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DETECTION OF SARS-COV-2 IN MILK FROM COVID-19 POSITIVE MOTHERS AND FOLLOW-UP OF THEIR INFANTS

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Abstract

Background In the current SARS-Coronavirus-2 (SARS-CoV-2) pandemic little is known about SARS-CoV-2 in human milk. It is important to discover if breast milk is a vehicle of infection.

Objective Our aim was to look for the presence of SARS-CoV-2 RNA in the milk of a group of SARS-CoV-2 positive mothers from North-West Italy.

Methods This is a prospective collaborative observational study where samples of human milk from 14 breastfeeding mothers positive for SARS-CoV-2 were collected. A search of viral RNA in breast milk samples was performed by RT-PCR (Real-Time reverse-transcriptase-Polymerase-Chain-Reaction) methodology tested for human milk. All the newborns underwent a clinical follow up during the first month of life or until the finding of two sequential negative swabs.

Results In thirteen cases the search for SARS-CoV-2 RNA in milk samples resulted negative and in one case it was positive. Thirteen of the fourteen newborns were exclusively breastfed and closely monitored in the first month of life. Clinical outcome was uneventful. Four newborns tested positive for SARS-CoV-2 and were all detected in the first 48 hours of life, after the onset of maternal symptoms. Also the clinical course of these 4 infants, including the one who received mother's milk positive for SARS-CoV-2, was uneventful, and all of them became SARS-CoV-2 negative within 6 weeks of life.

Conclusion Our study supports the view that SARS-CoV-2 positive mothers do not expose their newborns to an additional risk of infection by breastfeeding.

1 Introduction:

In the current SARS-Coronavirus-2 (SARS-CoV-2) pandemic it is important to identify every possible route of transmission to prevent the spread of the disease. To date, all reports suggest that lack of evidence for vertical transmission of SARS-CoV-2 is unlikely [1-6]. However, the possible transmission of the virus via breastfeeding is still under debate. Providing reliable data on this issue may have significant practical implications for the management of the SARS-CoV-2 positive

44 mother-infant dyad and breastfeeding support. Based on current knowledge from clinical studies, it
45 appears unlikely that the virus is transmitted through breast milk. [1-2, 4, 7-11]. However the data on
46 the detection of SARS-CoV-2 in breast milk available in the literature, have a low sample size
47 [13-15] and in most cases methodological limitations [16].

48 Here, we report the results of a prospective collaborative observational study whose primary aim
49 was to look for the presence of SARS-CoV-2 RNA in the milk of a group of SARS-CoV-2 positive
50 mothers from North-West Italy, an area with a very high incidence for Coronavirus disease 19 (COVID-
51 19) at the time of sample collection. The study was specifically designed to analyze human milk, with
52 the adoption of a real-time PCR method tested to effectively detect SARS-COV-2 nucleic acid in
53 human milk, a fluid that is difficult to analyze due to its complex composition. The secondary aim of
54 the study was to evaluate clinical outcome during the first month of life in infants exclusively
55 breastfed by their positive mothers.

56 **2 Methods:**

57 **2.1 Study design**

58 This is a prospective collaborative observational study coordinated by the Neonatal Care Unit and
59 the Laboratory of Molecular Virology of the University of Turin, Italy.

60 The study was approved by the local Ethical Committee (protocol number 0039684), and informed
61 consent to participate in the study was obtained from each mother.

62 The study centers were located in North-West Italy. From April 1st 2020 to July 31st 2020, these
63 centers collected samples of milk from 14 breastfeeding mothers diagnosed with Covid-19.

64 All the mothers were diagnosed with COVID-19 by real-time reverse-transcriptase–polymerase-
65 chain-reaction (RT-PCR) assays of nasal and pharyngeal swabs, in accordance with WHO guidance
66 [17]. We collected clinical records, breastfeeding history, and laboratory findings related to SARS-
67 CoV-2 of 14 mothers and their infants during hospital stay and in the six weeks after discharge.

