the glycoprotein
64 (G) gene sequence (3). Outbreaks are commonly produced by both subgroups, and
65 currently the most frequent genotypes worldwide are ON1 for RSV-A and BA for RSV-B,
66 characterised by duplications of a 72-nt and a 60-nt in the G gene, respectively (2,4,5).

67 In December 2019, the world experienced the beginning of the COVID-19 pandemic, caused
68 by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The global impact
69 at the public health level was catastrophic, with millions of hospitalisations and deaths.
70 Argentina's first COVID-19 case was detected on 3 March 2020 when the country had
71 already prepared for its arrival, including rapid detection of cases, patient's isolation, tracing
72 and quarantine of contacts (6,7). Nevertheless, COVID-19 cases increased, and
73 consequently mitigation measures were taken, including the closure of borders, educational
74 institutions, public places, stores, and strict social isolation as part of a comprehensive
75 lockdown. Up to 6 November 2021, a total of 5.3M cases and 116K deaths were reported in
76 Argentina due to COVID-19 (8).

77 Community mitigation measures to fight against COVID-19 also affected the circulation of
78 other respiratory viruses, such as RSV. The decrease or even the absence of cases of RSV
79 during 2020, and the appearance of delayed annual RSV outbreaks in both 2020 and 2021
80 have been reported in other countries (9,10,11). In this context, the question that arises in
81 Argentina is: what happened with RSV during 2020 and what genetic characteristics do the
82 reemerged RSV strains have after the mitigation measures were taken due to the COVID-19
83 pandemic?

84

85 Objective:

86 Perform a molecular epidemiological analysis of the RSV circulating strains before and
87 during the COVID-19 pandemic in Buenos Aires, Argentina.

88

89 **Methodology:**

90 Samples, G gene sequencing and phylogenetic analysis.

91 Viral RNA was extracted from nasopharyngeal RSV-positive samples collected from
92 hospitalised paediatric patients (range 0-12 years-old) due to ALRTI at the Ricardo Gutierrez
93 Children's Hospital in Buenos Aires, from 2018 to 2021. Samples were randomly selected
94 throughout each annual outbreak. The full G gene was sequenced as previously described
95 (4).

96 Sequence alignments were performed with MUSCLE (12). Phylogenetic analyses were
97 performed by Maximum Likelihood using IQ-TREE v.2.1 with ultrafast bootstrap and SH-
98 aLRT (1000 replicates each) to assess the phylogenetic clades statistical support (13).

99 Genotyping analyses were performed according to Goya et al 2019, using the ReSVidex
100 online tool and checked by phylogenetic analyses (3,14).

101 When needed, reference sequences with a collection date from 2017 onwards from other
102 countries were downloaded from the GISAID and GenBank databases considering the best
103 ten BLAST hits sequences (Supplementary Table 1).

104

105 **Results:**

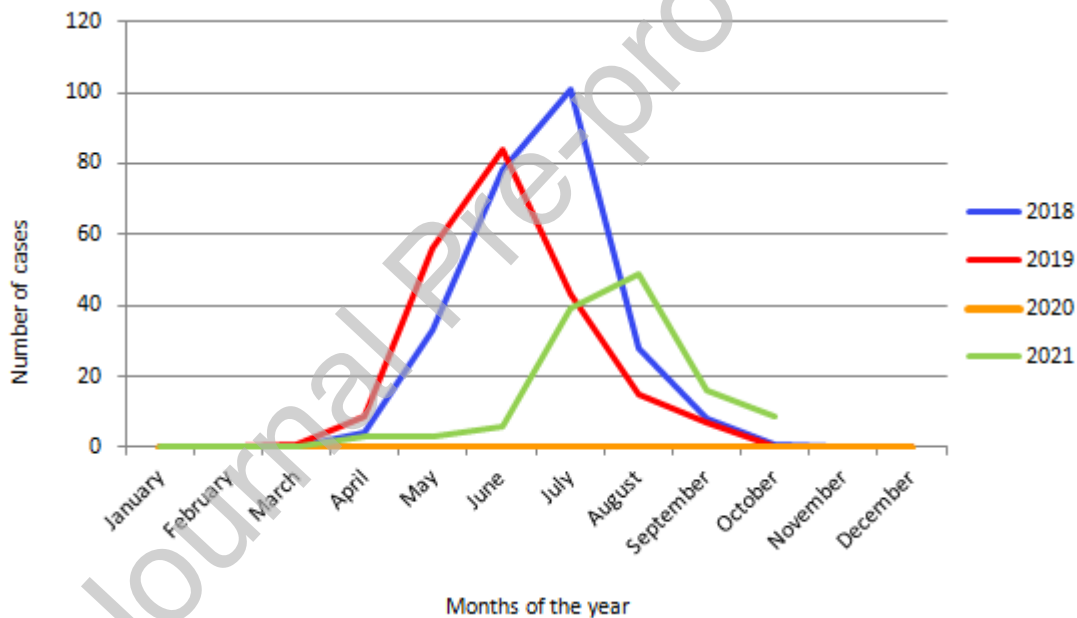
106 1. RSV epidemiology.

