	Inventaris Wob-verzoek W21-04								
		wordt verstrekt				**weigeringsgronden**			
nr.	**Aanvraagdossier 14680**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
1	AVD14680 aanvraagformulier 11 maart 2021				x		x	x	
2	NTS			x					
3	Projectvoorstel				x		x	x	
4	dierproef 1				x		x	x	
5	dierproef 2				x		x	x	
6	Ontvangsbevestiging aanvraag en factuur				x		x	x	
7	E-mail-wisseling over aanvraagdocumenten				x		x	x	
8	E-mail-wisseling over aanvraagdocumenten				x		x	x	
9	DEC-advies 25 maart 2021				x			x	
10	Projectvoorstel herzien				x		x	x	
11	dierproef 1 herzien				x		x	x	
12	dierproef 2 herzien				x		x	x	
13	DEC-advies herzien				x			x	
14	NTS	x							
15	dierproef 1 herzien				x		x	x	
16	dierproef 2 herzien				x		x	x	
17	Reactie op vragen CCD				x		x	x	
18	Adviesnota CCD				x		x	x	x
19	E-mail-wisseling over ondertekening beslissing				x		x	x	
20	E-mail-wisseling over ondertekening beslissing				x		x	x	
21	E-mail beschikking				x		x	x	
22	Beslissing en vergunning 10 mei 2021				x		x	x	
23	Terugkoppeling DEC-advies				x		x	x	

Aanvraag
Projectvergunning Dierproeven
Administratieve gegevens

- U bent van plan om één of meerdere dierproeven uit te voeren.
- Met dit formulier vraagt u een vergunning aan voor het project dat u wilt uitvoeren. Of u geeft aan wat u in het vergunde project wilt wijzigen.
- Meer informatie over de voorwaarden vindt u op de website www.centralecommissiedierproeven.nl. of in de toelichting op de website.
- Of neem telefonisch contact op. (0900-2800028).

1 Gegevens aanvrager

1.1 Heeft u een deelnemernummer van de NVWA?
Neem voor meer informatie over het verkrijgen van een deelnemernummer contact op met de NVWA.

☒ Ja > *Vul uw deelnemernummer in* 10.2.g
☐ Nee > *U kunt geen aanvraag doen*

1.2 Wat voor aanvraag doet u?

☒ Nieuwe aanvraag > Ga verder met vraag 1.3

☐ Wijziging > Vul hiernaast het AVD nummer van uw vergunde project in en ga verder met vraag 2.1

☐ Melding > Vul hiernaast het AVD nummer van uw vergunde project in en ga verder met vraag 2.2

1.3 Vul de gegevens in van de instellingsvergunninghouder die de projectvergunning aanvraagt.

Naam instelling of organisatie	10.2.g			
Titel, voorletters en achternaam van de portefeuillehouder	Titel	Voorletters	Achternaam	☒ Dhr. ☐ Mw
	10.2.e			
E-mailadres contactpersoon	10.2.e			
Titel, voorletters en achternaam van de diens gemachtigde (indien van toepassing)	Titel	Voorletters	Achternaam	☒ Dhr. ☐ Mw
	10.2.e			
E-mailadres gemachtigde	10.2.e			

Vul de gegevens van het postadres in.

Straat en huisnummer	10.2.g	10.2.g
Postcode en plaats	10.2.g	10.2.g
Postbus, postcode en plaats	10.2.g	

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	10.2.e	☐ Dhr. ☒ Mw.
Functie	10.2.e	
Afdeling	Virology	

		Telefoonnummer	10.2.e
		E-mailadres	
1.5	*(Indien van toepassing)* Vul hier de gegevens in van de plaatsvervangende verantwoordelijke onderzoeker.	(Titel) Naam en voorletters	☒ Dhr. ☐ Mw.
		Functie	10.2.e
		Afdeling	Virology
		Telefoonnummer	10.2.e
		E-mailadres	10.2.e
1.6	*(Indien van toepassing)* Vul hier de gegevens in van de persoon aan wie de portefeuillehouder de verantwoordelijkheid inzake de algemene uitvoering van het project en de overeenstemming daarvan met de projectvergunning heeft gedelegeerd.	(Titel) Naam en voorletters	☐ Dhr. ☐ Mw.
		Functie	
		Afdeling	
		Telefoonnummer	
		E-mailadres	
1.7	*(Optioneel)* Vul hier de gegevens in van de Instantie voor Dierenwelzijn	Telefoonnummer	
		E-mailadres	
1.8	Is er voor deze projectaanvraag een gemachtigde?	☒ Ja > *Stuur dan het ingevulde formulier Melding Machtiging mee met deze aanvraag*	
		☐ Nee	

2 Over uw aanvraag

2.1	Gaat uw aanvraag over een *wijziging* op een vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn?	☒ Nee > Ga verder met vraag 3
		☐ Ja > Geef hier onder kort de wijziging en de onderbouwing daarvan weer. Geef in de originele formulieren (niet-technische samenvatting, projectvoorstel en bijlage dierproeven) duidelijk aan (bij voorbeeld in een andere kleur) waar de projectaanvraag wijzigt. Ga daarna verder met vraag 6.
2.2	Gaat uw aanvraag over een *melding* op een vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn?	☒ Nee > Ga verder met vraag 3
		☐ Ja > Geef hier onder weer wat deze melding inhoudt en ga verder met vraag 6

3 Over uw project

3.1	Wat is de geplande start- en einddatum van het project?	Startdatum	01 - 06 - 2021
		Einddatum (t/m)	31 - 05 - 2026
3.2	Wat is de titel van het project?	Evaluation of novel influenza vaccine candidates for immunogenicity and capacity to protect against influenza infection in macaques	
3.3	Wat is de titel van de niet-technische samenvatting?	Evaluation of novel influenza vaccine candidates for immunogenicity and capacity to protect against influenza infection in macaques	
3.4		Naam DEC	10.2.g
		Postadres	

Wat is de naam van de Dierexperimentencommissie (DEC) van voorkeur?

E-mailadres 10.2.g

4 Factuurgegevens

4.1 (indien factuuradres afwijkt van de gegevens uit vraag 1.3) Vul de gegevens van het factuuradres in.

Naam: Afdeling:

Straat: Huisnummer:

Postcode: Plaats:

Postbus: Postcode: Plaats:

E-mail:

4.2 (optioneel) Vul hier het ordernummer van de instelling in.

