	Inventaris Wob-verzoek W21-04									
		wordt verstrekt				weigeringsgronden				
nr.	document NTS20174224-1	reeds openbaar	niet	geheel	deels	5.1, lid 1c	5.1, lid 2e	5.1, lid 2f	5.1, lid 2h	5.2, lid 1
1	Aanvraagformulier en toelichting op melding, d.d. 12 februari 2020				x		x		x	
2	Begeleidende e-mail bij ontvangstbevestiging, d.d. 13 februari 2020				x		x			
3	Ontvangstbevestiging, d.d. 13 februari 2020				x		x			

Centrale Commissie Dierproeven

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 bel met 0900-2800028 (10 ct/min).

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.

X Ja > *Vul uw deelnemernummer in*	50200
☐ Nee > *U kunt geen aanvraag doen*	

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

Naam instelling of organisatie	Biomedical Primate Research Centre	
Naam van de portefeuillehouder of diens gemachtigde	10.2.e	
KvK-nummer	41146967	
Straat en huisnummer	Lange Kleiweg	161
Postbus	3306	
Postcode en plaats	2288 GJ Rijswijk	
IBAN	NL87ABNA0515544779	
Tenaamstelling van het rekeningnummer	Stichting Biomedical Primate Research Centre	

1.3 Vul de gegevens van het postadres in.
Alle correspondentie van de CCD gaat naar de portefeuillehouder of diens gemachtigde en de verantwoordelijke onderzoeker.

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	10.2.e	X Dhr. ☐ Mw.
Functie	Section Head Molecular Virology	
Afdeling	Virology	
Telefoonnummer	10.2.e	
E-mailadres	10.2.e	

1.5 *(Optioneel)* Vul hier de gegevens in van de plaatsvervangende verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	10.2.e	☐ Dhr. X Mw.
Functie	Researcher Section Molecular Virology	
Afdeling	Virology	
Telefoonnummer	10.2.e	
E-mailadres	10.2.e	

1.6	*(Optioneel)* Vul hier de gegevens in van de persoon die er verantwoordelijk voor is dat de uitvoering van het project in overeenstemming is met de projectvergunning.	(Titel) Naam en voorletters	☐ Dhr. ☐ Mw.
		Functie	
		Afdeling	
		Telefoonnummer	
		E-mailadres	
1.7	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	Wat voor aanvraag doet u?	☐ Nieuwe aanvraag > Ga verder met vraag 3
		☐ Wijziging op (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn Vul uw vergunde projectnummer in en ga verder met vraag 2.2
		X Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn Vul uw vergunde projectnummer in en ga verder met vraag 2.3: AVD5020020174224
2.2	Is dit een *wijziging* voor een project of dierproef waar al een vergunning voor verleend is?	☐ Ja > Beantwoord dan in het projectplan en de niet-technische samenvatting alleen de vragen waarop de wijziging betrekking heeft en onderteken het aanvraagformulier
		☐ Nee > Ga verder met vraag 3
2.3	Is dit een *melding* voor een project of dierproef waar al een vergunning voor is verleend?	☐ Nee > Ga verder met vraag 3
		X Ja > Geef hier onder een toelichting en ga verder met vraag 6
		De originele aanvraag was gericht op de evaluatie van Riftdalkoorts/Rift Valley Fever virus (RVFV) vaccines die d.m.v. actieve immunisatie bescherming tegen toekomstige infectie moeten voorkomen. Wij willen dit project verbreden met het evalueren van passieve immunisatie tegen RVFV. Hierbij krijgen marmosets neutraliserende antilichamen toegediend die infectie moeten voorkomen, of na infectie, de RVFV infectie moeten bestrijden. In muizenmodellen zijn deze antistoffen reeds effectief gebleken. Doordat wij gedurende de projectperiode minder dieren zullen gaan gebruiken voor de evaluatie van actieve immunisatietechnieken, zal het eerdere geschatte aantal van 134 marmosets worden verminderd tot 124. Deze aanpassing van het project zal niet leiden tot een toename van het cumulatief ongerief, zoals dit is beschreven in de projectaanvraag. De ingangsdatum van deze aanpassing van het project is 01-04-2020

