Centrale Commissie Dierproeven

Form
Project proposal

- This form should be used to write the project proposal for animal procedures.
- The appendix 'description animal procedures' is an appendix to this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed.
- For more information on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	50200
1.2	Provide the name of the licenced establishment.	Biomedical Primate Research Centre
1.3	Provide the title of the project.	Evaluation of vaccines and antiviral therapeutics against coronavirus infections

2 Categories

2.1 Please tick each of the following boxes that applies to your project.

- ☐ Basic research
- ☒ Translational or applied research
- ☐ Regulatory use or routine production
- ☐ Research into environmental protection in the interest of human or animal
- ☐ Research aimed at preserving the species subjected to procedures
- ☐ Higher education or training
- ☐ Forensic enquiries
- ☐ Maintenance of colonies of genetically altered animals not used in other animal procedures

3 General description of the project

3.1 Background

Describe the project (motivation, background and context) with respect to the categories selected in 2.1.

Coronaviruses (CoVs) are a group of enveloped viruses with non-segmented, single-stranded, and positive-sense RNA genomes. CoVs are important pathogens as they infect a variety of economically

important vertebrates, such as pigs and chickens, but have also been described in birds, bats, mice, and various wild animals. In addition, CoVs can also infect the respiratory, gastrointestinal, hepatic,and central nervous system of humans. Until the end of 2019, six human coronaviruses (HCoV) were described; HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, severe acute respiratory syndrome (SARS-CoV), the Middle East respiratory syndrome (MERS)-CoV (1-3).
Until 2002, CoVs were only known to cause mild respiratory infections in humans. However, in November 2002, a severe respiratory disease first appeared in southern China and quickly spread to other countries, leading to over 8,000 confirmed cases at the end of the pandemic in June 2003, with a mortality rate of~9.6% (4). The causative agent was identified and named SARS-CoV, a zoonotic coronavirus. SARS-CoV originated in horseshoe bats, and had later adapted to infect the intermediate host, the palm civet, and ultimately infected humans (5). After an incubation period of 4–6 days, SARS patients develop flu-like symptoms and pneumonia, which in severe cases lead to fatal respiratory failure, and acute respiratory distress syndrome (6). No cases of SARS have been reported since 2004, but a rich gene pool of SARS-related coronaviruses was discovered in bats living in a cave in Yunnan, China, highlighting the necessity to prepare for future re-emergence (7).
Eight years after the SARS pandemic, in June 2012, MERS-CoV emerged in Saudi Arabia as the causative agent of a SARS-like respiratory disease (8). Although human-to-human transmission is considered limited, MERS-CoV has caused two major outbreaks in Saudi Arabia (2012) and in South Korea (2015), with the number of confirmed cases exceeding 2,000, and with a mortality rate of ~35% (9). Similar to SARS-CoV, MERS-CoV originated in bats, but it later adapted to dromedary camels as intermediate hosts (10). Currently, no vaccines or specific antiviral drugs are available for either SARS-CoV or MERS-CoV.

In December 2019, a cluster of 27 patients were hospitalized in Wuhan, China, with respiratory symptoms of unknown etiology. Cases showed symptoms such as fever, dry cough, dyspnoea, and radiological findings of bilateral lung infiltrates. The causative agent was quickly identified as a coronavirus, initially called 2019-nCoV (2019 new coronavirus)(11), and on 10 January its genome sequence was made publicly available (GenBank acc. no. MN908947). On 11 February, the virus was officially named SARS-CoV-2, and the disease caused by the virus, COVID-19 (Coronavirus disease 2019).Since the first publications, the pandemic spread rapidly and on 30 January 2020, the World Health Organization declared the SARS-CoV-2 outbreak a public-health emergency of international concern (PHEIC) (12). SARS-CoV-2 spread very quickly across the globe, and within the first two years of the COVID-19 pandemic, more than 450 million cases were reported worldwide, more than 100 million in the EU/EEA alone (13), with a mortality rate exceeding 1% before vaccines became available. The COVID-19 pandemic disrupted supply chains around the world, tested the resilience of global healthcare systems, and exposed weaknesses in public procurement processes (14).
The fast-track availability of vaccines (Adenovirus-based Astra Zeneca, and mRNA-based Pfizer/BioNTech and Moderna), relying on knowledge gained during the development of MERS vaccines (e.g. stabilization of Spike antigen in pre-fusion state), was essential to reduce the impact of the SARS-CoV-2 pandemic. For their proven potential to cause global pandemics, and the abovementioned lack of vaccines and antivirals, SARS and MERS are prioritized by the WHO on their R&D blueprint (12, 15). The WHO R&D Blueprint focuses on severe emerging diseases with potential to generate a public health emergency, and for which insufficient, or no preventive (vaccines) and curative solutions (antiviral therapeutics) exist (12, 15). The WHO list of diseases to be prioritized is:

