

Algemene gegevens / General Information

Programma / Programme : COVID-19 Programma
 Subsidierronde / Subsidy round : Bottom-up ronde COVID-19 aandachtsgebied 2
 Projecttitel / Project title : COVID-MILK-2
 Projecttaal / Project language : Engels / English
 Geplande startdatum / Planned start date : 15-06-2020
 Geplande duur / Planned duration : 7 maanden / months
 Datum indienen / Date of application : 14-05-2020
 Projecttype / Project type : Toegepast onderzoek
 Vervolg eerder ZonMw-project / Continuation previously funded project : Nee / No
 ZonMw

Projectleden / Project members**Prof. dr. J.B. van Goudoever (Main applicant)**

Functie / Position: kinderarts hoofd Emma Kinderziekenhuis | *Opleiding / Education:*

Studierichting / Subject:

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Prof. dr. J.B. van Goudoever MD PhD (Projectleader and secretary)

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Amsterdam UMC -Emma Kinderziekenhuis
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Prof. dr. J.A. Romijn (Administrative responsibility)

Functie / Position: voorzitter Raad van Bestuur | *Opleiding / Education:*

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Prof. dr. C.J. de Groot (Co-Applicant)

Functie / Position: gynaecoloog-perinatoloog | *Opleiding / Education:*

Studierichting / Subject:

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Prof. dr. A.J.R. Heck (Co-Applicant)

Functie / Position: Prof Utrecht Institute for Pharmaceutical Sciences | *Opleiding / Education:*

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Faculteit Scheikunde
Biochemie van Lipiden
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Aanvraagformulier GGG_digitaal / Applicationform GGG digital

Dossier nummer / Dossier number: (10)(2g)

(10)(2e)

Functie / Position: voedingskundige, biochemicus | *Opleiding / Education:**Studierichting / Subject:*

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Wageningen Universiteit
 Agrotechnology & Food Sciences Group
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 Postbus 17
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(10)(2e) 0(2e)

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Functie / Position: immunoloog | *Opleiding / Education:**Studierichting / Subject:*

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Sanquin Bloedbank
 Onderzoek en Ontwikkeling
 Plesmanlaan 125
 1066 CX AMSTERDAM

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Functie / Position: immunoloog | *Opleiding / Education:**Studierichting / Subject:*

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Projectgegevens / Project information**Aandachtsgebieden / Focus**

- 2.1 Thema's aandachtsgebied 2
- Transmissie en epidemiologie
 - Zorg en preventie voor kwetsbare burgers
- 2.4 Subthema's transmissie en epidemiologie
- Verspreiding van virus in meest getroffen gebieden of bevolkingsgroepen

Samenvatting / Summary

To date, no cure for SARS-CoV-2 is available. Plasma from cured COVID 19 blood donors is hypothesized to help, but still lacks evidence and widespread application (1). Preventive actions have slowed down the pandemic, but vaccines are not yet available.

Breastmilk, but not formula, prevents infants from respiratory tract infections via antimicrobial and immunological activity by 30% (2). Milk immunoglobulins (and mainly IgA) are considered the major molecular factor (3).

Just recently, we and others detected anti-SARS-CoV-2 Spike IgA antibodies in most raw milk samples of cured COVID 19 milk donors, present in their more stable secretory sIgA form. Pasteurization (Heat or Pressure) of breastmilk yielded similar results. Neutralizing activity tests are currently performed as well as isolation and identification of specific SARS-CoV-2 milk sIgA by de novo sequencing by mass spectrometry.

sIgA facilitates pathogenic microbial clearance from the mucosa by prevention of microbial binding to host receptors of mucosal epithelial cell layers in mouth and throat. We hypothesize that human milk derived secretory IgA from lactating, cured COVID 19 women with a natural surplus of milk prevents SARS-CoV-2 infections in vulnerable populations.

We propose a three-phase approach:

Phase 1: Quantification of secretory SARS-CoV2 IgA in donor milk of proven COVID 19 mothers, collected via use of existing (inter)national networks of human milk banks.