Ordernummer:

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

☒ Projectvoorstel Aantal bijlage(n) dierproeven 2

☒ Niet-technische samenvatting

Overige bijlagen, indien van toepassing

☐ Melding Machtiging

☐

6 Ondertekening

6.1 Print het formulier uit, onderteken het en stuur het inclusief bijlagen via de beveiligde e-mailverbinding naar de CCD en per post naar de Centrale Commissie Dierproeven (voor adresgegevens zie website)

Ondertekening door de portefeuillehouder namens de instellingsvergunninghouder of gemachtigde (zie 1.8). De ondergetekende verklaart:

- dat het projectvoorstel is afgestemd met de Instantie voor Dierenwelzijn.
- dat de personen die verantwoordelijk zijn voor de opzet van het project en de dierproef, de personen die de dieren verzorgen en/of doden en de personen die de dierproeven verrichten voldoen aan de wettelijke eisen gesteld aan deskundigheid en bekwaamheid.
- dat de dieren worden gehuisvest en verzorgd op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van richtlijn 2010/63/EU, behalve in het voorkomende geval de in onderdeel C van de bijlage bij het bij de aanvraag gevoegde projectvoorstel gemotiveerde uitzonderingen.
- dat door het ondertekenen van dit formulier de verplichting wordt aangegaan de leges te betalen voor de behandeling van de aanvraag.
- dat het formulier volledig en naar waarheid is ingevuld.

Naam 10.2.e

Functie Adjunct Directeur

Plaats 10.2.g

Datum 11 - 03 - 2021

Handtekening 10.2.e

NON-TECHNICAL PROJECT SUMMARY

Country	NL		*
Language	nl		*
EU submission	yes [1]		*
Title of the project	Evaluation of novel influenza vaccine candidates for immunogenicity		*
NTS identifier			
NTS national identifier			
Duration of the project	60	**(in months)**	*
Keywords			*
Keyword 1	Influenza		
Keyword 2	Vaccin		
Keyword 3	Effectiviteit		
Keyword 4	Infectie		
Keyword 5	non-humane primaten		

Purpose(s) of the project *

Objectives and predicted benefits of the project

Objectives of the project *

Griep wordt veroorzaakt door het influenzavirus. Jaarlijks krijgen tussen 2,5 en 10% van de mensen de griep. Meestal volgt na infectie een spoedig herstel, maar met name bij jonge kinderen, ouderen of bij mensen met longproblemen kan de infectie ernstig verlopen. Naar schatting overlijden jaarlijks tussen de 250.000 en 500.000 mensen wereldwijd aan de griep. Daarom krijgen kwetsbare groepen mensen, waaronder ouderen, jaarlijks een vaccin; de griepprik. Het probleem bij de griep is dat het virus dat de griep veroorzaakt constant verandert. Doordat het maken van een nieuw vaccin minimaal een half jaar duurt kan het gebeuren dat het griepvirus in de tussentijd veranderd is en het vaccin niet goed meer beschermt. Bovendien kunnen ook heel nieuwe varianten ontstaan door overdracht van griepvirussen tussen vogels, varkens en de mens. Omdat mensen nog niet eerder met dergelijke

Potential benefits likely to derive from this project *

Het uiteindelijke doel van de experimenten die in dit project worden uitgevoerd is om een vaccin te verkrijgen dat kan beschermen tegen een groot aantal griepvarianten en indien mogelijk ook tegen vogelgriep. Een dergelijk vaccin kan veel levens redden bij een nieuwe pandemie en biedt een betere bescherming van kwetsbare groepen in de samenleving. Bovendien is de jaarlijkse griepprik dan niet meer nodig en kan volstaan worden met een griepprik eens in de 5 jaar.

Predicted harms

In what procedures will the animals typically be used *

Sedatie, bij iedere handeling (gedurende 15-60 minuten). Implanteren / verwijderen van implantaat voor meten temperatuur, chirurgisch 1x in 1 x uit (± 60 minuten). Vaccinatie (maximaal 6x, 10 minuten). Bloedafnames (maximaal 4x na iedere vaccinatie en 10x na elke virusinfectie, 10 minuten). Bronchoalveolaire lavage (maximaal 3x na iedere vaccinatie en 5 x na elke virusinfectie, 30 minuten). CT scan (maximaal 5x na elke virusinfectie, 15min). Virusinfectie (maximaal 2x, 10 minuten). Bepaling virusload (neus, keel swabs maximaal 10x na infectie).

Expected impacts/adverse effects on the animals *

De dieren zullen stress ondervinden ten gevolge van een verandering in de huisvesting en de biotechnische handelingen. Ook kunnen de dieren pijn ondervinden door de biotechnische handelingen. Deze handelingen worden echter onder verdoving uitgevoerd. Ook kunnen de dieren ziek worden door de experimentele infectie met het griepvirus. Het nadelig gevolg van de biotechnische handelingen duurt over het algemeen 1 dag, met uitzondering van de chirurgische ingreep, waarvan het effect maximaal 1 week kan duren.

Expected harms *

Fate of animals kept alive

Reasons for the planned fate of the animals after the procedure *

Na afloop van het experiment zullen de dieren over het algemeen hersteld zijn van de infectie en kunnen dan deel blijven uitmaken van de experimentele dieren op het instituut en worden hergebruikt voor niet griep-gerelateerde studies. Echter, als moet worden nagegaan of het vaccin eventueel schadelijke effecten op het lichaam kan hebben of indien de dieren ten gevolge van de griepinfectie blijvende schade aan de longen krijgen, dan zullen ze op een humane wijze worden gedood.

Application of the Three Rs

1. Replacement *

Het is nog niet mogelijk om de beschermende werking van vaccins zonder gebruik van proefdieren te bepalen. Het afweersysteem is dermate ingewikkeld dat dit nog niet in het laboratorium kan worden nagebootst. Ook de beschermende werking van het vaccin tegen virusinfectie is complex en wordt bepaald door hoe de diverse componenten van het afweersysteem op lokaal niveau in de long de juiste type cellen kunnen beschermen tegen infectie en het individu kunnen beschermen tegen griepverschijnselen.

2. Reduction *

Voordat een griepvaccin in apen wordt getest is het al uitgebreid getest in het laboratorium en in andere diersoorten, bijvoorbeeld in muizen. Uit dit eerdere onderzoek moet zijn gebleken dat het vaccin veilig is en dat het vaccin voldoende werkzaam is om in een afweerreactie op te roepen na injectie. Hierna worden alleen de meest belovende vaccinkandidaten in apen getest. Het aantal benodigde dieren wordt per experiment bepaald aan de hand van statische analyses. Dit aantal zal afhangen van de eigenschappen van het vaccin en van het te gebruiken testvirus. Waar mogelijk zullen meerdere vaccins tegelijk getest worden, waardoor maar één controlegroep nodig is. Het aantal dieren in de controlegroep zal zoveel mogelijk worden beperkt door gegevens te gebruiken van dieren die reeds eerder geïnfecteerd zijn bij het opzetten van de virusinfectie modellen.