68 Newborn follow up was performed during the first month of life or until the finding of two sequential
69 negative swabs.

70 **2.2 Sample Collection**

71 Milk collection was performed with a dedicated breast-pump in ten of the mothers and in four with
72 manual expression. All mothers were advised to follow strict hygienic rules according to
73 international recommendations [18,19]. For milk expression mothers wore a disposable surgical face
74 mask, washed their hands with water and soap for at least 20 seconds, cleaned their breast with warm
75 water and soap and then dried it with a clean paper towel. Immediately after breast milk was collected
76 and transferred in sterile single-use plastic containers and stored in a freezer at -20°C. Eight mothers
77 expressed their milk in the hospital (cases 4, 6, 8, 9,11,12,13,14), five mothers expressed it at home
78 (cases 1, 2, 5, 7, 10), and one mother (case 3) expressed the first two samples in the hospital and the
79 following four samples at home.

80 **2.3 Detection Methods of SARS-CoV-2 in human milk by rt-PCR**

81 Analysis of the milk samples was carried out by the Laboratory of Clinical Microbiology at the
82 San Luigi Gonzaga Hospital, Orbassano, Italy. Positivity to SARS-CoV-2 in milk samples from Case
83 3 was confirmed by the Microbiology and Virology Unit at City of Health and Science of Turin,
84 Italy. Before analyzing milk samples, the analytical methods were tested for use in milk. To this aim,
85 the SARS-CoV-2 positive control plasmid provided by the manufacturer of the analytical kits, was
86 added to a milk sample collected in February 2019 from an Italian woman, almost one year before the
87 onset of the COVID-19 outbreak in Italy and therefore surely negative for SARS-CoV-2. In both
88 laboratories the positive control was properly detected by RT-PCR generating a standard
89 amplification curve and a threshold cycle similar to that obtained when the analytical methods were
90 used in routine diagnostic procedures. At the San Luigi Gonzaga Hospital RNA was extracted from
91 milk specimens using the BD MAX™ ExK™ TNA-3 and determined by rRT-PCR targeting the
92 SARS-CoV-2 S gene by using the VIASURE SARS-CoV-2 Real Time PCR Detection Kit (CertTest,

93 Zaragoza, Spain). Real-time qPCR reactions were performed on BD MAX RT-PCR apparatus
94 (Becton Dickinson). At the Microbiology and Virology Unit of City of Health and Science of
95 Turin, extraction was carried out with the QIA Symphony DSP Virus/Pathogen Midi Kit (Qiagen)
96 and amplification with the Seegene Allplex™ 2019-nCoV Assay (target genes: E, N, RdRp).

97 **3 Results:**

98 The main clinical characteristics of our population and the results of SARS-CoV-2 search in milk
99 samples are summarized in Table 1.

100 **Eleven out of fourteen mothers were symptomatic at the moment of the test for SARS-CoV-2,**
101 **performed in eight cases just before delivery and in three cases after delivery and presented at least**
102 **one of following signs: fever, myalgia, rhinitis, cough, dyspnea, sore throat, conjunctivitis, diarrhea,**
103 **chest pain, anosmia, ageusia. In three asymptomatic mothers (cases 9, 11, 14), the nasopharyngeal**
104 **swab for SARS-CoV-2 was performed as a routine test at hospital admission (required in two centers).**

105 In thirteen cases the assays were negative for SARS-CoV-2 RNA and in one case it was positive
106 (Case 3).

107 **3.1 Neonatal Outcome**

108 In our population, four newborns tested positive for SARS-CoV-2 (cases 1, 3, 7, 9 in Table 1), and
109 were all detected in the first 48 hours of life, after the onset of maternal symptoms and the positivity
110 of her swab test. The clinical course of these four infants was carefully monitored and was uneventful
111 and all of them became negative by six weeks of life. All of them received exclusive breastfeeding
112 during the observational period.

