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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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The impact of COVID-19 lockdown on infants' coronavirus exposure and routine healthcare access in Ireland: The CORAL birth cohort study at 6 months.

The public health strategy during the SARS-CoV-2 (COVID-19) pandemic of 2020–2021 led to profound changes in social behaviour, affecting not only familial but also wider social interactions. These changes may have altered viral and bacterial interchange.

A significantly decreased diversity of gut and skin microbiota has been demonstrated in allergic individuals compared with non-allergic subjects.¹ The CORAL study is a longitudinal study of the impact of the coronavirus pandemic on allergic and autoimmune dysregulation of infants born from March to May 2020, during Ireland's first

lockdown (Ireland formally entered national lockdown on 27 March 2020, although severe restrictions had been gradually escalated for the 2 weeks prior to that date. The first release of lockdown was on 8 June 2020). CORAL will follow the cohort initially until the children are 2 years old, with stool sampling at 6, 12 and 24 months for microbiome diversity analysis and allergy testing and blood RNA and epigenomic testing at 12 and 24 months.

Now, in the immunization phase of the pandemic response, attention is shifting from immediate population protection to the

consequences of both the pandemic and the societal disruption created by lockdown. There are particular concerns that important medical care (such as infant immunizations) may have been delayed, or even refused, due to patient or family concerns about infection risk when attending healthcare facilities also managing COVID-19.²⁻⁴

We report here the impact of COVID-19 lockdown on feeding practices, healthcare access, completion of immunization and COVID-19 infection rates in Irish babies to 6 months of age enrolled in the CORAL study.

Ethical approval was obtained from the National Research Ethics Committee (20-NREC-COV-067) and the participating hospitals' ethics committees. Informed written parental consent was obtained.

There were 3773 infants born in the 2 participating major maternity hospitals in Dublin in March, April and May 2020. Invitations were sent to the families of 3065 term babies who were eligible for inclusion. Exclusion criteria were pre-birth PCR-proven SARS-CoV-2 infection in a parent or co-dwelling person, intravenous antibiotics in the neonatal period, multiple births or major congenital anomaly.

At 6 months (September–November 2020), families were sent a questionnaire and a stool sample collected for microbiome analysis (results will be reported elsewhere). Families were also offered point-of-care SARS-CoV-2 antibody testing (VivaDiag™ SARS-CoV-2 IgM/IgG Rapid Test) for the infants. However, an escalation of national travel restrictions in response to the second surge of COVID-19 in September and October 2020 restricted access to this testing for some infants.

A total of 365 infants were enrolled. One hundred and ninety-five babies (53.4%) were male, 94.5% were of "White Irish" or "Any Other White" background, and 94% of mothers were educated to third level or higher. The average birthweight was 3.5 kg. Most babies were either firstborn (45%) or secondborn (37%). In infants with siblings ($n = 201$), atopic dermatitis was the most common allergic condition reported in siblings (15.4%), followed by asthma (12.4%) and food allergy (11.4%). Allergic rhinitis was the most commonly reported atopic condition in both mothers (36%) and fathers (30%).

A total of 360 6-month questionnaires were returned. Exclusive breastfeeding rate at 6 months was 38%; a further 15% were receiving both breastmilk and infant formula. 92% were having regular solid foods with a median weaning age of 5 months (range 3–6 months). The rates of introduction of allergenic foods such as egg and peanut are outlined in Table 1.

99% of infants had attended their general practitioner for scheduled immunizations at 2, 4 and 6 months of age,⁵ and 7.5% of babies had been admitted to hospital in the first 6 months. Antibiotics had been prescribed for 25 infants (7%) and 5 of those infants had received more than one course. Possible allergic reactions to a food or environmental substance were reported by 47 children (13%), but on elaboration most described a flare of eczema without identifying a particular allergen.