Phase 2: Optimizing immunological quality and safety of the human milk product via already established partnerships

Phase 3: Initiate a randomized clinical trial in elderly individuals with the hypothesis that gargling/ingesting modified donor human milk, or purified IgA fractions thereof, from COVID 19 mothers will reduce the incidence of proven COVID disease in

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vulnerable populations by 30%. Assuming an incidence of 3% with a power of 80%, 3815 elderly subjects and 1526 cured COVID 19 milk donors are needed.

Trefwoorden / Keywords

"breastmilk", "antibodies", "elderly", "COVID", "IgA", "Immunoglobulins"

Samenwerking / Collaboration

Samenwerking tussen onderzoek en praktijk / Cooperation between research and practice:

Ja / Yes

Inhoud / Content

Disciplines / Disciplines

- Infecties, parasitologie, virologie / Infections, parasitology, virology
- Preventieve gezondheidszorg, GVO / Preventive health care, health education and promotion
- Longziekten / Pulmonology
- Kindergeneeskunde / Paediatrics
- Bioinformatica/biostatistiek, biomathematica, biomechanica / Bioinformatics/biostatistics, biomathematics, biomechanics

Financiële gegevens / Financial data

ZonMw budget

Kostenpost	Jaar / Year								Totaal / Total
	1	2	3	4	5	6	7	8	
Personeel									
Materieel									
Implementatie									
Apparatuur									
Overig									
Totaal / Total									

Co-financiering / Cofinancing

Naam co-financier / Name of cofinancier	Bedrag / Amount	Status

Bijzondere gegevens / Additional information

Vergunningen / Permits

	Verklaring nodig / Statement required?		Status verklaring / Statement status		
	Ja / Yes	Nee / No	Verkregen / Acquired	Aangevraagd / Applied	Nog niet aangevraagd / Not applied yet
METC	X				X
DEC		X			
WBO		X			

Onderschrijvingen / Assents

	Ja / Yes	Nee / No	N.v.t. / N.A.
Code biosecurity / Code Biosecurity			X
Code openheid dierproeven / Code Transparency of Animal Testing			X

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Andere vergunningen / Other permits

Er wordt gewerkt met Viroclinics, een BSL 3 lab voorzien van alle vergunningen

AANVRAAGFORMULIER PROJECTIDEE – BOTTOM-UP RONDE

COVID 19 programma

Deadline voor indiening: 14 mei 2020 (14:00 u)

**LEES ALSTUBLIJFT ALLE INSTRUCTIES IN BIJLAGE "TOELICHTING
INDIENING PROJECTIDEE" VAN DE OPROEPTEKST ZORGVULDIG!**

Wanneer u het formulier heeft ingevuld:

1. Zet het formulier om naar een PDF file en controleer de details
2. Upload het complete formulier als een bijlage bij uw indiening in Projectnet
(Let op: dit zijn twee verschillende links, gebruik maar 1 van de 2!)
ProjectNet: [Aandachtsgebied 1 \(voorspellende diagnostiek en behandeling\)](#)
ProjectNet: [Aandachtsgebied 2 \(zorg en preventie\)](#)

BASISGEGEVENS (voorpagina)

NAAM VAN DE HOOFDAANVRAGER:

(10)(2e)

ORGANISATIE:

Emma Kinderziekenhuis – Amsterdam UMC

PROJECTTITEL:

COVID Milk 2

DATASTEWARD:

Wie is de datasteward die de open science en FAIR data planning in uw project ondersteunt? Zie de webinars op de [ZonMw website](#) om de datastewards te informeren en ondersteunen.

Ik betrek een datasteward bij mijn project:

Naam: (10)(2e)

Instituut: amsterdamUMC - AMC

E-mail: [10\(2e\)@amsterdamumc.nl](mailto:10(2e)@amsterdamumc.nl)

Was aanwezig bij de webinar: Ja Nee

Ik heb nog geen datasteward.