3 Over uw project

3.1	Wat is de geplande start- en einddatum van het project?	Startdatum	1 - 4 - 2018
		Einddatum	31 - 3 - 2023
3.2	Wat is de titel van het project?	Evaluation of Rift Valley Fever Virus vaccines in common marmosets	
3.3	Wat is de titel van de niet-technische samenvatting?	Onderzoek naar de werkzaamheid van Rift Valley Fever virus vaccins in penseelapen	
3.4	Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?	Naam DEC	10.2.g
		Postadres	Postbus 10.2.g
		E-mailadres	10.2.g

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

☐Nieuwe aanvraag Projectvergunning Lege

X Wijziging € 1389 Lege

4.2 Op welke wijze wilt u dit bedrag aan de CCD voldoen.
Bij een eenmalige incasso geeft u toestemming aan de CCD om eenmalig het bij 4.1 genoemde bedrag af te schrijven van het bij 1.2 opgegeven rekeningnummer.

☐ Via een eenmalige incasso

X Na ontvangst van de factuur

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

X Projectvoorstel

X Niet-technische samenvatting

Overige bijlagen, indien van toepassing

☐ Melding Machtiging

X 2 bijlagen

6 Ondertekening

6.1 Print het formulier uit, onderteken het en stuur het inclusief bijlagen via de beveiligde e-mailverbinding naar de CCD of per post naar:

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag

Ondertekening door de instellingsvergunninghouder of gemachtigde (zie 1.7). 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 F 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 10.2.e
Functie	
Plaats	
Datum	06 - 02 - 2020
Handtekening	10.2.e

Format
Niet-technische samenvatting

- Dit format gebruikt u om uw niet-technische samenvatting te schrijven
- Meer informatie over de niet-technische samenvatting vindt u op de website www.centralecommissiedierproeven.nl.
- Of neem telefonisch contact op. (0900-2800028).

1 Algemene gegevens

1.1	Titel van het project	**Onderzoek naar de werkzaamheid van Riftdalkoorts virus vaccins en antivirale antilichamen in penseelapen**
1.2	Looptijd van het project	1 april 2018 – 31 maart 2023 (5 jaar)
1.3	Trefwoorden (maximaal 5)	Rift Valley Fever virus, vaccin, marmoset, pathogeniciteit, antilichamen

2 Categorie van het project

2.1 In welke categorie valt het project.

U kunt meerdere mogelijkheden kiezen.

- ☐ Fundamenteel onderzoek
- X Translationeel of toegepast onderzoek
- ☐ Wettelijk vereist onderzoek of routinematige productie
- ☐ Onderzoek ter bescherming van het milieu in het belang van de gezondheid
- ☐ Onderzoek gericht op het behoud van de diersoort
- ☐ Hoger onderwijs of opleiding
- ☐ Forensisch onderzoek
- ☐ Instandhouding van kolonies van genetisch gemodificeerde dieren, niet gebruikt in andere dierproeven

3 Projectbeschrijving

3.1 Beschrijf de doelstellingen van het project (bv de wetenschappelijke vraagstelling of het wetenschappelijk en/of maatschappelijke belang)

Riftdalkoorts wordt veroorzaakt door het Rift Valley fever virus (RVFV) en is een ernstige ziekte bij vee die ook op mens kan worden overgedragen (zoönose). Riftdalkoorts bij mensen kenmerkt zich meestal door "griepachtige" ziekteverschijnselen, maar 1-3% van de geïnfecteerden ontwikkelt ernstige ziekte waaraan tot 50% van deze mensen overlijdt. Riftdalkoorts komt momenteel voornamelijk voor in Afrika, maar gevreesd wordt dat RVFV Europa zal binnenkomen via geïnfecteerde muggen, dieren of mensen. De Wereldgezondheidsorganisatie is groot pleitbezorger voor de ontwikkeling van vaccins of geneesmiddelen tegen RVFV-infectie. In dit