3. Refinement *

Alle handelingen worden uitgevoerd onder verdoving. Waar nodig wordt bovendien pijnstilling gegeven. De dieren worden getraind om zoveel mogelijk vrijwillig mee te werken aan de verdoving. Tijdens de studie worden de dieren dagelijks geobserveerd en tijdens de griepinfectie tweemaal daags. De ziekteverschijnselen worden genoteerd op een scorelijst. Wanneer ernstige ziekteverschijnselen optreden of wanneer een bepaalde score overschreden wordt, worden de dieren direct op een humane wijze gedood om verder ongerief te voorkomen.

Explain the choice of species and the related life stages *

Onderzoek naar de werkzaamheid van griepvaccins kan in diverse dieren worden uitgevoerd. Alleen in de laatste fase van de vaccinontwikkeling is testen in apen nodig, omdat deze dieren wat betreft de anatomie van de luchtwegen, het afweersysteem en vatbaarheid voor influenza het meest op de mens lijken. Andere proefdieren, zoals muizen zijn in deze fase van het onderzoek niet geschikt omdat humane griepvirussen in muizen vaak niet een goede infectie geven en zowel muizen als fretten wat betreft hun afweersysteem op diverse punten afwijken van de mens. Daarom is in apen de kans het grootst dat eventuele onverwachte nadelige effecten alsnog opgespoord kunnen worden en een goede voorspelling gedaan kan worden wat betreft werkzaamheid bij de mens. Dit geldt met name

Project selected for Retrospective Assessment

Project selected for RA?	no [0]
Deadline for RA	

*

Reasons for retrospective assessment

Contains severe procedures	
Uses non-human primates	
Other reason	

Explanation of the other reason for retrospective assessment

Additional fields

National field 1	
National field 2	

National field 3	
National field 4	
National field 5	
Project start date	
Project end date	
Project approval date	
ICD code 1	
ICD code 2	
ICD code 3	
Link to the previous NTS version outside the EC system	

Describe the objectives of the project (for example, addressing certain scientific unknowns, or scientific or clinical needs).

What are the potential benefits likely to derive from this project? Explain how science could be advanced, or humans, animals or environment may ultimately benefit from the project. Where applicable, differentiate between short-term benefits (within the duration of the project) and long-term benefits (which may accrue after the project is finished).

In what procedures will the animals typically be used (for example, injections, surgical procedures)? Indicate the number and duration of these procedures.

What are the expected impacts/adverse effects on the animals, for example pain, weight loss, inactivity/reduced mobility, stress, abnormal behaviour, and the duration of those effects?

What species and numbers of animals are expected to be used? What are the expected severities and the numbers of animals in each severity category (per species)?

What will happen to the animals kept alive at the end of the procedure?

Please provide reasons for the planned fate of the animals after the procedure.

State which non-animal alternatives are available in this field and why they cannot be used for the purposes of the project.

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce the number of animals to be used, and principles used to design studies. Where applicable, describe practices that will be used throughout the project to minimise the number of animals used consistent with scientific objectives. Those practices may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms to take up emerging refinement techniques during the lifetime of the project.

Applicable to those MS where this information is required by the legislation.

Compulsory!

Compulsory!

Compulsory! Field will not be published. Indicate if project is falling inside the scope of the Directive or not

Compulsory! Maximum length is 500 characters.

Unique NTS identifier assigned by the central EC system. To be filled-in only when updating NTS already sto

Field will not be published. National identifier of the NTS. Maximum length is 500 characters.

Compulsory! Project duration expressed in months. Must be a whole number between 1 and 60!

Compulsory! At least one keyword must be entered!

Maximum length is 50 characters including spaces. May consist of more than one word.

Maximum length is 50 characters including spaces. May consist of more than one word.

Maximum length is 50 characters including spaces. May consist of more than one word.

Maximum length is 50 characters including spaces. May consist of more than one word.

Maximum length is 50 characters including spaces. May consist of more than one word.

Compulsory! Please fill-in 'Purpose of the project' sheet. At least one purpose must be chosen!

Compulsory! Maximum length is 2500 characters.

Compulsory! Maximum length is 2500 characters.

Compulsory! Maximum length is 2500 characters.

Compulsory! Maximum length is 2500 characters.

Compulsory! Please fill-in 'Expected harms' sheet. At least one row must be filled-in!

Please fill-in 'Fate of animals kept alive' sheet if applicable.

Compulsory! Maximum length is 2500 characters.

Compulsory! Maximum length is 2500 characters.

Compulsory! Maximum length is 2500 characters.

Compulsory! Maximum length is 2500 characters.

Compulsory! Maximum length is 2500 characters.

To be filled-in by competent authority!

Compulsory!

Whether the particular field is applicable or not, depends on national rules.
Field will not be published. Maximum length is 2500 characters.

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To be used in transition period where the previous version of the NTS might not exist in the EC system. Ma

ɔred in the EC system.

27-05-2022 or 27/05/2022)
27-05-2022 or 27/05/2022)
'epending on your system settings (e.g. 27-05-2022 or 27/05/2022)
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ximum length is 500 characters.

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 (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10.2.g
1.2	Provide the name of the licenced establishment.	10.2.g
1.3	Provide the title of the project.	Evaluation of novel influenza vaccine candidates for immunogenicity and capacity to protect against influenza infection in macaques

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.

Influenza epidemics are estimated to result in infection of 2,5-10% of the world population every year, causing 2-5 million cases of severe illness and 250.000-500.000 deaths (1). Vaccination is considered the

most effective measure against the influenza disease and, as such, it is recommended by the European Council (2) and implemented in all EU/European Economic Area member states. The main problems of the current influenza vaccines are; a) they are not very effective in the elderly, b) they only protect against highly homologous strains, while circulating influenza virus strains constantly evolve as a result of antigenic drift, c) they do not protect against new pandemic strains that emerge as a result of recombination between different viral strains found in animal reservoirs, d) they do not protect against highly pathogenic avian influenza virus (3-5). These problems are amplified by the cumbersome current production methods, which involves growing the virus on eggs to prepare inactivated- or live attenuated- influenza vaccines. The 6 months required for vaccine manufacture means that the vaccines have to be based on predictions about which virus strains will circulate during the next influenza season. A mismatch between the vaccine and the actually circulating influenza strain(s) however, results in lower vaccine effectiveness as shown for the 2014-15 influenza season with regard to the H3N2 strain (6). New vaccine strategies that can provide broader protection and cover a range of seasonal influenza strains as well as pandemic and avian influenza virus strains are urgently needed (7). These so called "universal" influenza vaccines are directed at either a) inducing broadly neutralizing antibodies by targeting the relatively conserved stem region of the haemagglutinin (HA) subunit, which is responsible for virus entry into the target cell, b) induction of antibodies to the neuraminidase (NA) surface glycoprotein (8), c) inducing protective T-cell responses that are usually directed against more conserved proteins of the virus and therefore provide broad recognition (9-12). Retrospective epidemiological studies as well as studies in experimentally infected volunteers indicate that in the absence of antibodies, cellular immune responses can have a protective effect (9, 13, 14). Their role in cross-protection was demonstrated in a H1N1 infection study in non-human primates (NHP) (12). More recently the appreciation of the importance of non-neutralizing anti-influenza antibodies in conferring broad protection against variant strains, especially in the case of avian influenza viruses, has prompted research into their mechanism of action (via antibody dependent cellular cytotoxicity (ADCC), antibody dependent phagocytosis (ADP) (15) or complement activation (16-18) and vaccine strategies to induce these antibodies. New methods for faster vaccine production, the induction of T-cell responses and improvement of vaccine responses in the elderly have involved application of DNA, virus like particles (VLP), recombinant viral vectors and strategies to target vaccines to the appropriate antigen presenting cells (5, 19-23) and more recently the advent of mRNA-vaccines which facilitate fast responses as exemplified by COVID-19 vaccines from Pfizer and Moderna (24). Evaluation of the immunogenicity of these vaccines requires additional methods, besides the standard antibody ELISA, micro-neutralization and haemagglutination inhibition assays. Especially, proper assessment of adaptive cellular immune responses and function of the innate immune system in relation to non-neutralizing antibody effector function and induction of immune responses by these new vaccine modalities is needed (7).