ONDERZOEKSVORSTEL
max 3 pagina's A4
(inclusief literatuurreferenties)

(voorpagina met basisgegevens niet meegerekend -
font type Arial 10 pts)

1. PROBLEEMSTELLING EN DOELSTELLING(EN):

To date, **no cure for SARS-CoV-2 is available**. Plasma from cured COVID 19 blood donors is hypothesized to help, but still lacks evidence and widespread application (1). Preventive actions have slowed down the pandemic, but vaccines are not yet available.

Breastmilk, but not formula, prevents infants from respiratory tract infections via antimicrobial and immunological activity by 30% (2). Milk immunoglobulins (and mainly IgA) are considered the major molecular factor (3).

Just recently, **we and others detected anti-SARS-CoV-2 Spike IgA antibodies in most raw milk samples** of cured COVID 19 milk donors, present in their more stable secretory sIgA form. Pasteurization (Heat or Pressure) of breastmilk yielded similar results. Neutralizing activity tests are currently performed as well as isolation and identification of specific SARS-CoV-2 milk sIgA by *de novo* sequencing by mass spectrometry.

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We propose a three-phase approach:

Phase 1: Quantification of secretory SARS-CoV2 IgA in donor milk of proven COVID 19 mothers, collected via use of existing (inter)national networks of human milk banks.

Phase 2: Optimizing immunological quality and safety of the human milk product via already established partnerships

Phase 3: Initiate a randomized clinical trial in elderly individuals with the hypothesis that gargling/ingesting modified donor human milk, or purified IgA fractions thereof, from COVID 19 mothers will reduce the incidence of proven COVID disease in vulnerable populations by 30%. Assuming an incidence of 3% with a power of 80%, 3815 elderly subjects and 1526 cured COVID 19 milk donors are needed.

2. PLAN VAN AANPAK:

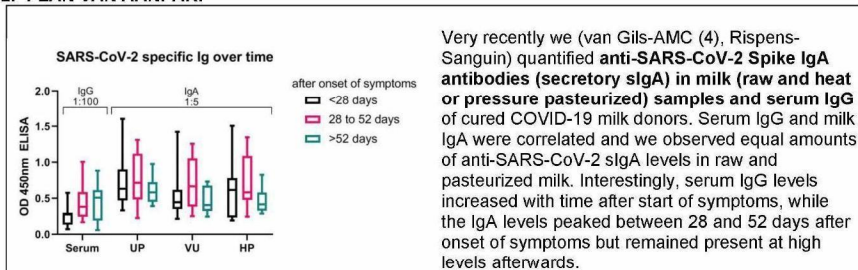


Fig Preliminary data (van Gils-AMC) on serum and milk IGs from cured 50 COVID-19 donors and controls. UP=unpasteurized, VU= Holder pasteurization (@VUmc, HP= High Pressure pasteurization @WJR.

Phase 1: Collection of donor milk of proven COVID-19

Organization of human milk bank

The Dutch Human Milk Bank supplies 50% of all Dutch Neonatal ICs in the Netherlands. The process of milk donation including screening the donors (Sanquin, partner) and **the milk bank is fully operational, follows international guidelines and is approved/controlled by the "Voedsel en Waren Autoriteit"**.

Acquisition of donor milk

We will use a two layered approach. When making use of one single request for donor milk via Facebook, we reached 127.480 interested people. Over 1400 donors volunteered to donate donor milk, while 40

contributors were sufficient. Of these 1400, there was a proven or high likelihood COVID-19 infection in 14%. The average supply of a regular milk donor is 7 liters, for a median duration of 12 weeks of donation. With the assumptions made for the RCT, 10.628 L donor milk is needed, corresponding with 1526 milk donors. **First, we will launch a national campaign with ambassadors targeted to lactating women.** Secondly, if the yield is not high enough, we will use networks like 'Landelijke Borstvoedings Raad', "moedermelknetwerk" and the KNOV (Midwives) with whom we have close contacts.