107 In 2018 and 2019, the outbreaks of RSV in hospitalised paediatric patients with ALRTI
108 showed the same seasonal pattern as the one that was seen during the last decade (2). A
109 total of 253 RSV-positive cases were detected in 2018 with peaks in July and 215 in 2019
110 with peaks in June (Figure 1). Surprisingly, in 2020 no hospitalisations due to RSV were
111 registered, coinciding with the implementation of community mitigation measures due to the
112 COVID-19 pandemic (Table 1). In this context, an additional study was conducted in 82
113 paediatric outpatients with respiratory symptoms with a negative diagnosis for SARS-CoV-2

114 between July and November 2020 to detect possible cryptic RSV circulation in the
115 mentioned population. No RSV-positive cases were found in the studied group.

116 In 2021, a total of 116 RSV-positive cases in hospitalised paediatric patients were
117 registered. Nevertheless, the total number of hospitalisations during the reemergence of
118 RSV in 2021 was lower in comparison to the 2018 and 2019 outbreaks. In addition, the 2021
119 outbreak was delayed, starting in April, peaking in July-August, and ending in November
120 (Figure 1). The patients' age distribution analysis showed that each year the highest
121 percentage of hospitalised patients corresponded to the 0-12 months range (>60% of cases)
122 (Table 1).

123



124

125 Figure 1. Seasonality of the RSV outbreaks between 2018 and 2021 at the Ricardo
126 Gutiérrez Children's Hospital, Buenos Aires, Argentina. Only hospitalised cases were
127 considered.

Year	Total No. of hospitalised patients due to RSV	Age group (months)	No. of cases by age (%)
2018	253	0 to 6	103 (40.70%)
		6 to 12	86 (34%)
		12 to 24	36 (14.23%)

		>24	28 (11.07%)
2019	215	0 to 6	76 (35.35%)
		6 to 12	58 (26.98%)
		12 to 24	42 (19.53%)
		>24	39 (18.14%)
2021	116	0 to 6	41 (34.34%)
		6 to 12	37 (31.90%)
		12 to 24	13 (11.21%)
		>24	25 (21.55%)

128 Table 1. RSV Epidemiology. The total number of hospitalised paediatric patients per year
129 and age distribution in months are detailed.

130

131 2. RSV genotyping.

132 A total of 57 G-gene sequences were obtained from 2018, 44 from 2019 and 46 from 2021.

133 RSV-A predominated in 2019 whereas RSV-B were more frequent in 2018 and 2021. All the
134 RSV-A sequences were ON1-like, comprising two genetic lineages GA2.3.5 and GA2.3.6b.

135 In addition, the RSV-B sequences were BA-like associated to the genetic lineage GB5.0.5a
136 (Table 2).

Year	No. of hospitalised cases due to RSV	Subgroup	Sequences per subgroup per year (%)	Genetic lineage	No. of G-gene sequences	Cases sequenced per total RSV-positive cases (%)
2018	253	A	21.05	GA2.3.5	10	22.53
				GA2.3.6b	2	
		B	78.95	GB5.0.5a	45	
2019	215	A	84.10	GA2.3.5	21	20.46
				GA2.3.6b	16	
		B	15.90	GB5.0.5a	7	
2021	116	A	28.26	GA2.3.5	5	39.65
				GA2.3.6b	8	
		B	71.74	GB5.0.5a	33	