Despite progress in the development of universal influenza vaccines, only few universal influenza vaccine candidates have progressed to clinical trials (25-27). In order to improve progress in the field of (universal) influenza vaccines, the National Institute of Allergy and Infectious Diseases (NIAID) has prepared a strategic plan outlining areas in our knowledge about influenza infection and immunity that require further investigation (7). Animal models have played an important role in preclinical evaluation of candidate influenza vaccines (28-30) and are still required during clinical development (7). While a number of species have been used, the most commonly used models to assess immunogenicity and efficacy against influenza virus infection are the mouse, ferret and NHP models. There are important differences between these species in immune function and susceptibility to influenza virus infection. Mice are not naturally susceptible to influenza and usually lethal infection models are used, that are based on extremely adapted human viruses. Ferrets are naturally susceptible to most human influenza viruses and recapitulate the natural course of infection. However, infection is often restricted to the upper airways and this model has the disadvantage that limited immunological tools are available, especially to study innate and cellular adaptive immune responses, which are especially important in the evaluation of "universal" vaccine strategies (31). NHP play an important role in influenza virus research and have been used to study pathogenesis as well as efficacy of preventive and therapeutic intervention strategies (32). Of the different animal models used in influenza virus research, NHP have a unique close homology to humans in most components of their immune system (33-35). For instance, similar T and B-cell subsets have been described in NHP (36). Moreover, the immunoglobulin gene germline repertoire is highly conserved between macaques and humans, which is important when induction of broadly neutralizing antibodies by new "universal" influenza vaccine strategies is studied (37, 38). In addition, structure and function of Fc receptors, which are

essential for the function of non-neutralizing antibodies, show many homologies between macaques and humans (39). Only very limited information is available on Fc receptors in ferrets and only reagents to detect the IgA receptor are available (31). Finally in NHP the innate immune system, including molecular pathways and antigen presenting cell subsets, are much more homologous to humans than what is seen in mice (34). NHP not only most closely reflect the human physiology, but also resemble humans in their clinical symptoms, limited pathology, pattern of viral replication, fever and cytokine and chemokine responses following influenza virus infection (40).
In conclusion, the strong immunological and physiological resemblances to humans make NHP a unique model in pre-clinical safety, immunogenicity and efficacy evaluation, particularly in relation to the new influenza virus vaccine delivery platforms being developed and for the evaluation of the important broadly neutralizing antibody, non-neutralizing antibody and cellular broadly protective immune responses. Evaluation in NHP is essential before the new "universal" influenza candidates can be evaluated in humans. Moreover, although challenge studies have been performed in humans (41), these are limited to the milder influenza strains and hampered by pre-existing immunity caused by previous exposures to influenza virus (42) limiting the value of the vaccine efficacy data that can be obtained.

Under project licence AVD10.2.g experiments were performed to refine the influenza virus infection model in macaques by evaluating aerosol delivery for infection with pandemic H1N1 (pH1N1) (43) influenza virus and highly pathogenic avian H5N1 influenza virus10.2.g
Experimental infection in NHP is typically performed by either intra-tracheal, or a combination of intra-tracheal, intra-nasal and intra-ocular virus inoculation. However, influenza virus infection in humans is assumed to be mainly caused by exposure to aerosols or droplets that enter the airways either via respiration, inhalation or via contact with contaminated surfaces (42). Our studies showed that aerosol delivery resulted in infection of the upper as well as lower respiratory tract for both pH1N1 and H5N1 influenza virus. However, infection with pH1N1 after aerosolized exposure resulted in lower levels of immune activation and inflammation than infection via combined intra-bronchial, intra-nasal and oral delivery. For H5N1 infection via aerosol exposure led to less severe disease than combined-route exposure. Hence, the route of exposure has clear consequence for disease pathogenesis. This allows for a fine tuning of the applied infection model in relation to the research question. When vaccine mediated protection against infection is studied then aerosol exposure would be the best option as it mimics best the situation in humans and allows adequate detection of reduction in virus replication. However, if protection against infection is difficult to achieve then the second objective should be protection against disease and in this case a combined-route exposure model should preferably be used. In conclusion aerosol delivery is now a well established infection model. However, it will still be needed to set up infection models for new influenza viruses that have not been used in NHP before.

The current project licence AVD10.2.g runs until 10.2.g 2022. However, the institute has recently been granted a project in which a novel mucosal influenza vaccine strategy will be evaluated, consisting of a systemic immunization with DNA followed by an oropharyngeal spray immunization with an adenovirus expressing the vaccine antigens. The hypothesis is that with this method strong local immune responses will be induced in the lungs. This prime/boost strategy involves in total a 83 week study period, which falls beyond the end date of the current project licence.

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 goal of this project is to evaluate novel influenza virus vaccine candidates for occurrence of adverse effects, immunogenicity and capacity to protect against influenza virus infection in macaques. Both the capacity of new vaccine candidates to elicit a broad immune response, that not only protects against a homologous virus that is similar to the vaccine but also against heterologous viruses, as well as the immunogenicity of new influenza vaccine delivery platforms will be evaluated under this project application. The ultimate goal is to develop an influenza vaccine that can induce an immune response that is sufficiently broad to provide protection against seasonal influenza virus variants over a 5 year period (to obviate the need to vaccinate every year), is effective in elderly and can provide a degree of heterogeneous protection

that would lead to reduced morbidity and mortality caused by pandemic as well as highly pathogenic avian influenza viruses.
The main goal can be divided in 2 sub-goals:
1. Vaccine evaluation. Immunogenicity and efficacy to protect against infection will be evaluated using an appropriate influenza virus challenge strain in relation to the type of vaccine being used.
2. Set-up infection model for influenza viruses that have not yet been used in NHP at our institute and that are needed for vaccine evaluation.