Contingency plan: In addition, we can extend our trial abroad as the Dutch Milk Bank is member of the European Milk Banking Association (220 milk banks) and the Collaborative Network of Human Milk Banks and Associations (85 milkbanks) (5). Several sites already expressed their huge interest (Imperial College, London, University Hospital Warsaw, Mount Sinai, New York)

Phase 2: **Optimizing immunological quality and safety of the human milk product**

Utrecht University (group of Spinoza laureate Albert Heck, partner) revealed by affinity purification coupled to top-down mass spectrometry that the IgG and IgA antibody repertoire circulating in plasma of individual donors is dominated by a few dozen unique clones. These immunoglobulin repertoires respond strongly upon onset of disease/infection and are dominated by immunoglobulins responding to the physiological/disease state.

Immunoglobulins from human milk of individual donors can be affinity purified and profiled by mass spectrometry (preliminary collaborative work). In this study, the goal is to detect how many SARS-CoV-2 antibodies (and which ones) are present in human milk (and serum) of individual donors, and in a next phase how this may be changing during lactation and correlates with the seriousness of the COVID-19 disease symptoms and other clinical parameters.

The affinity purification assays for either total IgGs or IgAs from either serum or milk are very efficient. Next, we can affinity purify either SARS-CoV-2 directed antibodies (using e.g. spike protein epitope) or profile the complete repertoire preferably by combining these two approaches to identify which clones respond to affinity enrichment/depletion. **Individual COVID-19 responsive clones will be isolated and identified and de novo sequenced by mass spectrometry. These purified immunoglobulins will be functionally tested by using virus neutralization assays** (Stittelaar, Viroclinics). The most effective antibodies will be sequenced in further detail, and this information can and will be used to recombinantly produce them. This may lead to a new and powerful set of COVID-19 virus neutralizing IgG and IgA antibodies.

Fortified donor milk needs to be safe and thus contain as little pathogens as possible. This is routinely achieved with Holder pasteurization (30 min at 62.5°C). Although robust, this leads to inactivation of milk immune factors, including antibodies. Previously, we (Hettinga, WUR) compared several thermal and non-thermal pasteurization processes bacterial inactivation, overall level of protein denaturation, inactivation of specific immune-active proteins (by LC/MSMS), and functionality of these proteins by bacterial growth assays. High pressure (HP) pasteurization seems the most suitable non-thermal process and is industrially available for large-scale application. As we were able to show comparable anti-SARS-CoV-2 sIgA levels in breast milk after both the Holder and HP pasteurization method, we will further optimize the HP pasteurization method for large-scale immunoglobulin production.

Moreover, the mass spectrometry-based analysis of the IgG and IgA repertoire will also be used to monitor what effect the two approaches of pasteurization have upon the human milk samples, whereby the focus is on the retainment of active IgA and IgG clones (even after application of the Holder and HP pasteurization methods). As human milk IgA is present predominantly in the very stable secreted form of sIgA, we expect especially these IgA immunoglobulins to retain their anti-SARS-CoV-2 neutralizing activity, even after pasteurization. Finally, the quality at the end of shelf life will be compared based on IgA retention, bacterial growth, and flavor development (by Gas Chromatography-Mass Spectrometry: GC-MS).

Phase 3: Randomized controlled trial

We hypothesize that human milk derived sIgA from lactating, cured COVID-19 women with a natural surplus of milk prevents SARS-CoV-2 infections in vulnerable populations by 30%.

We will initiate an (inter)national, randomized clinical trial in which we will investigate whether donor milk from COVID-19 cured mothers can have a preventive role for COVID-19 disease in elderly vulnerable individuals. Ingestion of modified donor human milk, or purified sIgA fractions thereof, from COVID-19 mothers will prevent binding of SARS-CoV-2 to mucosal layers in the upper respiratory tract and thereby preventing the development of COVID-19 disease.