By the age of 6 months, 30 (8.3%) had had community testing for SARS-CoV-2 PCR due to symptoms or known close contact, and 2

TABLE 1 Introduction of allergenic foods at 6 months

Allergenic foods at 6 months	Introduced food (%) Total N = 360
Egg	95 (26%)
Peanut	49 (13.6%)
Tree nut	12 (3.3%)
Wheat	151 (42%)
Fish	59 (16.4%)
Dairy	166 (46%)
Dairy alternatives	17 (4.7%)

(0.5%) were PCR-positive. One of these 2 babies attended the 6-month review; the other was unable to attend due to national travel restrictions. A total of 268 (73%) of babies had point-of-care lateral flow immunoassay testing at 6 months as part of the study, of whom 3 (1.1%) returned positive results for IgM and IgG SARS-CoV-2 antibodies. One infant (as above) had known prior PCR-proven SARS-CoV-2 infection but there were two further cases with no known COVID-19 exposure. Of these, 1 was PCR-positive (indicating very recent or current infection) and the other was PCR-negative (indicating remote infection). Overall, 4 babies (1%) were shown to have immunological evidence of exposure to SARS-CoV-2 infection before the age of 6 months.

It is reassuring that this population of infants born during lockdown had been presented as advised for routine health care such as immunizations, actually at even higher rates than national norms. Weaning rates were in line with national trends in Ireland, and despite the low rates of egg and peanut introduction, these are in line with national data, so weaning to allergenic foods did not seem to have been impacted by heightened fear of adverse outcomes or risk of needing to emergency care during the pandemic. In contrast, the overall rate of attendance at hospital for any reason (7.5%) or any use of antibiotics were lower than expected, supporting our previous data about significant changes in circulation of virus-related illnesses in the early stages of the 2020 lockdown.⁶

This self-selected cohort of infants is largely representative of the overall Irish population.^{7,8} The proportion of firstborns (45%) is higher than the national average (38.8%) but these larger maternity hospitals (more than 8000 deliveries each per annum) have high rates of primigravida patients.⁸ A notable difference is the level of maternal education. In the 2016 Irish census, 43.2% of women were educated to third level compared with 94% of mothers in this study.⁹ This, along with mothers working from home during lockdown, is likely contributing to the atypically high percentage of Irish infants still breastfed at 6 months; in Ireland, this figure is usually approximately 15%.¹⁰ The rate of reported food allergy in siblings of this cohort (sourced from unconfirmed parental reports rather than from healthcare record searches) is higher than the general population, which may have contributed to parental desire to enrol in this study.¹¹

The rare (1%) positive SARS-CoV-2 antibody tests at 6 months suggesting recent or current infection imply that in Ireland, at least, newborn babies, traditionally considered to be particularly vulnerable to viral infection, appear to have been largely protected from SARS-CoV-2 exposure during the first COVID-19 lockdown in 2020.

The next stage of the CORAL study commenced in February 2021. It involves a review of the participants at 12 months with skin prick testing for common food and aeroallergens, a further questionnaire, stool sample and SARS-CoV-2 antibody test and peripheral blood sample for RNA sequencing to determine host immune responses to the COVID-19 lockdown.

CONFLICT OF INTEREST

JO'BH is a board member of Clemens Von Pirquet Foundation. No other conflicts declared.

AUTHOR CONTRIBUTIONS

Marguerite Lawler: Data curation (equal); Formal analysis (equal); Investigation (equal); Writing-original draft (lead); Writing-review & editing (supporting). **Ruth Franklin:** Data curation (equal); Investigation (supporting); Methodology (equal); Project administration (equal); Resources (equal); Writing-review & editing (supporting). **Meredith Kinoshita:** Conceptualization (supporting); Project administration (supporting); Writing-review & editing (supporting). **Aideen Byrne:** Conceptualization (supporting); Writing-review & editing (supporting). **John Fitzsimons:** Conceptualization (supporting); Writing-review & editing (supporting). **Martin White:** Conceptualization (supporting); Methodology (supporting); Project administration (supporting); Resources (equal); Supervision (equal); Writing-review & editing (supporting). **Naomi McCallion:** Conceptualization (supporting); Data curation (supporting); Funding acquisition (supporting); Investigation (supporting); Methodology (supporting); Project administration (supporting); Resources (supporting); Supervision (supporting); Writing-review & editing (supporting). **Liam O Mahony:** Conceptualization (equal); Data curation (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (lead); Methodology (equal); Project administration (equal); Resources (equal); Writing-review & editing (supporting). **Jonathan Hourihane:** Conceptualization (lead); Data curation (lead); Formal analysis (lead); Funding acquisition (lead); Investigation (lead); Methodology (lead); Project administration (lead); Resources (lead); Supervision (lead); Validation (lead); Writing-original draft (equal); Writing-review & editing (lead).

