ZonMw

Aanvraagformulier GGG_digitaal / Applicationform GGG_digital

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

DEFINITIEF

Algemene gegevens / General Information

Programma / Programme	:	**COVID-19 Programma**
Subsidieronde / Subsidy round	:	**Bottom-up ronde COVID-19 aandachtsgebied 1**
Projecttitel / Project title	:	**The role of prior exposure to livestock-associated coronaviruses in severity of COVID-19 through antibody-dependent enhancement (ADE)**
Projecttaal / Project language	:	**Engels / English**
Geplande startdatum / Planned start date	:	**13-07-2020**
Geplande duur / Planned duration	:	**24 maanden / months**
Datum indienen / Date of application	:	**14-05-2020**
Projecttype / Project type	:	**Fundamenteel onderzoek / Fundamental research**
Vervolg eerder ZonMw-project / Continuation previously funded project ZonMw	:	**Nee / No**

Projectleden / Project members

Dr. (10)(2e) (10)(2e)
Functie / Position: onderzoeker | *Opleiding / Education:* WO
Studierichting / Subject: virologie
T: (10)(2e) | *F:* | *E:* (10)(2e)@rivm.nl

Rijksinstituut voor Volksgezondheid en Milieu
Centre for Infectious Disease Control (CIb)
Antonie van Leeuwenhoeklaan 9
3721 MA BILTHOVEN

(10)(2e) (10)(2e)
Functie / Position: onderzoeker | *Opleiding / Education:* WO
Studierichting / Subject: diergeneeskunde, veterinaire epidemiologie
T: (10)(2e) | *F:* | *E:* (10)(2e)@rivm.nl

Rijksinstituut voor Volksgezondheid en Milieu
Centre for Infectious Disease Control (CIb)
Antonie van Leeuwenhoeklaan 9
3721 MA BILTHOVEN

(10)(2e) (10)(2e)
Functie / Position: directeur Centrum Infectieziektebestrijding | *Opleiding / Education:* WO
Studierichting / Subject: geneeskunde
T: (10)(2e) | *F:* | *E:* (10)(2e)@rivm.nl

Rijksinstituut voor Volksgezondheid en Milieu
Centre for Infectious Disease Control (CIb)
Antonie van Leeuwenhoeklaan 9
3721 MA BILTHOVEN

(10)(2e) (10)(2e)
Functie / Position: onderzoeker | *Opleiding / Education:* WO
Studierichting / Subject: viroloog
T: (10)(2e) | *F:* | *E:* (10)(2e)@uu.nl

Universiteit Utrecht
Faculteit der Diergeneeskunde
Infectieziekten en Immunologie
Postbus 80163
3508 TD UTRECHT

(10)(2e) (10)(2e)
Functie / Position: onderzoeker | *Opleiding / Education:* WO
Studierichting / Subject: Diergeneeskunde

T: (10)(2e) *F:* | *E:* (10)(2e)@rivm.nl

Rijksinstituut voor Volksgezondheid en Milieu
Centre for Infectious Disease Control (CIb)
Antonie van Leeuwenhoeklaan 9
3721 MA BILTHOVEN

(10)(2e) (10)(2e)
Functie / Position: arts-microbioloog | *Opleiding / Education:* WO
Studierichting / Subject: Geneeskunde, Medische microbiologie
T: (10)(2e) | *F:* | *E:* (10)(2e)@etz.nl

Elisabeth-TweeSteden Ziekenhuis
Postbus 90151
5000 LC TILBURG

(10)(2e) (10)(2e)
Functie / Position: arts-microbioloog | *Opleiding / Education:* WO
Studierichting / Subject: Geneeskunde, Medische microbiologie
T: (10)(2e) | *F:* | *E:* (10)(2e)@dz.nl

Deventer Ziekenhuis
Postbus 5001
7400 GC DEVENTER

(10)(2e) (10)(2e)
Functie / Position: onderzoeker | *Opleiding / Education:* WO
Studierichting / Subject: diergeneeskunde, veterinaire microbiologie
T: (10)(2e) | *F:* | *E:* (10)(2e)@rivm.nl