		project zullen we de beschermende werking van vaccins en effektiviteit van antistoffen tegen riftdalkoorts testen in penseelapen.
3.2	Welke opbrengsten worden van dit project verwacht en hoe dragen deze bij aan het wetenschappelijke en/of maatschappelijke belang?	**Er zijn nog geen RVFV vaccins of geneesmiddelen beschikbaar voor gebruik in de mens. In dit project zullen wij nieuwe vaccins en antivirale antistoffen testen op hun vermogen om penseelapen te beschermen tegen (profylactisch) /of te genezen van (therapeutisch) infectie met RVFV. Wij verwachten dat dit onderzoek zal bijdragen aan de ontwikkeling van een vaccins en behandelingen tegen Riftdalkoorts die kunnen worden gebruikt om toekomstige epidemieën bij mens en dier te voorkomen**
3.3	Welke diersoorten en geschatte aantallen zullen worden gebruikt?	**Maximaal 124 penseelapen**
3.4	Wat zijn bij dit project de verwachte negatieve gevolgen voor het welzijn van de proefdieren?	De dieren ondervinden ongerief door biotechnische handelingen, en het plaatsen van een meetinstrument in de buikholte. Daarnaast kunnen de dieren ziek worden door de virusinfectie.
3.5	Hoe worden de dierproeven in het project ingedeeld naar de verwachte ernst?	Door toepassing van een humaan eindpunt wordt de welzijnsaantasting beperkt tot matig.
3.6	Wat is de bestemming van de dieren na afloop?	De dieren worden aan het einde van het experiment geëuthanaseerd.

4 Drie V's

4.1	**Vervanging** Geef aan waarom het gebruik van dieren nodig is voor de beschreven doelstelling en waarom proefdiervrije alternatieven niet gebruikt kunnen worden.	Het is nog niet mogelijk om de beschermende werking van vaccins zonder gebruik van proefdieren te bepalen. Het afweersysteem is dermate ingewikkeld dat de beschermende werking van een vaccin tegen RVFV infectie nog niet in het laboratorium kan worden nagebootst. Vanwege hun grote immunologische overeenkomsten met de mens zijn apen het meest geschikt als proefdiermodel voor dit vaccinonderzoek. Dit maakt een optimale vertaling van bevindingen naar de mens mogelijk.
4.2	**Vermindering** Leg uit hoe kan worden verzekerd dat een zo gering mogelijk aantal dieren wordt gebruikt.	Alleen vaccinkandidaten die eerst in vee op veiligheid en werkzaamheid zijn getest zullen in apen worden getest op hun werkzaamheid. **Verder worden alleen die antistoffen in penseelaapjes getest, die al in het laboratorium en knaagdieren bewezen hebben goed aan het virus te kunnen binden en het virus te kunnen neutraliseren.**

	Het aantal benodigde dieren wordt per experiment bepaald aan de hand van statistische analyses. Waar mogelijk zullen meerdere vaccins tegelijk getest worden, waardoor maar één controlegroep nodig is. Bij vaccinstudies wordt gebruik gemaakt van een twee-fase benadering: als het vaccin geen immuunreactie opwekt, of als het nadelig is voor de gezondheid van de dieren, zal niet worden overgegaan op het infecteren met RVFV
4.3 **Verfijning** Verklaar de keuze voor de diersoort(en). Verklaar waarom de gekozen diermodel(len) de meest verfijnde zijn, gelet op de doelstellingen van het project.	Penseelapen zijn zeer gevoelig voor RVFV-infectie, en het infectieverloop is in hoge mate vergelijkbaar met ernstige ziekte bij de mens. In deze apensoort is de kans het grootst dat eventuele nadelige effecten van de vaccins kunnen worden opgespoord. Omdat hun afweersysteem grote gelijkenis vertoont met dat van de mens, kan een gedegen voorspelling worden gedaan wat betreft werkzaamheid bij de mens.
Vermeld welke algemene maatregelen genomen worden om de negatieve (schadelijke) gevolgen voor het welzijn van de proefdieren zo beperkt mogelijk te houden.	Alle handelingen worden uitgevoerd onder verdoving. Waar nodig wordt pijnstilling gegeven. De dieren worden getraind om zoveel mogelijk vrijwillig mee te werken aan de toediening van de verdoving. De dieren worden intensief geobserveerd zodat wanneer ziekteverschijnselen optreden zeer snel actie kan worden ondernomen. Om de dieren zo veel mogelijk natuurlijk gedrag te laten vertonen is op het onderzoeksinstituut een uitgebreid programma voor diertraining en kooiverrijking opgezet.