137

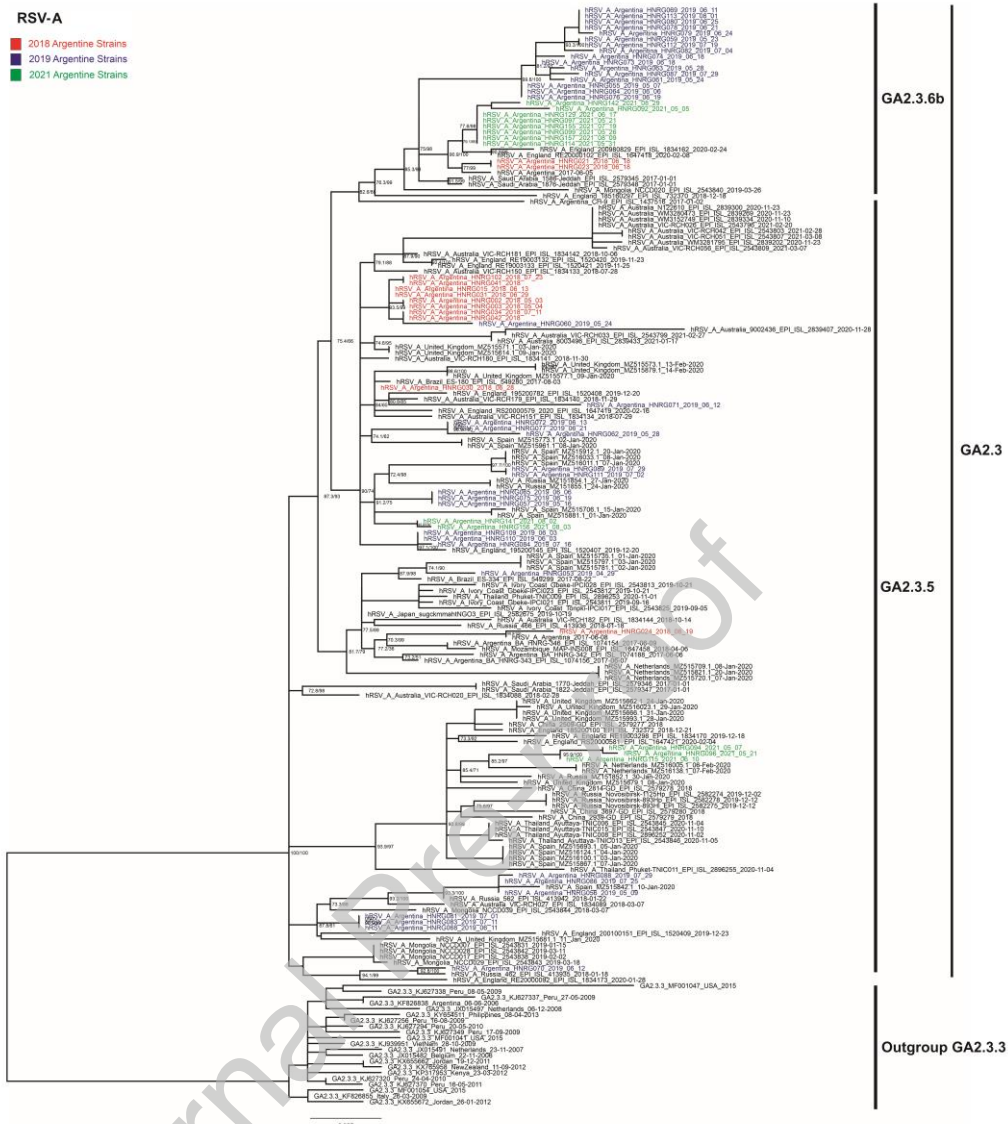
138 Table 2. G-gene sequences analyses. The subgroup and genetic lineages were obtained
139 from the genotyping analyses. The classification of genetic lineages was established
140 according to Goya et al (3).

141

142 Phylogenetic analyses to assess the relationships among Argentine 2021-sequences with
143 locally and globally sequences from 2017-2021 reported in public databases were
144 performed. The results suggest that the Argentine sequences detected in 2021
145 corresponded to multiple new viral introductions to our country. The GA2.3.5 2021-
146 sequences shown in Figure 2 are distributed in two different phylogenetic clades, one shares
147 a most recent common ancestor (MRCA) with sequences that circulated in Spain in 2020
148 and in Argentina in 2019. The other one shares an MRCA with sequences from the
149 Netherlands in 2020. The GA2.3.6b 2021-sequences are associated in a well-supported
150 monophyletic clade and share an MRCA with sequences from England 2020.

151 Regarding RSV-B, GB5.0.5a 2021-sequences shown in Figure 3 are associated with a well-
152 supported monophyletic clade with two sequences from Chile 2021, unrelated to previous
153 Argentine sequences.