- Crimean-Congo Hemorrhagic Fever
- Ebola and Marburg Viral Disease
- Lassa Fever
- Nipah and Henipa viral diseases
- Rift Valley Fever
- Zika disease
- SARS-CoV-1, MERS-CoV, and SARS-CoV-2
- Disease X

The WHO put SARS and MERS on the 2018 list of priority diseases and added SARS-CoV-2 in 2020 (12, 15). Although SARS-CoV-2 vaccines have been essential in the reduction of the impact of the SARS-CoV-2 epidemic, there is still room for improvement. Current SARS-CoV-2 vaccines protect against severe disease and hospitalization, but do not prevent transmission, do not fully protect against variants of concern and do not provide long-lived protection.
Novel coronaviruses with Disease X characteristics can join the MERS- and SARS-CoVs on the list. The ongoing SARS-CoV-2 epidemic, as well as the outbreaks of pathogenic SARS- and MERS-CoVs in the last two decades, have shown the urgency of developing vaccines and anti-viral therapeutics against the known emerged coronaviruses, but also points towards the need for preparedness for future outbreaks of yet unknown CoVs. It is highly likely that new CoV outbreaks will occurin the future due to the increased interactions of human with animals. To diminish the impact of such new outbreaks, there is an urgent need to develop effective therapies and broadly-acting vaccines against coronaviruses.
The current proposal concerns a continuation of CCD project AVD5020020209404. In project AVD5020020209404 10 vaccines and one monoclonal antibody were evaluated for efficacy against SARS-CoV-2. Of the 10 evaluated vaccines one vaccine was registered and several others are currently in clinical development (16-19).

3.2 Purpose

3.2.1 Describe the project's immediate and ultimate goals. Describe to which extent achieving the project's immediate goal will contribute to achieving the ultimate goal.

- If applicable, describe all subobjectives

The immediate goal of this research project is to test the immunogenicity, efficacy, and safety (absence of serious adverse events) of coronavirus vaccines, and to determine the pharmacokinetics, safety, and efficacy of anti-viral therapeutics that are developed for use in humans, in nonhuman primates (NHP). The focus of the project will be on coronavirus vaccines and antivirals designed to protect against infection with coronaviruses.
The ultimate goal of this research project is to accelerate the development of vaccines and anti-viral therapeutics capable of protecting humans from (novel) coronavirus infections.

3.2.2 Provide a justification for the project's feasibility.

The institute has extensive and long-standing expertise in conducting vaccination studies using nonhuman primates. Since 2012, researchers at the institute have been working on respiratory infections, like influenza, RSV, and SARS-CoV-2, in macaques and marmosets, and on the evaluation of vaccines against these viruses. With this they are thus skilled in working with pathogenic, airborne viruses (16-18, 20-24). The institute has the appropriate facilities and experience to work with airborne pathogenic viruses at DM-III and ML-III biosafety conditions. In addition, the institute has the appropriate virological knowledge and expertise for assessment of vaccine efficacy, and to determine absence of pathogenicity. The experience with respiratory viruses guarantees that these animal studies will be adequately performed.

3.2.3 Are, for conducting this project, other laws and regulations applicable that may affect the welfare of the animals and/or the feasibility of the project?

☒ No

☐ Yes > Describe which laws and regulations apply en describe the effects on the welfare of the animals and the feasibility of the project.

3.3 Relevance

3.3.1 What is the scientific and/or social relevance of the objectives described above?

Coronavirus epidemics can cause considerable morbidity and high mortality world-wide, and especially can affect vulnerable groups, like the elderly and people with underlying diseases. Also, the economic

and societal impact of coronavirus epidemics can be substantial (as exemplified by SARS-CoV-2 pandemic) (13, 14). Therefore, the availability of (broadly-protecting) vaccines or anti-viral therapeutics against coronaviruses will have great societal impact. Novel vaccines, new vaccine delivery methods, and anti-viral therapeutics require evaluation in appropriate animal models such that the level as well as the mechanism of protection can be adequately established and understood, before these new vaccines can be evaluated in clinical studies (25, 26).