3.2.2 Provide a justification for the project's feasibility.

At our institute we have been performing vaccine evaluation studies in NHP for over 20 years. Most vaccine candidates were directed against human immunodeficiency virus, hepatitis B virus, hepatitis C virus, and tuberculosis. Since 2012 we have been working on influenza virus infection in macaques and the evaluation of vaccines against influenza (43-48). We have the appropriate facilities and experience to work with pathogenic viruses, including influenza virus, at DM-3 and ML-3 biosafety conditions. In addition, we have the appropriate immunological assays for assessment of cellular, humoral and innate immune responses against influenza. Our long-standing experience with pathogenic viruses, including influenza, and with vaccine evaluation guarantees that the animal studies describe in this proposal 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?

Annual influenza virus epidemics cause considerable morbidity and mortality world-wide and especially affect more vulnerable groups like young children, the elderly and people with pulmonary diseases. In addition, there is the continuous threat of the emergence of new viral recombinants that can cause a pandemic. Previous pandemics, especially the 1918 pandemic, have caused millions of deaths. Finally, avian influenza viruses are widely spread and can occasionally infect humans. Mutations that lead to a strong increase in transmission have been described (49), indicating that also these viruses pose a continuous threat to the human population. Current influenza vaccine strategies and vaccine production methods are not adequate to deal with such emergencies. Even for protection against the current seasonal influenza viruses, annual vaccination of risk groups is necessary. Hence a vaccine that could offer protection against a broader range of viruses, including yet unknown recombinants and avian influenza would be of great benefit to the community. In addition, annual vaccination would no longer be necessary since a broadly protective vaccine would be effective over a period of at least five years against newly emerging variants. This has led the EU and the USA to invest in the development of so called "universal" influenza vaccines that would fulfil these criteria. Both the application of new delivery methods, for instance in the form of DNA, mRNA or viral vectors, as well as new vaccine modalities, such as mucosal delivery, require evaluation in appropriate animal models so that the level as well as the mechanism of protection can be adequately established, before these new vaccines can be tested in clinical studies.

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

The stakeholders for an influenza vaccine are the aforementioned target groups for whom protection from influenza infection and disease would increase their health and well-being. The vaccination of risk-groups and the resulting decrease in influenza burden would also be of great societal benefit. The animals involved in the experiments will not benefit and will experience moderate discomfort as a result of the experiments.

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.

In order to evaluate that; a new influenza virus vaccine candidate is immunogenic, has the capacity to protect against infection and that no adverse effects occur, a vaccine evaluation experiment will be performed according to well established procedures, as described in Appendix 1. Typically, one or a number of immunizations are given over a certain period of time. After immunization the induction of T-cell and antibody immune responses is measured. The strength of these responses as well their breadth, i.e. the capacity to recognize not only homologous viruses that are similar to the vaccine but also heterologous viruses, is determined. Subsequently, the capacity of the vaccine to protect against infection is tested by experimental infection of the animals with influenza virus. The choice of the virus strain to be used for experimental infection will depend on the outcome of the evaluation of the immune responses and the stage of development of the vaccine. An entirely new vaccine concept may require that in first instance protection against infection by a homologous virus strain is tested. However, for most vaccine candidates protection against infection with a heterologous virus will be tested. Experimental infection will only be performed when the immunization has induced virus inhibiting antibody and cellular immune responses against the virus used for experimental infection so that protection against infection is possible. Whether protection is actually 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 requires experimental infection of an animal. Also mucosal delivery methods and combinations of systemic as well as mucosal delivery can only be evaluated in a complex multi-organ environment. Ideally, the vaccine should provide a robust level of protection and be able to reduce disease and virus multiplication in animals that receive a standard virus dose via aerosol delivery. If protection against infection is unlikely and protection against disease or early immune inflammation needs to be established then combined exposure to the upper respiratory tract and lungs needs to be applied. A virus dose must be chosen that is not unrealistically high (above 10^7 infectious particles), but high enough to lead to infection of all control animals.
In case proper evaluation of the capacity of a vaccine to protect against infection requires that a virus has to be used that has not been tested before in macaques at our institute then this virus will first be tested in a small number of animals. This to determine if all animals become infected and what the amount of virus multiplication is (Appendix 2). Either aerosol, intra-bronchial, oral, intranasal and intraocular inoculation is used, matching the method that will be used for the vaccine evaluation (Appendix 1).

3.4.2 Provide a justification for the strategy described above.

Vaccine candidates that fulfil the criteria for evaluation in NHP may be directly tested in a vaccine evaluation study (Appendix 1), if the influenza virus that will be used for establishing capacity of the vaccine to protect against infection has already been used in NHP at our institute. If this is not the case, the virus has to be tested first in an influenza virus infection study (Appendix 2). Also when efficacy against low dose aerosol infection has to be tested, a preceding influenza virus infection study (Appendix 2) is necessary.
Vaccine evaluation in macaques.
For this type of experiment animals will be immunized either once or they will receive a number of immunizations over a certain period of time. During the study animals will be monitored for adverse effects of the vaccine, including monitoring of general behaviour and health. Blood and occasionally nasal washes and lung lavages will be taken to measure induction of systemic as well as local immune responses. When adequate immune responses are induced that indicate that protection against infection might be achieved, the efficacy against infection will be tested by experimental infection with influenza virus. A group of non-vaccinated animals will be included as infection controls.
Vaccines that will be tested in NHP have to be in the final stages of preclinical development and require this last validation step in order to assure that there are no adverse effects that were missed in the preliminary studies in other species and that they are effective in an animal species that has an immune system closely related to humans. Additional criteria for vaccine evaluation are: a) the vaccine strategy must be novel, for instance with regards to choice of antigen, formulation, route of application, that have not been tested before in similar NHP studies, b) demonstration that the vaccine or vaccine components are non-toxic, c) when specific host molecules are targeted then cross recognition of macaque homologues must have been demonstrated, d) the vaccine cannot be adequately tested in other than NHP animal models, for instance due to the mechanism of action or the type of immunological assessment needed, e) preferably immunogenicity of vaccine candidates should have been proven in other species, unless this is not possible because the specific vaccine modality used does not work in other species.