5 In te vullen door de CCD

Publicatie datum	
Beoordeling achteraf	
Andere opmerkingen	

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 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'.	50200
1.2	Provide the name of the licenced establishment.	Biomedical Primate Research Centre
1.3	Provide the title of the project.	Evaluation of Rift Valley Fever Virus vaccines **and antiviral antibodies** in common marmosets

2 Categories

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

- ☐ Basic research
- X Translational or applied research
- ☐ Regulatory use or routine production
- ☐ Research into environmental protection in the interest of human or
- ☐ 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.

- For legally required animal procedures, indicate which statutory or regulatory requirements apply (with respect to the intended use and market authorisation).
- For routine production, describe what will be produced and for which uses.
- For higher education or training, explain why this project is part of the educational program and describe the learning targets.

Rift Valley fever virus (RVFV) is a mosquito-borne virus and the causative agent of Rift Valley fever (RVF). RVFV belongs to the Order Bunyavirales (genus Phlebovirus, family Phenuiviridae). Its genome

is composed of 3 negative-sense RNA segments, referred to as large (L), medium (M), and small (S). The L segment encodes for the viral RNA polymerase (L protein). The M segment encodes the structural glycoproteins Gn and Gc, the non-structural protein NSm, and a large 78-kDa glycoprotein (LGp). The small segment encodes for the nucleocapsid protein (N), and the interferon antagonist NSs, which is a major determinant of virulence (6).

RFV is a zoonotic disease affecting wild and domesticated ruminants, and humans. In ruminants and humans, RVFV causes markedly different disease syndromes, reflecting the differences in immune system and physiology between the target species.
In ruminants, the disease is characterized by neonatal mortality and an increased incidence of abortions and fetal malformations. Sheep are the animal species that are most susceptible to severe disease. Infection of adult sheep usually results in about 20% mortality, but the mortality rate in newborns can reach 100%. An important feature of RVF outbreaks are the so-called "abortion storms" in which almost all pregnant ewes abort. Clinical symptoms in other ruminants (goats, cattle) are usually less severe, although abortions and death also occurs in herds of these animals. The most important clinical symptoms in adult animals are fever, drop in milk production, respiratory disease, diarrhea and anorexia.
RVFV is able to cause disease in humans and can have serious consequences. Humans can be infected via mosquito bites, but human cases can also be attributed to contact with tissues and blood during slaughtering of RVFV-infected animals, or to handling of aborted fetuses. Human infection usually manifests as a transient, flu-like febrile illness with symptoms including fever, severe headaches, muscle pains, nausea and general weakness. A common additional complication is temporary or permanent blindness as a result from retinal damage. A minority of patients (1-3%), develop severe RVF disease, exhibiting early symptoms of acute hepatitis with associated jaundice, renal failure, encephalitis, and hemorrhagic complications. The fatality rate in this group can be as high as 50% (2,6,16). In contrast to the disease in ruminants, abortions are not a common RVFV disease symptom in humans.

RVFV is endemic to Africa, the Arabian Peninsula, and several islands located off the coast of Southern Africa, including Madagascar. The largest epidemics generally occur in East-Africa (8,13). Eradication of RVFV is difficult because the virus circulates between wild- and domesticated ruminants, and is transmitted by several common species of mosquitos, including *Aedes* and *Culex* mosquito species (9). Animal movements, legal or illegal, strongly contribute to viral spread, and there is serious concern among both veterinary authorities and human health authorities that the virus via these routes will reach other geographic regions, including Europe, where vectors are abundantly present (3).
Experimental infection of the common European mosquito *Culex pipiens* (in Dutch the 'gewone steekmug'), but also the invasive *Aedes albopictus* ('Tijgermug') confirmed their competence to transmit RVFV (1). Thus, there is an increasing urgency that effective RVFV vaccines **and antiviral therapies become available to prevent or control potential future outbreaks worldwide**, in both animals and humans.