154



155
 156 Figure 2. Maximum Likelihood phylogenetic analysis of RSV-A. Argentine sequences from
 157 2018 to 2021 were analysed with sequences from the same period downloaded from
 158 GISAID and GenBank databases. Argentine sequences are highlighted in colours according
 159 to the year. The genotypes are indicated on the right. The model established by IQTREE
 160 was GTR + F + G4. Nodes supports: SH-aLRT support (%)/ultrafast bootstrap support (%)
 161 (1000 replicates each). Only supports over 70/70 are shown.



162

163 Figure 3. Maximum Likelihood phylogenetic analysis of RSV-B. Argentine sequences from
 164 2018 to 2021 were analysed with sequences from the same period downloaded from
 165 GISAID and GenBank databases. Argentine sequences are highlighted in colours according
 166 to the year. The genotypes are indicated on the right. The model established by IQTREE

167 was TIM3 + F + G4. Nodes supports: SH-aLRT support (%)/ultrafast bootstrap support (%)
168 (1000 replicates each). Only supports over 70/70 are shown.

169

170 **Discussion:**

171 Epidemic outbreaks of respiratory viruses depend on regional factors (demography, age
172 distribution, susceptibility, etc.), but also on global factors (e.g., transmission due to long-
173 distance air travel) (15). Isolation, social distancing measures and border closures or limited
174 international travel in response to the COVID-19 pandemic since March 2020 in Argentina
175 seriously affected the circulation of RSV. Usual RSV outbreaks in Argentina were disrupted
176 in 2020 when no RSV cases were detected. In fact, cases of bronchiolitis in children under
177 two years of age decreased by 84.5% from 2019 to 2020 in Argentina, evidencing the effect
178 of lockdown on respiratory infections (7).

179 Furthermore, 2021 underwent a delayed RSV outbreak with a lower total number of cases.
180 Since January 2021, the lockdown in Argentina began to decrease, borders reopened for the
181 Argentines who travelled abroad, educational institutions resumed later that year under the
182 'bubbles' approach (children attended in consistent non-overlapping groups) and there was a
183 concomitant social relaxation. Nevertheless, the children were the last to leave the lockdown
184 in Argentina. In this context and considering the importance of household transmission (16),
185 it is plausible that RSV has reemerged in Argentina with such a delayed outbreak.

186 After a year of strict confinement, those 1-year-olds not exposed to RSV could have been
187 more susceptible to severe infections the following year. However, this possible increase in
188 hospitalised cases was not registered (Table 1) with the majority of those under 12 months.
189 This reinforces the idea that the implementation of any prophylaxis (vaccine, monoclonal
190 antibodies) should target children under 1 year.

191 The performed genotyping analyses suggest that the same three genetic lineages co-
192 circulated during the three-year period, but the evolutionary evidence together with the
193 epidemiological data support the idea that the lineages that spread in 2021 might be new

194 introductions to our country. Moreover, to the extent that RSV sequence-databases grow
195 and there is more global representativeness in geographic and temporal terms, it will be
196 possible to determine from which countries these RSV strains could have been introduced.
197 Finally, it would be important to continue a deep global molecular surveillance to
198 characterise RSV strains and other respiratory viruses in the pre- and post-pandemic era to
199 analyse the effect of the mitigation measures with an impact in the implementation of future
200 vaccines.

201

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212 (Supplementary Table 1).

213

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219 Competing interests

220 The authors declare no competing interests.

221 Ethical statement

222 The project was reviewed and approved by the Medical Ethics and Research Committees of
223 the Ricardo Gutierrez Children's Hospital, Buenos Aires, Argentina (IRB No. 17.21). Parental
224 informed consent was not obtained because patient information was anonymised before
225 analysis.

226 Author contributions

227 Conceptualisation: Goya Stephanie, Mistchenko Alicia S., Viegas Mariana

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229 Formal analysis: Acuña Dolores, Goya Stephanie, Nabaes Jodar Mercedes S., Mischenko
230 Alicia S., Mariana Viegas

231 Funding acquisition; Mistchenko Alicia S., Viegas Mariana

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233 Mischenko Alicia S., Mariana Viegas

234 Writing - original draft: Acuña Dolores, Goya Stephanie

235 Writing - review & editing: Acuña Dolores, Goya Stephanie, Nabaes Jodar Mercedes S.,
236 Mistchenko Alicia S., Viegas Mariana

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