113 The other ten infants tested negative for SARS-CoV-2. Nine of them were breastfed, and one received
114 artificial formula from fourth day of life (the breast milk sample was collected previously). None of
115 them turned out to be positive and/or symptomatic during the first month of life.

116 **3.2 Case 3 report**

117 Case 3 resulted positive for SARS-CoV-2 at the first two milk collections, based on two different

118 samples collected with manual expression with one-hour interval at day five after delivery. Further
119 samples were collected at day 18, 28, 31, and 36 after delivery. Of these samples, only the one
120 collected at 28 days resulted weakly positive for SARS-CoV-2 (Figure 1). The mother and her infant
121 were kept together immediately after delivery and practiced rooming-in in the first two days of life
122 without any specific precautions, until the first clinical signs of COVID-19 appeared in the mother
123 (fever, cough, ageusia, anosmia) **one day after delivery**. A maternal nasopharyngeal swab was
124 performed and resulted positive for SARS-CoV-2. The day after a nasopharyngeal swab was
125 performed in the newborn, with a positive result for SARS-CoV-2 as well. Maternal fever and
126 coughing disappeared in one week, with residual anosmia and ageusia still persisting after one month.
127 The newborn was exclusively breastfed for the first month of life, closely monitored during this period
128 and the clinical outcome was uneventful. The newborn was found negative for SARS-CoV-2 at two
129 consecutive nasopharyngeal swabs at 16 and 26. One more swab was performed and resulted negative
130 at 38 days of life (because the mother was again positive at day 26).

131 **4 Discussion**

132 In this case series we found that milk samples of 13 out of 14 SARS-CoV-2 positive mothers were
133 negative for the virus. In one case the milk samples tested positive. Thirteen out of the fourteen
134 newborns were exclusively breastfed during the observation period, and clinical outcome in all of
135 them was carefully monitored and was uneventful. Thus, we have no evidence to consider
136 breastfeeding in SARS-CoV-2 positive mothers as unsafe for their newborns. A recent cohort study
137 [11] concerning breastfed infants of SARS-CoV-2 positive mothers in the first month of life, revealed
138 that they remained SARS-CoV-2 negative on nasopharyngeal swab. However, the presence of the
139 virus in human milk was not evaluated. **Recently the presence of SARS-CoV-2 in breastmilk was
140 evaluated in 18 infected women from delivery to 19 months, with only one sample found positive
141 [12]. These data are in accordance with our results.**

142 We suppose that in the four newborns who tested positive for SARS-CoV-2, the virus passed from the
143 mother to the infant through intrapartum or airborne exposure. The four infants remained

144 asymptomatic during the observation period including case 3, in whom, irrespective of the reason for
145 milk positivity, careful monitoring did not reveal any clinical symptoms. Of particular interest is that
146 this infant at 16 and 26 days tested negative although the mother was positive again at 26 days and
147 the milk was again positive at 27 days (Figure 1). This supports the view that breast milk, even when
148 positive, is not a route of contagion.

149 A recently published letter [13] describes a case where milk samples of a mother with COVID-19 were
150 positive for SARS- CoV-2, and the newborn after discharge was re-hospitalized with icterus and
151 respiratory symptoms. As this newborn tested positive for both Respiratory Syncytial Virus (RSV)
152 and SARS-CoV-2, it is possible that the symptoms for re-hospitalization were due to RSV and not
153 to SARS-CoV-2, being the former a common cause of bronchiolitis. However, even if the newborn
154 symptoms were Covid-19 related, this does not mean that the oral route of transmission poses an
155 additional risk respect to the respiratory route. In our study, we hypothesize three possible
156 explanations for the positivity of SARS-CoV-2 in breast milk of case 3.

157 The first is that the virus could have shed in the milk of the infected mother during lactation. The
158 argument supporting this hypothesis is that the virus was consistently detected in samples from
159 three independent collections (Figure 1).