Rift Valley Fever virus vaccines and antiviral antibody therapies

Currently, no licensed vaccines or effective therapies are available to treat RVFV disease in humans. At present, there are three licensed RVFV vaccines only for use in animals. One inactivated-virus vaccine, and two vaccines based on live-attenuated viruses (LAV), are currently being used in Africa to vaccinate livestock. The inactivated-virus vaccine can be applied safely during all life-stages, including pregnancy, but requires repeated vaccinations for optimal efficacy, which makes it unsuitable for controlling outbreak situations. One LAV-vaccine is based on the RVFV Smithburn strain, which contains attenuating mutations across its genome, and another live-attenuated vaccine is based on the Clone 13 RFV virus, which lacks 70% of the NSs gene (the most important virulence factor of the

virus). Although both vaccines are very effective, and are being used to controls RVF outbreaks, both cannot be used safely in pregnant animals, due to residual pathogenicity and their potential to induce abortions (5). During an outbreak of RVF, it is logistically impossible to vaccinate all RVFV-susceptible animals in a very short time-period, and thereby also fully protect humans against RVFV infection. In this context, it is important to realize that not only farm animals play a role in the transmission, but also wild animals such as deer, buffalos, and possibly also rodents are reservoirs for RVFV. The zoonotic RVFV is recognized as an important pathogen, both by the world organization for animal health (OIE), as well as the World Health Organization (WHO).
Importantly, RVF is one of the diseases that are prioritized by the WHO on their R&D blueprint (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 compounds) exist (See Executive Summary attached to this paragraph).

In addition, zoonotic infections, like RVF, are likely candidates for a One Health approach to disease control:

> 'One Health recognizes that the health of people is connected to the health of animals and the environment. The goal of One Health is to encourage the collaborative efforts of multiple disciplines-working locally, nationally, and globally-to achieve the best health for people, animals, and our environment' (https://www.cdc.goc/onehealth/index.html)

Future 'One Health-based' RVFV vaccines and antivirals should be able to protect or cure both animals as well as humans against infection. The vaccines and antiviral antibodies that will be evaluated in this project are developed in the framework of partnerships between veterinary industry and (inter)national health organizations, or publicly-funded research collaborations.
Inherent to the broad spectrum of protection, efficacy of One Health-based test substances need to be evaluated both in models that represent disease in livestock animals, and in animal models that best mimic human infection.

Nonhuman primate models for RVFV research
For the veterinary use of such RVF vaccines, vaccine efficacy is commonly evaluated in ruminant models for natural RVFV infection, like sheep, goats and cattle (12). However, for the efficacy evaluation of novel RVF vaccines intended for use in humans, non-human primates (NHP) are the preferred species because they are the species that most closely reproduces disease symptoms observed in humans and have an immune system that is comparable to that of humans.
Several nonhuman primate infection models have been evaluated to assess the human RVFV vaccines (12). Rhesus macaques (*Macaca mulatta*) and cynomolgus monkeys (*Macaca fascicularis*) can be infected with RVFV and develop viremia after experimental infection, but the disease pattern observed in these animals differs from that in humans with severe disease. In addition, disease is only seen in a minority (20%) of the infected macaques (7,10,11,12,14). Therefore, the macaque infection model is not considered an optimal RVFV disease model (17).
More recently, African green monkeys (AGM; *Chlorocebus aethiops*), and common marmosets (*Callithrix jacchus*) were used to develop an animal model for human RVF (4,14). In both species, 100% of the animals became infected and developed fever with a biphasic pattern that is also found in humans. They also developed clinical illness with clear signs of encephalitis.
Additionally, marmosets were more susceptible to infection at lower doses than AGM (4,14). Equally, marmosets showed higher morbidity, mortality, and viremia, and displayed marked aberrations in hematological and chemistry values. These animals exhibited acute-onset hepatitis, delayed-onset encephalitis, and hemorrhagic disease, which are dominant features of severe human RVF (14). Together with an immune system and physiology that are highly similar to that of humans, this makes

marmosets the most optimal animal model to study human-like pathogenicity and immunology and development of human RVF vaccins. In addition, this makes marmosets the best model to study absence of residual pathogenicity of human vaccines.