Rijksinstituut voor Volksgezondheid en Milieu
Centre for Infectious Disease Control (CIb)
Antonie van Leeuwenhoeklaan 9
3721 MA BILTHOVEN

(10)(2e) (10)(2e)
Functie / Position: onderzoeker | *Opleiding / Education:* WO
Studierichting / Subject: virologie, immunologie
T: (10)(2e) | *F:* | *E:* (10)(2e)@rivm.nl

Rijksinstituut voor Volksgezondheid en Milieu (RIVM)
Centre for Immunology of Infectious diseases and Vaccines (IIV)
Postbus 1
3720 BA BILTHOVEN

(10)(2e) (10)(2e)
Functie / Position: (10)(2e) | *Opleiding / Education:* WO
Studierichting / Subject: Geneeskunde, Medische microbiologie
T: (10)(2e) | *F:* | *E:* (10)(2e)@sanquin.nl

Sanquin Research
Postbus 9892
1006 AN AMSTERDAM

Projectgegevens / Project information

Aandachtsgebieden / Focus

1.1 Thema's aandachtsgebied 1
- Risicoanalyse en prognostiek
- Virus, immuniteit, immuunrespons en pathogenese

1.3 Setting
- Anders
- Ziekenhuiszorg

Samenvatting / Summary

The current SARS-CoV-2 pandemic demonstrates large discrepancies in incidence of severe disease depending on geographic distribution and age. A possible biological explanation might be that individuals suffering the most have been primed by one or more prior coronavirus exposures, are experiencing the effects of antibody dependent enhancement (ADE) of viral infection. This phenomenon has been demonstrated and characterized for SARS-CoV and postulated for SARS-CoV-2.

By comparing maps of livestock density in The Netherlands with maps depicting hospitalized COVID-19 patients striking patterns can be observed, which are supported by preliminary data from spatial analyses, indicating that severe disease occurs more in patients living in close proximity to certain livestock farms.
We hypothesize that livestock-associated coronaviruses are priming viruses that induce antibodies in humans, putatively mediating ADE of SARS-CoV-2 and subsequently enhancing infection and severity of disease. To test our hypothesis the following research questions will be addressed:
I. Does (part of) the Dutch population have antibodies to animal coronaviruses?
II. Do these antibodies to animal coronaviruses cross-react with and mediate ADE of SARS-CoV-2 in vitro?
III. Is presence of these antibodies to animal coronaviruses related to severity of COVID-19 disease?
IV. Is anti-animal-CoV seroprevalence in humans associated with the regional distribution of severe COVID-19 cases?

If prior exposure to animal coronaviruses indeed has an effect on COVID-19 this would have important implications for identification of risk groups with regards to mitigation and exit strategies as well as vaccine safety. Moreover, closer monitoring of patients with potential ADE-inducing antibodies and development of specific treatment regimens may improve clinical outcome.

Trefwoorden / Keywords

antibody-dependent enhancement; cross reactivity; one health; serology; animal coronaviruses; spatial analysis;

Samenwerking / Collaboration

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

Ja / Yes

Organisaties

Deventer Ziekenhuis
Postbus 5001
7400 GC DEVENTER

Elisabeth-TweeSteden Ziekenhuis
Postbus 90151
5000 LC TILBURG

Rijksinstituut voor Volksgezondheid en Milieu
Centre for Infectious Disease Control (CIb)
Antonie van Leeuwenhoeklaan 9
3721 MA BILTHOVEN

Rijksinstituut voor Volksgezondheid en Milieu (RIVM)
Centre for Immunology of Infectious diseases and Vaccines (IIV)
Postbus 1
3720 BA BILTHOVEN

Sanquin Research
Postbus 9892
1006 AN AMSTERDAM

Inhoud / Content

Disciplines / Disciplines

- Bioinformatica/biostatistiek, biomathematica, biomechanica / Bioinformatics/biostatistics, biomathematics, biomechanics
- Immunologie, serologie / Immunology, serology
- Infecties, parasitologie, virologie / Infections, parasitology, virology
- Epidemiologie / Epidemiology
- Geneeskunde, overig / Medicine, other
- Diergeneeskunde / Veterinary medicine