Influenza virus infection in macaques.
In order to establish infectivity and pathogenicity of a new virus that has not been tested previously in NHP at our institute, a small number of animals will be infected and monitored for clinical symptoms, fever, body weight and changes in blood parameters. Development of lesions in the lungs will be monitored by (PET)-CT analysis. Nasal and tracheal swabs will be taken to determine if the animals have become infected and determine the magnitude of virus multiplication. Proper application in vaccine evaluation requires that in these infection studies > 80% of the animals become infected and that the amount of virus produced in the trachea over the infection period is clearly measurable and that the variation between the animals is sufficiently low to allow measurement of reduction in virus load in vaccinated animals with less than 10 animals per group. In case these parameters are not achieved then the experiment will be repeated with a 10-100 times higher dose. In case any of the animals reaches the humane endpoint within the first four days after infection then a 10-100 times lower virus dose will be evaluated. The same criteria will be used to determine whether the aerosol infection model is sufficiently robust.

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	Influenza vaccine evaluation in macaques
2	Establishment of a new influenza infection model in macaques
3	
4	
5	
6	
7	
8	
9	
10	

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Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedur
- For more information on the project proposal, see th Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10.2.g
1.2	Provide the name of the licenced establishment.	10.2.g
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 1 — Type of animal procedure: Influenza vaccine evaluation in macaques

2 Description of animal procedures

A. Experimental approach and primary outcome parameters
Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

We have developed a general study protocol for the evaluation of influenza vaccines in macaques. Temperature and potentially movement and heart rate will be measured by telemetry. A device that records and transmits these parameters is surgically placed in the abdominal cavity at least 4 weeks before the infection. Subsequently the animals are immunized either once or they receive a number of immunizations over a certain period of time. Although the vaccines that are used in these studies have already been extensively evaluated in other animals and are expected to give no or only very limited adverse effects, macaques will be monitored for possible changes in general behaviour and health. The site of immunization will be checked for local reactions and blood will be drawn to measure clinical chemistry and haematology parameters. Before, between and after immunizations, blood and occasionally nasal washes and lung lavages will be taken to measure induction of systemic as well as local immune responses. Both T-cell and antibody responses will be measured against several influenza viruses in order to establish the strength and breadth of the immune response. When adequate immune responses are induced that indicate that protection against infection might be achieved, the efficacy against infection will be tested by experimental infection with influenza virus. A group of non-vaccinated animals will be included as infection controls. Usually only one virus will be used for experimental infection. The choice of the virus strain to be used will depend on the outcome of the evaluation of the immune responses and the stage of development of the vaccine. An entirely new vaccine concept may require that in first instance protection against infection by a homologous virus strain is tested. However, for most vaccine candidates protection against infection with a heterologous virus will be tested. All animals in a specific vaccine group will be challenged with the same virus. If protection against two different viruses needs to be established than two groups of animals are immunized with the same vaccine and subsequently group 1 will be challenged with virus 1 and group 2 with virus 2. If animals are protected against infection, then a second challenge with a different virus may be performed. In this case an additional challenge control group is needed. In this manner the breadth of the immune response is both measured *in vitro;* via the evaluation of the induced immune response and *in vivo* via experimental infection with different virus strains. The infection will be performed as described in appendix 2.

The primary outcome parameters are:

- Absence of unexpected reactogenicity of the vaccine: effects of the vaccine on general behaviour, health, local reactions and blood parameters.
- Immunogenicity: Induction of cellular and humoral immune responses. The type and strength of the induced responses will determine if the objectives of the vaccine strategy are achieved and whether immune responses are sufficiently strong to proceed with viral challenge.
- Efficacy: Capacity to protect against viral challenge will be established in terms of: reduction in clinical symptoms, fever, virus multiplication and changes in blood parameters.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

A telemetry device is surgically placed in the abdominal cavity at least 4 weeks before the infection takes place. This time frame is necessary for full recovery of the animals and to allow adequate recording of body temperature and/or heart rate and activity during a two to three-week period to establish normal values before immunizations start. Animals will receive one or more immunizations. If multiple immunizations are given then typically a 4 to 8-week time interval is used, although occasionally a longer time frame is needed between immunizations when different vaccine modalities are used for priming and boosting of the immune response. In rare occasions these limits may have to be exceeded, with a maximum of 6 immunizations. Specific rationale will then be presented to the animal welfare body (AWB). Immunizations will be done either by intradermal injection, intramuscularly, subcutaneously, intravenously, intranasally, intra-tracheally, intra-bronchially using a bronchoscope or via aerosol using a nebulizer. All vaccines have to be sterile and have to be given under aseptic conditions. At regular time intervals, usually two and four weeks after every immunization, blood is drawn to measure systemic adverse effects, which includes measurement of clinical chemistry and haematology, and to measure induction of cellular and humoral immune responses. The two-week interval after immunization is chosen, because this is optimal for measuring cellular immune responses, while four weeks is optimal for measuring humoral immune responses. In some studies, nasal washes and lung lavages (BAL) are taken after immunization in order to measure induction of local immune responses. BAL will be maximally collected three times after each immunization, resulting in total in 18 BAL collections if animals are immunized six times. Immune responses recorded after the final immunization will be used to decide whether protection against viral challenge can be reasonably expected. If these responses are inadequate then the study will be stopped and animals may be re-used in other non-influenza virus related experiments. Otherwise, experimental challenge will usually take place between 4 and 8 weeks after the last immunization. This time period is needed to allow immunological memory to form after the last immunization. Influenza infection may be done by intra-bronchial or intra-tracheal inoculation alone or in combination with oral, intranasal and intraocular inoculation or via aerosol using a nebulizer (as described in appendix 2). Clinical symptoms will be monitored twice daily during the infection phase. Nasal and tracheal swabs will be taken before infection, frequently during the first days after infection (daily and then every other day) and subsequently less frequently until day 21, to measure virus multiplication in the upper airways. Lung lavages may be taken at selected time points (max 5 times) to measure virus multiplication in the lungs. Blood is taken simultaneously with the swabs, to monitor changes in clinical chemistry and haematology parameters, leucocyte subsets and cytokine production. At the same time points body weight and physiological parameters (for instance pulse rate, blood pressure) are recorded and imaging (for instance CT or PET-CT) is performed to measure lung infiltration. After the virus is cleared (usually at day 21 after infection) animals may be exposed to a second challenge virus. All handling will then be repeated. However, this will be rarely the case and specific argumentation will be presented to the AWB. In general animals are either returned to the experimental stock or they are euthanized and a full necropsy is performed in order to establish lung pathology. Euthanasia is only performed when assessment of lung pathology is required to establish vaccine safety and in case pathogenic influenza viruses are used that are known to cause persistent lung pathology. Some telemetric devices, that have been designed to measure multiple parameters, can be quite large and require a substantial operation. These will not be removed and the animal will be euthanized instead. A small telemetric device can be surgically removed and animals may be re-used. A specific rational has to be provided to the AWB for using these devices. In case an animal should reach the humane endpoint during the study it will be immediately euthanized and a full necropsy will be performed to establish lung pathology and virus multiplication in the respiratory tract.