3.3.2 Who are the project's stakeholders? Describe their specific interests.

The stakeholders for coronavirus vaccines and anti-viral therapeutics are:

- The world population: The reduction of the impact of (future) coronavirus epidemics will be of great societal and economic importance. Most notably, the most vulnerable people, those with underlying diseases and the elderly, for whom protection from coronavirus infection will increase their quality of life.
- The scientific community: Information obtained from the planned research described in this proposal will increase knowledge on coronavirus infection, efficacy of vaccines and antiviral compounds, correlates of protection, and as such will contribute to the preparedness to reduce the impact of future CoV epidemics.
- Pharmaceutical companies: Proof of concept for vaccines and antiviral compounds obtained in the studies described in this proposal will contribute to the clinical development and ultimately registration of their products.
- For the animals as stakeholders, there are no direct benefits and they will experience moderate discomfort as a result of the experimental procedures.

3.4 Strategy

3.4.1 Provide an overview of the overall design of the project (strategy). If applicable, describe the different phases in the project, the coherence, the milestones, selection points and decision criteria.

The project comprises five separate components:

1. Coronavirus infection in NHP
2. Coronavirus vaccine evaluation in NHP
3. Coronavirus vaccine evaluation under the "animal rule"
4. Pharmacokinetics (PK) of CoV antivirals in NHP
5. CoV antiviral efficacy study in NHP

Coronavirus infection in NHP. Before vaccines or anti-viral therapeutics can be evaluated a coronavirus challenge model in NHP must be established. To confirm infectivity and pathogenicity of a new virus, or virus stock that has not been tested previously in NHP at our institute, animals will be infected and monitored for clinical symptoms, development of fever, loss of body weight, and changes in blood parameters. Nasal and tracheal swabs, as well as bronchoalveolar lavages (BAL) will be collected to confirm that the animals have become infected and to determine the amount of virus produced. To evaluate a new virus, the virus is inoculated via (a combination of routes); i.e. intra-bronchial, oral, ocular, intranasal, or via an aerosol as described in Appendix 1. The virus challenge dose needs to be established at a level where all animals become infected, i.e. show virus replication.

Vaccine evaluation: For the evaluation of the safety, immunogenicity, and efficacy of a vaccine concept, a vaccine evaluation experiment will be performed according to well established procedures, as described in Appendix 2. Typically, several immunizations are given over a certain time-period. Following immunization, induction of T-cell and antibody immune responses are measured, systemically in the blood, as well as locally, in the upper and lower respiratory tract. The strength of these responses as well as their duration are determined. Subsequently, the capacity of the vaccine to protect against infection is evaluated by experimental challenge of the animals with the coronavirus under investigation (Appendix 1).

Experimental infection will only be performed when the immunization has induced immune responses against the virus that is to be used for experimental infection, such that protection against infection is to be expected (i.e. a significant increase in post-vaccination immune responses, either humoral and or

cellular, must be observed). Whether protection is achieved depends on local interaction between cells of the immune system and local anti-viral antibodies with the virus and virus infected cells in the respiratory tract. This cannot be adequately modelled in an *in vitro* system and thus requires experimental infection of an animal. Ideally the vaccine should provide a robust level of protection and be able to reduce disease and virus replication in animals infected with a standard virus dose via delivery to the (upper) respiratory tract.

Vaccine evaluation under the "Animal Rule": The Food and Drug Administration (FDA) Animal Rule was devised to facilitate approval of candidate vaccines and therapeutics using animal survival data when human efficacy studies are not practical or ethical (25-29). This regulatory pathway is critical for candidates against pathogens with high case fatality rates that prohibit human challenge trials, as well as candidates with low and sporadic incidences of outbreaks that make human field trials difficult (25, 26). Important components of a vaccine development plan for Animal Rule licensure are the identification of an immune correlate of protection and immunobridging to humans (25, 26), see below for a more detailed description of the identification of a CoP and immunobridging to humans. The relationship of vaccine-induced immune responses to survival after vaccination and challenge must be established in validated animal models and then used to infer predictive vaccine efficacy in humans via immunobridging (26). The experiments performed under the Animal Rule are similar to those described in appendices 1 and 2, but because the Animal Rule applies to the licensure of a vaccine, the criteria are more stringent (i.e. require more animals, as well as validation experiments) than those outlined in appendices 1 and 2, this is described in Appendix 3.
As a first step a virus challenge dose that reproducibly yields a pre-defined virus load needs to be established, where a number of challenge doses (e.g. 3 doses) are evaluated as described in Appendix 1. The virus challenge dose, as established in the initial step, then must be validated in an independent second challenge study, as described in Appendix 1. The next step is the identification of a correlate of protection (CoP), to this end several vaccine doses will be tested (as described in appendix 2), such that a range of, potentially protective, immune responses are induced (26). The animals are then infected using the validated virus challenge dose and virus loads are determined in the target tissues. Following statistical analysis of the data, a CoP is then established. The CoP then needs to be validated in a second independent study, as described in Appendix 2, where a vaccine dose is used that reliably yields protective immune responses (i.e. higher than the established CoP). A group of vaccinated animals will then be compared with a group of placebo vaccinated animals in a challenge study, as described in Appendix 1, to validate the CoP. The CoP thus established will then be used as a (surrogate) CoP in an immunobridging clinical trial. When the vaccine is capable of inducing immune responses exceeding the established CoP in human volunteers the vaccine potentially qualifies for registration with the FDA and European Medicines Agency (EMA) (27, 28). Because of these specific conditions in the study setup, these experiments are described in Appendix 3.