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Bacterial microbiota composition of fresh unpasteurized cow's milk and home-made and commercially available fermented milk products

To the Editor,

The anti-allergic properties of raw and fermented cow's milk are being explored globally.^{1,2}

Traditionally fermented milk (isiXhosa—"amasí") is consumed regularly by rural South African communities. To produce *amasí*, unpasteurized milk is left for three to five days to naturally ferment at room temperature. Commercially fermented *amasí* (CFA) is produced from pasteurized milk reseeded by microorganisms (commercially available starter cultures) and allowed to ferment. In South Africa, the process of commercial milk pasteurization is standardized and comprised heating to 72°C for at least 15 s and not longer than 25 s followed by rapid cooling.

The South African Food Sensitisation and Food Allergy (SAFFA) study demonstrated that children living in an urban environment had significantly higher rates of allergic diseases compared to their rural counterparts, and the consumption of fermented milk was associated with lower rates of allergic rhinitis, atopic dermatitis, and self-reported asthma.³ Lactic acid-producing bacteria produce important by-products and end-products during milk fermentation which may contribute to their anti-inflammatory and anti-allergic properties.⁴ In this study, we characterize and compare the bacterial microbiota in raw cow's milk (collected from urban and rural farms) and home-made and commercially fermented milk products by high-throughput 16S rRNA gene amplicon sequencing. This study received ethical approval from the University of Cape Town (animal ethics: 018_033).

Rural fresh cow's milk samples were collected from three farms in rural South Africa. Urban samples were collected from a farm in Cape Town. Before milking commenced, the cows and the udders were declared in a "heathy state" by each farmer. The udders were not cleaned or washed prior to milking, as these procedures were not included in the normal milking routine on the farms. Three samples

from urban farms and two samples from rural farms were frozen within 1 hour of collection and transported frozen to the laboratory. A further sample of milk obtained from a separate farm in the rural area was sealed and left for five days at ambient temperatures to allow it to ferment naturally into *amasí*. Three different brands of commercially fermented *amasí* were obtained. All samples were analyzed by the Centre for Proteomic and Genomic Research (CPRG), Cape Town, South Africa.

DNA was extracted from the milk samples using the ZymoBIOMICS® DNA Miniprep Kit (Zymo Research). The V3-V4 variable region of the 16S rRNA gene was amplified from 2.5 ng to 25 ng of purified DNA by 25 cycles of PCR and barcoded for multiplexing using the Nextera® XT Index kit (Illumina) and KAPA HiFi DNA Polymerase (Roche®). The nine milk product samples, a positive control (ZymoBIOMICS® Microbial Community DNA standard), and a negative control (DNA suspension buffer) were included in the library preparation. The size of the libraries was verified using an Agilent® 2100 Bioanalyzer (Agilent). Library concentration was evaluated using the KAPA Illumina Library Quantification Kit (Roche). The libraries were sequenced on an Illumina MiSeq sequencer at the CPRG using MiSeq Reagent Kit v2 (Illumina®) to produce paired-end 250 base pair reads.

Illumina MiSeq read quality assessment and taxonomic profiling were performed on a high-performance compute cluster using a custom Nextflow pipeline [<https://github.com/h3abionet/TADA>], implementing FastQC⁵ and MultiQC⁶ for quality control, dada2⁷ for ASV prediction, and the RefSeq-RDP 16S database (v3 May 2018) for taxonomic annotation.⁸ All downstream analyses were performed in R, with custom functions [<https://gist.github.com/kviljoen/97d36c689c5c9b9c39939c7a100720b9>]. Taxa (merged at the lowest available taxonomic level, tax_glom.kv function) were deemed significantly different (in terms of abundance and/or absence/presence)