The details of each study, regarding the interval between the immunizations, the number and time points of sampling, the specific criteria to proceed with a viral challenge, the time interval between the last immunization and viral challenge will depend on the actual type of vaccine that is being tested and this will be submitted to the AWB.

Table. Maximum number of repeats per procedure.

Procedure	Maximum	Duration
Sedation	60	15-60 min
Recorder in/out	2	60 min
Vaccination	6	10 min
Blood sample	44	10 min
Bronchoalveola lavage (BAL)	28	30 min
Infection	2	10 min
Swabs	20	10 min
CT-scan	10	15 min

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

The number of animals will be based on statistical power analysis. Calculations take into account the number of animals needed to measure statistically significant induction of immune responses in relation to unvaccinated controls or, in case different vaccines are compared, whether significant differences between the vaccine groups can be obtained. In addition, calculations are performed to establish the number of animals needed to obtain a significant reduction in virus load in the trachea between the vaccine groups and the challenge control group. Experience in previous influenza vaccine evaluation studies performed at our institute indicates that statistically significant reductions in virus multiplication can be obtained with six animals per group. However, in order to be able to measure differences between the vaccine groups themselves, more animals per group may be needed. Only the minimum number of animals required will be used. Since historical data are available on infection in non-vaccinated animals (appendix 2), usually less animals can be used in the non-vaccinated challenge control group than in the vaccine groups.

B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
1	Rhesus or cynomolgus macaque	Purpose bred	adult	90	M / F	no	Not applicable

Provide justifications for these choices

Species	Macaque species have been used in several influenza vaccine studies (1-6). The most frequently used species are the rhesus- (*Macaca mulatta*) and cynomolgus monkey (*Macaca fascicularis*). Both species are semi-permissive to influenza infection. Therefore, both rhesus and cynomolgus macaques can be used for influenza vaccine evaluation studies.
Origin	All animals are purpose bred. They are either bred at our institute or obtained from a certified supplier.
Life stages	Adult animals will be used because these allow larger volumes of blood to be collected.
Number	90 macaques in total (either cynomolgus or rhesus). The number of animals requested is based on the assumption that each study will contain two vaccine groups and 1 control group, with 10 animals per group. The group size will be determined per experiment, based on power calculation specific for the experiment. As stated above, probably fewer animals will be needed in the non-vaccinated challenge control groups and also in the vaccine groups in some of the studies. In all, we anticipate performing 3 such studies over a 5-year period.

Gender	Adult male and female animals can be used. Since there are immunological differences between males and females (7, 8), we prefer that for each individual experiment either all animals are male or all are female, in order to minimize the variation within the experimental test group, thereby increasing the probability of finding statistically significant differences between the experimental groups. This choice is also important with regard to the amount of blood needed to perform all assays. Male animals are usually larger than female animals and therefore more blood per time point can be drawn from males. In case assays need to be performed which consume large amounts of blood, male monkeys are preferred over females.
Genetic alterations	Not applicable
Strain	Not applicable

C. Accommodation and care

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

☒ Yes

☐ No > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

☒ Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

After placement of the recording device in the abdomen and after removal, which is needed in case the animal will not be euthanized after completion of the experiment, animals will receive analgesics for as long as necessary, typically 3 days. In previous studies we have observed that animals can experience some body temperature elevation during the first days after insertion of the recording device, but have recovered very well within 1 week after the operation. Pain relieve will also be applied when substantial induration is seen at the site of vaccine injection. In case of the latter, analgesics known not to interfere with the induction of the vaccine response will be used.

Describe which other adverse effects on the animals' welfare may be expected?

1. Discomfort because of insertion or removal of the temperature recording device
2. Discomfort due to injection
3. Discomfort due to lung lavages
4. Discomfort due to virus installation
5. Discomfort due to PET-CTs
6. Stress because of sedation and recovery
7. Reduced food intake during the first days after infection
8. Disease symptoms due to the infection

Explain why these effects may emerge.

1. The surgery needed for insertion and removal of the temperature recording device will cause pain and some local inflammation.
2. When vaccines are given by injection, this can cause local pain and irritation.
3. For the lung lavages a bronchoscope is used. Insertion will cause irritation.
4. When virus is given intra-bronchially a bronchoscope is used and this combined with the inoculum volume will cause irritation. Aerosol application implies that animals have to be sedated and forced to breathe via a nebulizer device and this can give irritation.
5. For the PET-CT animals are sedated, intubated and mechanical ventilation with a forced breathing pattern is applied. No adverse effects are expected from the scan itself
6. Animals will be repeatedly sedated for vaccine delivery, blood sampling, virus infection, collection of swabs, CTs and lung lavages. Nausea can sometimes be observed during recovery from the sedation.
7. Especially during daily sedation during the first 2 days after infection food intake might be reduced.
8. Influenza infection can cause fever, coughing, sneezing, nose discharge, laboured breathing, increased breathing rate, loss of appetite, inactivity.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

1. Surgery will be done under anaesthesia and after surgery analgesics will be applied.
2. Animals will be sedated for vaccine delivery. Only rarely are strong adverse effects seen. Should granuloma formation be observed, then the animal will be sedated, the wound will be cleaned and analgesics are applied if necessary following veterinary consultation.
3. For the lung lavages animals are first deeply sedated and then receive a local muscle relaxant.
4. The same procedure as described under 3 will be followed
5. The animals are first deeply sedated and then receive a local mucosal relaxant before intubation. The mechanical ventilation & forced breathing patterns will be monitored and adapted on each animals characteristics.
6. Recovery of the animals will be monitored and the veterinarian will be consulted in case of problems.
7. Animals will receive supportive feeding with dense "brokkenballen".
8. Animals are monitored twice daily and a weighed clinical scoring list is used to record the clinical symptoms. When a certain pre-determined clinical score is reached then the animal will be humanely euthanized and a full necropsy will be performed to establish the cause of the disease and viral distribution over the respiratory tract.

E. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question F

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

Animals are monitored twice daily and a weighed clinical scoring list is used to record the clinical symptoms (9). When a clinical score of 35 is reached this indicates that the maximum duration of severity is reached then the animal will be euthanized. Individual scores are added and the decision is based on the total daily score and veterinarian assessment of discomfort. Symptoms that lead to an immediate endpoint are: open mouth breathing or cyanosis, lethargy as defined by minimal response to human approach.

Indicate the likely incidence.

The likely incidence for reaching a clinical endpoint depends on the virus strain that is used for infection. Human influenza viruses only rarely cause severe disease (<1% of the animals). Highly pathogenic avian influenza viruses can cause lethal disease in up to 75% of the non-vaccinated control animals.

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

The total amount of discomfort is estimated as moderate. This is mainly caused by the surgical implantation and removal of the recording device and development of disease due to infection and the high maximum number of sedations.