Financiële gegevens / Financial data

ZonMw budget

	Jaar / Year								
Kostenpost	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	**Totaal / Total**
Personeel	(10)(2b)	(10)(2b)	0	0	0	0	0	0	(10)(2b)
Materieel	(10)(2b)	(10)(2b)	0	0	0	0	0	0	(10)(2b)
Implementatie	(10)(2b)	(10)(2b)	0	0	0	0	0	0	(10)(2b)
Apparatuur	0	0	0	0	0	0	0	0	0
Overig	(10)(2b)	(10)(2b)	0	0	0	0	0	0	(10)(2b)
Totaal / Total	(10)(2b)	(10)(2b)	**0**	**0**	**0**	**0**	**0**	**0**	(10)(2b)

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

Andere vergunningen / Other permits

AANVRAAGFORMULIER PROJECTIDEE – BOTTOM-UP RONDE

COVID 19 programma

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

LEES ALSTUBLIEFT 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:

RIVM, Centrum voor Infectieziektebestrijding

PROJECTTITEL:

The role of prior exposure to livestock-associated coronaviruses in severity of COVID-19 through antibody-dependent enhancement (ADE)

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: RIVM
E-mail: (10)(2e)@rivm.nl
Was aanwezig bij de webinar: ☐ Ja ☒ Nee

☐ Ik heb nog geen datasteward.

ONDERZOEKSVOORSTEL max 3 pagina's A4 (inclusief literatuurreferenties)	(voorpagina met basisgegevens niet meegerekend - font type Arial 10 pts)

1. PROBLEEMSTELLING EN DOELSTELLING(EN):

The current SARS-CoV-2 pandemic demonstrates large discrepancies in incidence of severe disease depending on geographic distribution and age. Also in the Netherlands, regional clusters of hospitalized patients (Fig. 1) and seroprevalence (Fig. 2) exist.

A biological mechanism putatively explaining the observed differences in disease severity might be that individuals suffering the most have been primed by one or more prior coronavirus exposures and are experiencing the effects of antibody dependent enhancement (ADE) of viral infection. This phenomenon has been demonstrated and characterized for SARS-CoV [1-5], causing similar disease and epidemiological observations during the 2003 epidemic. In ADE antibodies generated to protect the host against a homologous viral infection, play an opposite role; they seem to be hijacked by the related heterologous virus to facilitate host cell viral entry and replication. Whether SARS-CoV-2, which is approximately 79% homologous to SARS-CoV, is receiving ADE from other coronaviruses as well, is postulated [6] though not yet established.

When comparing maps of livestock density in The Netherlands (e.g. pigs in Fig. 3) with maps depicting hospitalized COVID-19 patients (Fig. 1) striking spatial patterns can be observed. Preliminary data from spatial analyses, indicate that severe COVID-19 occurs more in patients living in close proximity to certain livestock farms (Hogerwerf et al., publication in preparation). These patterns can be observed in other parts of the World (Italy, Wuhan, USA) as well. In recent years, outbreaks of coronaviruses have occurred in pigs (PED 2014, GD), horses [7], and many other animal coronaviruses are endemic [8]. Airborne transmission is considered a potential route for farm to farm transmission of PED [9, 10]. To date, it has not been described whether humans evoke serological responses upon exposure to animal coronaviruses, thus the anti-animal-coronavirus seroprevalence in the Dutch population is not known.

Altogether, this prompted us to hypothesize that livestock-associated coronaviruses are the priming viruses that by previous exposure or infection induce antibodies in humans, putatively mediating ADE of SARS-CoV-2 and subsequently enhancing severity of disease. To test our hypothesis of immune priming by exposure to animal coronaviruses we follow a stepwise approach by addressing the following research questions:

I. Does (part of) the Dutch population have antibodies to animal coronaviruses?
II. Do these antibodies to animal coronaviruses cross-react with and mediate ADE of SARS-CoV-2 *in vitro*?
III. Is presence of these antibodies to animal coronaviruses related to severity of COVID-19 disease?
IV. Is anti-animal-CoV seroprevalence in humans associated with the regional distribution of severe COVID-19 cases?