Pharmacokinetics (PK) of CoV antiviral therapeutics: For this type of study, animals will be administered with different dosages of an antiviral therapeutic as described in Appendix 4. If necessary, the therapeutic may be given at multiple time-points over a certain period. At regular time-points after administration, blood samples will be collected for measurement of the concentration of the compound, or active component thereof. In parallel, blood chemistry and hematology will be performed to monitor for adverse effects of the compound. General behaviour, health, body weight, and body temperature will also be monitored. Antiviral therapeutics are first tested *in vitro*, and in other animal models, like rodents, before they are evaluated for safety and efficacy in NHP.

Antiviral therapeutics: We will focus on the prophylactic and curative use of antiviral therapeutics as described in Appendix 5. In a therapeutic efficacy study protocol, we will make use of a standard viral inoculum (see Appendix 1), and an established antiviral compound dose will be used. In this protocol, the animals will first be infected, and at a later time-point be given the antiviral compound. In the protocol, the absence or decrease in viral load in nasal and tracheal swabs as well as Bronchoalveolar lavages (BAL) will be measured as an indicator of antiviral activity. Additionally, during the studies the animals will be monitored for adverse effects of the compound, including monitoring of general behaviour and

health. A group of non-treated animals will be included as infection controls. Antiviral therapeutics are first tested *in vitro*, and in other animal models, like rodents, and must have demonstrated some form of efficacy before they are evaluated for safety and efficacy in NHP.

The criteria to consider vaccine candidates or antiviral compounds for evaluation are:

- Demonstration that the vaccine or antiviral compounds are non-toxic,
- If specific host molecules are targeted, cross recognition of macaque homologues has been demonstrated,
- The vaccine or antiviral compound cannot be adequately tested in other than NHP animal models, for instance due to the mechanism of action (i.e. interaction with specific receptors), or the type of immunological assessment needed, This concerns only vaccines and antiviral compounds for which it is not possible to directly evaluate them in other species because interaction with specific host molecules is required that are only present in humans and in NHPs, but not in other species. In this case, we would like to add the additional requirement that for this type of vaccine or antiviral compound a similar strategy that targets slightly different molecules but uses the same mode of action has been evaluated and found to be effective in other species.
- The immunogenicity of vaccine candidates or effectiveness of antiviral compounds should have been proven in other species, unless this is not possible because the specific vaccine or antiviral compound modality used does not work in other species.

3.4.2 Provide a justification for the strategy described above.

Several research groups, including BPRC, have established nonhuman primate (NHP) models for infection with coronaviruses like SARS-CoV-1, SARS-CoV-2 and MERS CoV (16, 17, 20, 21, 30-46). Mostly widely used in CoV research are rhesus macaques and cynomolgus macaques and their susceptibility for infection with coronaviruses is well established.
Several animal species have been used to study coronavirus infection (31, 44, 47-50). However, of these different species, NHP have the advantage that they physiologically, anatomically, and immunologically most closely resemble humans. This has important implications, both for vaccine evaluation and antiviral therapeutics (Appendices 2, 3, 4 and 5), as well as for the interaction with coronaviruses since this is affected both by physiology and by the reaction of the innate and adaptive immune system. These aspects are important for the evaluation of vaccines and antiviral therapeutics. The proper evaluation of these vaccines and antiviral therapeutics requires adequate infection models in NHP, which is the purpose of the studies proposed here.

3.4.3 List the different types of animal procedures. Use a different appendix 'description animal procedures' for each type of animal procedure.

Serial number	Type of animal procedure
1	Development of a coronavirus infection model in NHP
2	Coronavirus vaccine evaluation in NHP
3	Coronavirus vaccine evaluation under the "Animal Rule"
4	Phamacokinetics (PK) of CoV antivirals in NHP
5	Antiviral efficacy of CoV antivirals in NHP

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