The primary objective: a reduced number of SARS-CoV-2 infections (-30%, defined by a positive SARS-CoV-2 RT-PCR obtained from a naso-pharyngeal swab) in a vulnerable population (elderly people aged > 70 years living in a nursing home)

Secondary objectives:

- Reduction of other respiratory infections
- Reduction in COVID-19 related mortality

In this randomized controlled trial, the intervention will consist of gargling and ingesting modified donor human milk from lactating women cured from a proven COVID-19 infection (defined by a positive SARS-CoV-2 RT-PCR obtained from a naso-pharyngeal swab) (10 ml 4 times per day) for 10 weeks. Controls will receive cow's milk in a similar fashion.

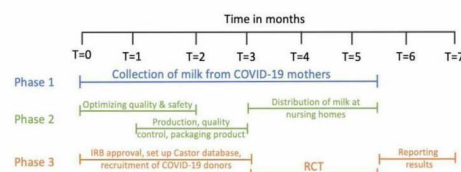
Reducing the incidence from 3% (placebo) to 2% (intervention) at a power of 80% requires 3815 subjects per arm. At present, we find a 2% incidence while screening (PCR) asymptomatic adults pre-surgery. Our network (Hertogh, VUmc) consist of >15.000 elderly within 24 organizations of elder care. For a sufficient supply of donor milk, we estimate the participation of 1526 COVID-19 milk donors supplying 7 L donor milk.

3. HAALBAARHEID VAN HET PROJECT:

We aim to conduct this project in 7 months as is depicted in Figure 2.

MOTIVATIE HAALBAARHEID

Chances of success are based on broad network of highly motivated experts in all the relevant fields (accessibility of milk donors via national milk bank, milk processing, immunological & viral knowledge and testing capacity of SARS-CoV-2, methodology (Bossuyt, AMC), elderly health). Ample opportunities for extension abroad.



4. RELEVANTIE VOOR DE PRAKTIJK:

Onderbouw de relevantie aan de hand van de in de subsidieoproep benoemde relevantiecriteria
Prevention is key. Optimization of the human milk product by the newest techniques, validated by measuring neutralizing antibodies and functional testing of the product in cell cultures will allow maximal potential to be effective. The selected subjects are highly prone for infection and belong to the high risk group with concomitant morbidity and mortality. **In contrast to newly produced vaccins or drugs, human milk will have no side effects.** In contrast to serum-derived antibodies or monoclonal antibodies, milk-derived antibodies will exhibit their inhibitory properties at the surface of buccal mucosa after oral non-invasive administration routes. **There is no need for trained health care personnel. When effective, this method can be applied worldwide at low costs.**

5. DEELNAME VAN DE STAKEHOLDER(S) (e.g. patiënten, zorgprofessionals, etc.):

Milk donors through Dutch Milk Bank (expansion possible via existing international networks), elderly participants through "academische werkplaats ouderenzorg (UNO VUmc)", pasteurization via Dutch Milk Bank and Wageningen University & Research, serology and milk Ig testing and purification via Amsterdam UMC, Sanquin and University Utrecht, neutralizing virus capacity via Viroclinics, RCT methodology and registry via AmsterdamUMC. All stakeholders are committed and worked together on other projects.

6. LITERATUURREFERENTIES (optioneel):

1. Bloch, E.M., et al., *Deployment of convalescent plasma for the prevention and treatment of COVID-19*. J Clin Invest, 2020.
2. Chantry, C.J., et al., *Full breastfeeding duration and associated decrease in respiratory tract infection in US children*. Pediatrics, 2006. 117(2): p. 425-32.
3. Mantis, N.J., et al., *Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut*. Mucosal Immunol, 2011. 4(6): p. 603-11.
4. Brouwer et al. *Potent neutralizing antibodies from COVID-19 patients define multiple targets of vulnerability*. 2020 pre-print bioRxiv: <https://doi.org/10.1101/2020.05.12.088716>.
5. Schenker, N et al. *Maintaining safety and service provision in human milk banking: a call to action in response to the COVID-19 pandemic*. Lancet Child Adolesc Health 2020. [https://doi.org/10.1016/S2352-4642\(20\)30134-6](https://doi.org/10.1016/S2352-4642(20)30134-6)