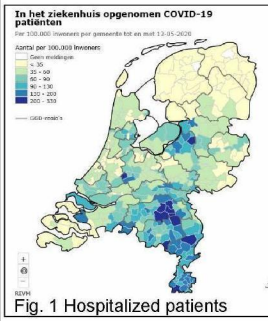


Fig. 1 Hospitalized patients

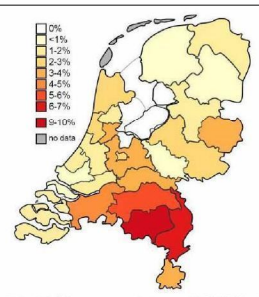


Fig 2. Seroprevalence (1-15 Apr)

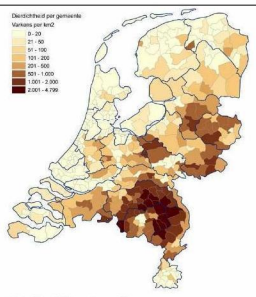


Fig 3. Pig density

2. PLAN VAN AANPAK:

I. Does (part of) the Dutch population have antibodies to animal coronaviruses?

Livestock-species of interest will be identified from spatial analysis of COVID-19 cases. Coronaviruses associated with these species will be identified from literature and by expert opinion. If needed, the selection will be reduced based on zoonotic potential of these animal coronaviruses to SARS-CoV-2. Spike

(S1) protein of prioritized animal viruses will be produced at Faculty of Veterinary Medicine, Utrecht University. These proteins will be incorporated in the multiplex protein array for all human coronaviruses, including SARS-CoV-2, serological testing at RIVM [11-13]. Available sera of people with intensive direct contact to animals (e.g. farmers, livestock traders, slaughterers, veterinarians), and people living in close proximity of livestock farms will be tested. Sera of people without intensive (direct or indirect) contact to animals and without COVID-19 will be used to determine variation of serological response in unexposed individuals. Since no reference sera or a predefined cut-off value for the response to animal coronavirus antigens is available, Bayesian mixture modelling [14] will be used to analyze serological results.

II. Do these antibodies to animal coronaviruses cross-react with and mediate ADE of SARS-CoV-2 *in vitro*?

Positive sera and appropriate controls identified in Q.I will be assessed for binding to SARS-CoV-2 and for neutralization capacity in a microneutralization assay. To assess whether cross-reactive antibodies enhance infection or - more importantly - the inflammatory response, freshly isolated PBMCs from healthy donors will be exposed to SARS-CoV-2 particles pre-incubated with varying concentrations of serum (and/or purified antibodies). Read-outs will be infection rate (FACS) and cytokine secretion (multiplex immunoassay)

III. Is presence of these antibodies to animal coronaviruses related to severity of COVID-19 disease?

In a case-control design, the antibody response to SARS-CoV-2 and the animal coronaviruses identified in Q.II will be compared in mild (i.e. controls) and severe (i.e. cases) PCR-proven COVID-patients. In addition to clinical data, data on comorbidities (e.g. cardiovascular diseases and diabetes), age, sex, BMI and home address will be collected. Antibody responses in cases and controls will be compared taking into account the days after onset of symptoms and the antibody titers.

IV. Is anti-animal-CoV seroprevalence in humans associated with the regional distribution of severe COVID-19 cases?

As antigens of the identified animal coronavirus(es) of interested will be incorporated in the multiplex protein array for SARS-CoV-2, data will become available for all tested individuals and additional sera will be obtained via Sanquin. Multiplex array outcomes will be used in spatial analyses to study associations with distance of home address to livestock farming of specific animals.