In case of an infection with RVFV, administration of anti-RVFV antibodies to clear or inhibit the virus can be used as therapeutic treatment. The earliest application of antibodies as a treatment for viral infections can be traced back to the early 20th century, using sera from infected humans who had recovered from the same infection. This serum therapy was gradually replaced by antibodies purified from pooled sera, intravenous immune globulin (IVIG). Since the mid-1980s, methods have been developed for the efficient isolation of monoclonal antibodies against viruses from humans and animals (23).
Marmosets have already been used to evaluate the use of monoclonal antibodies to prevent or cure viral infections. Passive immunisation studies, i.e. the administration of human antibodies pre- or post-experimental infection, have been succesfully performed in the marmoset infection models for orthopox viruses (21) and the MERS coronavirus (18-20,22). The RVFV infection model in marmosets for the evaluation of vaccines for use in humans can be applied to test human neutralizing antibodies for their efficacy to prevent or cure RVFV infection.

Marmosets are a suitable NHP species for the evaluation of RVF vaccines because other highly susceptible animal species, like sheep, show a disease pattern (neonatal mortality and increased incidence of abortions and fetal malformations) that is much unlike that seen in humans. Macaques, in contrast to marmosets, show disease symptoms in a minority of animals.
In sum, the common marmoset is chosen as the best animal model in this project because it represents a NHP model that is most sensitive to RVFV infection, that best mimics the severe manifestations of human RVF, and that can be utilized for the evaluation of potential therapeutics and vaccines.

References:

1. Brustolin, M., Talavera, S., Nunez, A., Santamaria, C., Rivas, R., Pujol, N., et al. (2017). Rift Valley fever virus and European mosquitoes: vector competence of *Culex pipiens* and *Stegomyia albopicta* (= *Aedes albopictus*). *Medical and Veterinary Entomology*, *356*, 1422.
2. CDC. (2013). *Rift Valley Fever (RVF)* (https://www.cdc.gov/vhf/rvf/RVF-FactSheet.pdf).
3. Chevalier, V. (2013). Relevance of Rift Valley fever to public health in the European Union. *Clinical Microbiology and Infection, 19(8), 705-708.*
4. Hartman, A. L., Powell, D. S., Bethel, L. M., Caroline, A. L., Schmid, R. J., Oury, T., & Reed, D. S. (2014). Aerosolized Rift Valley Fever Virus Causes Fatal Encephalitis in African Green mMonkeys and Common Marmosets. *Journal of Virology*, *88*(4), 2235–2245.
5. Makoschey, B., van Kilsdonk, E., Hubers, W. R., Vrijenhoek, M. P., Smit, M., Wichgers Schreur, P. J., et al. (2016). Rift Valley Fever Vaccine Virus Clone 13 Is Able to Cross the Ovine Placental Barrier Associated with Foetal Infections, Malformations, and Stillbirths. *PLoS Neglected Tropical Diseases*, *10*(3), e0004550.
6. Mansfield, K. L., Banyard, A. C., McElhinney, L., Johnson, N., Horton, D. L., Hernández-Triana, L. M., & Fooks, A. R. (2015). Rift Valley fever virus: A review of diagnosis and vaccination, and implications for emergence in Europe. *Vaccine*, *33*(42), 5520–5531.
7. Morrill, J. C., Jennings, G. B., Johnson, A. J., Cosgriff, T. M., Gibbs, P. H., & Peters, C. J. (1990). Pathogenesis of Rift Valley fever in rhesus monkeys: role of interferon response. *Archives of Virology*, *110*(3-4), 195–212.

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Executive summary

On 24-25 January 2017, the World Health Organization held an informal consultation in Geneva, Switzerland, to review the list of priority diseases for the WHO R&D Blueprint. The R&D Blueprint focuses on severe emerging diseases with potential to generate a public health emergency, and for which insufficient or no preventive and curative solutions exist. The original list of diseases that most readily meet these criteria and for which additional research and development is urgently required was agreed at an international consultation held in November 2015.

The January 2017 meeting brought together virologists, bacteriologists, vaccinologists, public and animal health professionals as well as infectious disease clinicians to review the list of priority diseases. These experts made use of a tailored prioritization methodology developed by WHO and validated at an informal consultation in November 2016. The methodology uses the Delphi technique, questionnaires, multi-criteria decision analysis, and expert review to identify relevant diseases.