G. Replacement, reduction, refinement	
Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.	
Replacement	The immune system is very complex and the *in vivo* interactions between virus and/or vaccine and host are not completely understood. At present there is no *in vitro* model available that can mimic the human immune system sufficiently to study the potential of a vaccine to induce protective immune responses. Due to the complex interaction of influenza virus with different tissues and the role of local immunity in eradication of the virus, the efficacy of an influenza vaccine to protect against infection can only be adequately established in an animal model. Several animal species have been used as a model for human influenza virus infection (10). However, mice are not naturally susceptible to influenza and usually lethal infection models are used, that are based on extremely adapted human viruses. Ferrets are naturally susceptible to most human influenza viruses and recapitulate the natural course of infection. However, infection is often restricted to the upper airways and this model has the disadvantage that limited immunological tools are available, especially to study innate and cellular adaptive immune responses, which are especially important in the evaluation of "universal" vaccine strategies. NHP have an immune system that most closely resembles that of humans. Also the availability of many cross reactive reagents makes it possible to study in detail the contribution of the innate immune system and to analyse vaccine induced immune responses and evaluate their role in control of infection. In addition, vaccine strategies that aim to trigger immune responses through targeting of specific cell surface molecules or innate immune receptors have either limited cross reactivity to other animal species or trigger different cell types or different responses because of differences in receptor expression pattern or cell signalling pathways. These aspects are essential for the evaluation of so called "universal" influenza vaccines. For this type of vaccines new vaccination strategies as well as vaccine modalities are used that aim for the induction of cross protective cellular immune responses or induction of broadly cross neutralizing antibody responses or non-neutralizing antibody responses that become effective through interaction with innate immune cells. Here, the close homology between the immune system in NHP and humans is essential for proper induction and adequate analysis of these responses, allowing a better extrapolation to the human situation. Vaccines that will be tested in NHP have to be in the final stages of preclinical development and require this last validation step in order to assure that there are no adverse effects that were missed in the preliminary studies in other species and that they are effective in an animal species that has an immune system closely related to humans.
Reduction	The number of animals needed per experiment will be based on statistical power calculation for achieving statistically significant induction of immune responses and a significant reduction in virus load in the respiratory tract between the vaccine groups and the challenge control group. Only the minimum number of animals needed will be used. Since historical data are available on infection in non-vaccinated animals (appendix 2), usually less animals can be used in the challenge control group than in the vaccine groups. Furthermore, we aim to evaluate multiple vaccine candidates in a single experiment, so that a single challenge control group can be used. This has to be weighed against the fact that in order to obtain significant differences in immune response between the vaccine groups, more animals per vaccine group may occasionally be required.

Refinement	The use of recording devices enables the monitoring of body temperature, heart- and breathing-rate every 15 minutes. We have designed a method that allows very precise calculation of fever induction caused by influenza infection (2). With this method we have observed a significant reduction in fever by influenza vaccine candidates (5). Such precise measurements are not possible with the traditional rectal temperature measurement. Placement and removal of the temperature responders will require a small surgical procedure, which will be done under anaesthesia. Subsequently, animals will receive analgesics as long as required. Animals are trained to cooperate as much as possible for the invasive handlings, such as receiving the sedation. Animals will be socially housed with a socially compatible animal. There is an extensive program for enrichment in our institute that consists of playing material and methods to present food. During the study animals will be observed daily by qualified and competent animal caretakers. Should changes occur in behaviour, appetite or stool then a veterinarian will be informed and measures will be discussed with the investigator and implemented. Possible local reactions on the injection site of the vaccine will be recorded at multiple time points using a scoring system that includes redness, swelling and induration. In case substantial induration is seen, then the wound will be treated and analgesics will be applied. During the infection phase, animals will be observed twice daily and clinical symptoms will be scored using a well-established clinical scoring list adapted from Brining et al.(9) . On the basis of the scoring system a humane endpoint is defined. In addition, a sudden strong decrease in body temperature is taken as a humane endpoint. When this endpoint is reached the animal will be immediately euthanized and a full necropsy will be performed to determine the cause of clinical disease. All handlings will be performed under sedation. On every time point where a handling is performed the animal will be weighed and closely examined. During the first 2 days of the infection the animal will receive supportive feeding with dense "brokkenballen". This is necessary, because animals have to fast the night before the sedation and since animals will be sedated daily during the first days after infection the food intake during this period would otherwise be very limited.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

☒ No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

☐ No > Continue with question I.

☒ Yes > Explain why re-use is considered acceptable for this animal procedure.

Animals that will be used in these experiments, have possibly been used in previous experiments. Animals that have been involved in previous influenza vaccine or influenza virus infection studies or that have pre-existing antibodies against influenza are not suitable. In view of the long life-span of the animals of this species re-use of animals will take place within the limitations described in art 1e of the Wet op de Dierproeven.

Are the previous or proposed animal procedures classified as 'severe'?

☒ No

☐ Yes > Provide specific justifications for the re-use of these animals during the procedures.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

Not applicable

J. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☒ No > Continue with question K.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

☐ No > Provide information on the destination of the animals.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

In most studies the animals will be re-used after the virus is cleared (usually at day 21 after infection). However, in cases where possible adverse effects of the vaccine have to be studied animals are euthanized and a full necropsy is performed.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☒ Yes > Will a method of killing be used for which specific requirements apply?

☒ No > Describe the method of killing.

Euthanasia is done by injection of an anaesthetic dose of ketamine followed by an overdose of barbiturate intravenously

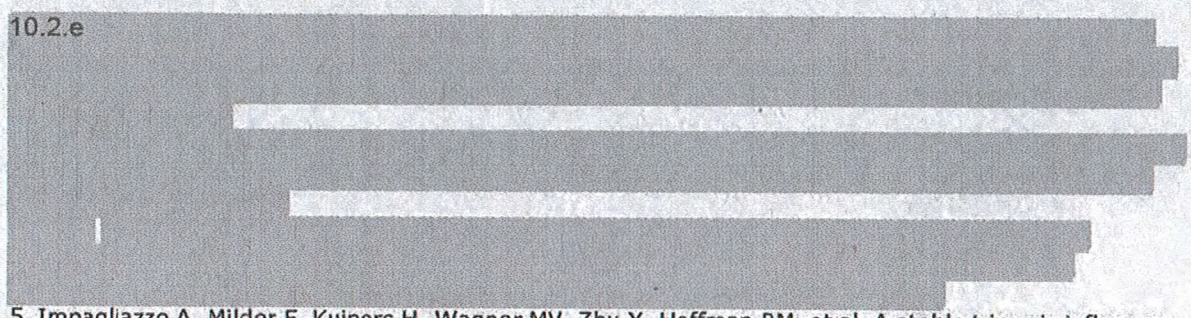
☐ Yes > Describe the method of killing that will be used and provide justifications for this choice.

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

References

10.2.e [redacted]

10.2.e



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