3. HAALBAARHEID VAN HET PROJECT:

The project will be planned in four, partially overlapping phases

Planning	Y1Q1	Y1Q2	Y1Q3	Y1Q4	Y2Q1	Y2Q2	Y2Q3	Y2Q4
I Select animal coronaviruses								
I Produce antigen and incorporate								
I Test exposure sera and analyze results								
II *In vitro* testing with positive sera from I								
III Study design and collect patient sera								
III Test patient sera and analyze results								
IV Test population sera								
IV Implementation in spatial analysis								
Reporting and dissemination of results								

The project group is perfectly suited to address the research questions. It includes experts on coronavirus serology and antigen production ((10)(2e) and (1 (10)(2e) and experts on *in vitro* experiments to determine cross-reactivity and ADE ((10)(2e), (1 (10)(2e), (10)(2e) (10)(2e) and (10)(2e)). (10)(2e), (10)(2e) and (10)(2e) are experts in the field of livestock-associated zoonoses and can provide sera and analyze data to take into account the association with livestock farming (Q.I and Q.IV). (10)(2e) is a mathematical modeler and expert analyzing serological results through Bayesian mixture modelling, as well as GIS and spatial statistics. (10)(2e), (10)(2e), (10)(2e) and (10)(2e) have access to patient and population sera (Q.III). (10)(2e) and (10)(2e) will be involved to align our patient study (Q.III) with ongoing epidemiological and immunological projects at RIVM. Since animal coronavirus antigens can be included in the multiplex protein array that is routinely used for serological testing at RIVM, testing op population sera (Q.IV) can be carried out efficiently.

Our project is based on finding antibodies to animal coronaviruses in the Dutch population. In case no antibodies to animal coronaviruses are detected (Q.I), we will still proceed with patient sera (Q.III) and include the most likely animal coronavirus S1 antigens in the multiplex protein array (and continue with in

vitro testing as described for Q.II in case cross-reactive antibodies are detected in the patient sera). Moreover, since the protein array also includes common human coronaviruses, our project will, as secondary goal, also provide insights in the possibility that previous exposure to common human CoVs has a role in COVID-19 severity, although this is unlikely to associate with the spatial distribution observed.

4. RELEVANTIE VOOR DE PRAKTIJK:

In case prior exposure to animal coronaviruses has effect on the course of COVID-19 this information can help identify risk groups for severe clinical manifestations of SARS-CoV-2 infections, either based on (professional) contact with specific animals species or living in close proximity to those farms, or based on the identification of previous infections with other CoV through serological testing. Mitigation and exit strategies could be tailored to these risk groups or regions to prevent infections in those at risk for a severe outcome. Moreover, the possibility of ADE in individuals with potential cross-reacting antibodies should be specifically considered in vaccine safety trials or in treatment regimens including e.g. convalescent plasma therapy. For patients with SARS-CoV-2 infection, testing for animal coronavirus antibodies, may have prognostic value. Closer monitoring of at risk patients and specific treatment regimens may improve clinical outcome.

To postulate the possibility of ADE [6], and other immune-mediated differences in the course of COVID-19 is not new and known for human coronaviruses in general. However, a possible role for animal coronaviruses in the human pandemic appears to be neglected. The Netherlands has a unique position to study those effects, as we are a livestock-dense country with a strong One Health network. From previous studies on livestock-associated diseases, there is a multitude of samples and data specifying the presence and distance between livestock farms and local residents. At this point, we do not aim to fully elucidate the immunological mechanism, but may either reject the hypothesis that animal coronaviruses play a role, or collect enough evidence for animal coronaviruses to be considered in other (international) projects focusing on the role of the immune response in SARS-CoV-2 infected patients.

The discussion of the association between livestock density and COVID-19 infections in the media (e.g. https://www.trouw.nl/cs-b2b5487d) highlights the societal impact of this research topic, which warrants spending public money to investigate the underlying mechanism. Currently, mainly the link of disease incidence with air quality or a possible role of zoonotic transmission of SARS-CoV-2 is under investigation. Our project focuses on an different hypothesis, which can relatively easily be studied and would have important implications as outlined above. Our research objectives are studied within the same network of One Health researchers and we aim to collaborate and discuss in order to balance the different approaches, and provide the most complete picture of the influential mechanisms.