160 Furthermore, the pattern of positivity in milk samples was consistent with that of the mother's swabs.
161 On the basis of the literature available so far, including this report, the presence of SARS-CoV-2 in
162 human milk is a rare event. Indeed, up to now there is no evidence of shedding, apart from a case
163 of SARS-CoV-2 in human milk that was detected, but not confirmed when retested two days after
164 [3] and three cases in which it was consistently found positive [13-15]. Data on other human
165 coronaviruses do not shed light on this issue. A single case report failed to detect SARS-CoV in the
166 milk of a SARS positive mother [20], while no data are available on MERS-CoV. A study on the
167 human coronavirus NL63, causing common cold, provided limited evidence of vertical transmission
168 and did not investigate the presence of viral RNA in milk samples [21].

169 The second hypothesis is that milk samples were contaminated in the laboratory during the assay
170 procedures. This possibility is highly unlikely since the virus was found in three samples from the
171 same mother analyzed independently.

172 The third hypothesis is that milk was contaminated due an inadequate compliance with hygiene
173 measures in expressing the milk.

174 Finally, detection of SARS-CoV-2 RNA does not necessarily imply the presence of an infectious and
175 active virus that can be transmitted via breastfeeding and to infect the infant [12].

176 In conclusion, our study supports the view that SARS-CoV-2 positive mothers do not expose their
177 newborns to an additional risk of infection by breastfeeding. This is an important message that should
178 be given to mothers and healthcare providers in this moment of uncertainty. This information is useful
179 also for all mothers living in areas with high prevalence of COVID-19 infection. In fact, our results
180 support the view that they should breastfeed, irrespective of swab test results, considering the
181 immunological and anti-infective properties of mother's milk. Clearly, the recommended hygiene
182 measures for the control of airborne exposure, for direct breastfeeding, as well as for milk expression
183 when a mother and her infant need to be separated, must be carefully followed.

184 Findings from our study are limited by small samples size and self-collection of milk samples so
185 future studies are needed to confirm our results.

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196 Table 1:

197 Characteristics of the study population and results of SARS-CoV-2 search in milk samples

	Maternal age (years)	Gestational age at delivery (weeks)	Method of delivery	Interval between delivery and milk collection (days)	Interval between maternal positive swab and milk collection (days) [°]	Maternal symptoms at milk collection	Breast milk test	First maternal positive swab (days before (-) or after (+) delivery)	First maternal negative swab (days after delivery)
Case 1	25	40	Vaginal	27	0	No	Negative	+3	41
Case 2 [□]	28	37	Vaginal	14	0	No	Negative	0	25
Case 3 [□]	26	38	CS	5	4	Yes	Positive	+1	34
Case 4 [*]	30	36	Vaginal	4	5	Yes	Negative	-1	23
Case 5	34	38	CS	21	6	No	Negative	0	24
Case 6	35	30	CS	3	0	Yes	Negative	-8	33
Case 7	30	39	CS	27	0	No	Negative	+1	45
Case 8 ^{**}	38	32	CS	2	12	Yes	Negative	-9	3
Case 9 [□]	24	34	CS	4	5	No	Negative	0	9
Case 10	29	41	Vaginal	13	10	No	Negative	-1	16
Case 11	37	38	CS	4	5	No	Negative	-11	16
Case 12	31	40	Vaginal	3	3	Yes	Negative	0	23
Case 13	37	37	CS	1	0	Yes	Negative	-18	20
Case 14	32	37	CS	3	0	No	Negative	-1	14

198 *Mother decided to stop breastfeeding at discharge (day five).

199 ** Mother with interstitial pneumonia. Ritonavir/Lopinavir therapy discontinued three days before
200 the milk collection.

201 [□]Manual expression

202 [°] We considered the time interval as 0 if the milk collection took place between the recording of
203 two positive maternal swabs

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