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

We aim to involve and inform stakeholders as needed and have currently identified the following groups:

- RIVM, Public Health Services (GGD), Ministry of Public Health (VWS) – implications for mitigation and exit strategies
- Healthcare professionals (hospital) – collection of patient sera (Q.III) and possible implications for treatment
- Sanquin – collection of patient (Q.III) and population sera (Q.IV)
- KNMvD, veterinarians – possibly participating as high exposure group (Q.I), potential risk group
- Livestock farmers and local residents, and representative organizations – tested based on already available sera with appropriate consent (Q.I), potential risk group
- Slaughterhouse personnel (e.g. VION) – tested in case sera are available (Q.1), potential risk group
- Animal Health Service (GD), Wageningen Bioveterinary Research – expert elicitation prevalence of livestock coronaviruses (Q.I)
- RIVM, Centre for Sustainability, Environment and Health (DMG) will be involved to account for other factors in spatial modelling (Q.IV)

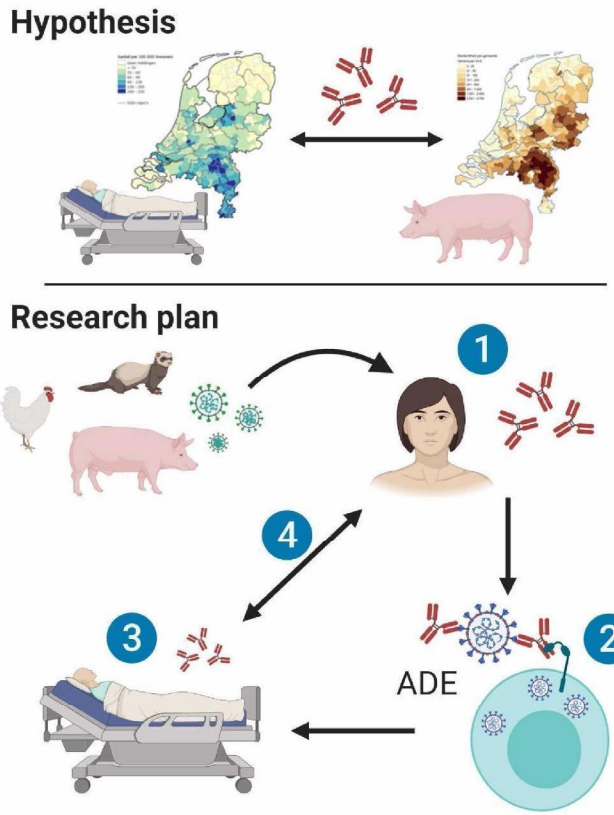
6. LITERATUURREFERENTIES (optioneel):

[1] Ho, et al., EID, 2005. 11: 1730. **[2]** Jaume, et al., J Virol, 2011. 85: 10582. **[3]** Wan, et al., J Virol, 2020. 94: e02015. **[4]** Yang, et al., PNAS, 2005. 102: 797 **[5]** Yip, et al., Virol J, 2014. 11: 82. **[6]** Tetro, Microbes and Infection, 2020. 22: 72 **[7]** Zhao, et al., Viruses, 2019. 11(12). **[8]** Decaro, et al., Res Vet Sci, 2020. 131: 21 **[9]** Alonso, et al., Vet Res, 2014. 45: 73. **[10]** Beam, et al., PLoS One, 2015. 10:e0144818. **[11]** Reusken, et al., Eurosurveillance, 2013. 18:20441. **[12]** Reusken, et al., EID, 2015. 21: 1422. **[13]** Reusken, et al., Lancet, 2013. 13: 859. **[14]** Opsteegh, et al., Prev Vet Med, 2012. 104: 317.

The role of prior exposure to livestock-associated coronaviruses in severity of COVID-19 through antibody-dependent enhancement (ADE)

Hypothesis

Livestock-associated coronaviruses are the priming viruses that by previous exposure or infection induce antibodies in humans, putatively mediating ADE of SARS-CoV-2 and subsequently enhancing severity of disease.



Research questions

I. Does (part of) the Dutch population have antibodies to animal coronaviruses?
II. Do these antibodies to animal coronaviruses cross-react with and mediate ADE of SARS-CoV-2 *in vitro*?
III. Is presence of these antibodies to animal coronaviruses related to severity of COVID-19 disease?
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