The 2017 annual review determined there was an urgent need for research and development for:[1]

- Arenaviral hemorrhagic fevers (including Lassa Fever)
- Crimean Congo Haemorrhagic Fever (CCHF)
- Filoviral diseases (including Ebola and Marburg)
- Middle East Respiratory Syndrome Coronavirus (MERS-CoV)
- Other highly pathogenic coronaviral diseases (such as Severe Acute Respiratory Syndrome, (SARS))
- Nipah and related henipaviral diseases
- Rift Valley Fever (RVF)
- Severe Fever with Thrombocytopenia Syndrome (SFTS)
- Zika

In addition, any disease identified using the R&D Blueprints decision instrument for new diseases.

Chikungunya virus was discussed during the meeting and a number of experts stressed the risks it poses. Along with a number of other pathogens, there was agreement that Chickungunya Virus continues to warrant further research and development.

Other pathogens were considered during the review and a wide range of additional relevant research and development initiatives encouraged. In particular, participants noted the importance of cross-cutting research and development which would help to address a range of different pathogens or diseases at the same time.

The meeting also stressed the importance of continuing research and development on diseases other than those on the priority list. Further research and development is needed on a wide range of diseases. Where there are already substantive efforts to develop

[1] The order of diseases on this list does not denote any ranking of priority.

relevant medical measures any necessary further actions for such diseases could usefully be coordinated through the disease-specific initiatives (such as existing major disease control initiatives, extensive R&D pipelines, funding streams, or established regulatory pathways for improved interventions).

The value of a One Health approach was recognized, as well as the importance of working more closely with animal health to identify priority diseases and develop relevant countermeasures. The meeting also noted that whilst anti-microbial resistance is an issue being dealt with by thematic initiatives at the international level, specific diseases with resistance might be considered for prioritization in the future.

Feedback from the meeting on the methodology used and opportunities for further strengthening this process will be fed into its next review to be conducted within two years.

Annex B: The 2017 Prioritization Committee

Dr. Celia ALPUCHE
Prof. Lucille BLUMBERG
Dr. David BRETT-MAJOR
Dr. Miles CAROLL
Dr. Inger DAMON
Dr. Peter DASZAK
Dr. Xavier DE LAMBALLERIE
Dr. Mourya DEVENDRA
Prof. Christian DROSTEN
Dr. Delia ENRIA
Prof. Sahr GEVAO
Prof. Stephan GUENTHER
Prof. Peter HORBY
Prof. Roger HEWSON
Dr. Nadia KHELEF
Prof. Gary KOBINGER
Dr. Linda LAMBERT
Dr. Dieudonne NKOGHE
Dr. George WARIMWE
Dr. Mark WOOLHOUSE
Dr. YOUNGMEE Jee
Dr. Stefano Messori
Dr. Cathy ROTH
Dr Heinz FELDMANN

Observers

Dr. Hinta Meijerink
Dr. Ben MCCORMICK
Dr. Stacey KNOBLER

3.2 Purpose

Describe the project's main objective and explain why this objective is achievable.

- If the project is focussed on one or more research objectives, which research questions should be addressed during this project?
- If the main objective is not a research objective, which specific need(s) does this project respond to?

The aim of this research project is:

- to test the immunogenicity, efficacy, and absence of pathogenicity of novel RVFV vaccines and **antiviral antibodies** that are developed for use in humans in the marmoset model for human RVFV infection

The institute has extensive and long-standing expertise in conducting studies using nonhuman primates. Since 2012, researchers at the institute have been working on mosquito-transmitted virus infections in macaques and marmosets, like West Nile virus, Zika virus, and dengue virus. The institute has the appropriate facilities and experience to work with pathogenic viruses at DM-III and ML-III biosafety conditions. In addition, they have the appropriate virological and immunological assays for assessment of the efficacy against RVFV, and to determine absence of pathogenicity. The experience with mosquito-borne viruses guarantees that these animal studies will be adequately performed.

3.3 Relevance

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

As RVFV is a zoonotic virus, outbreaks of RVF in animals often coincide with human infections and fatalities. In 1993, for instance, southern Egypt suffered an outbreak in which 600–1500 human infections were reported (2). Since 2000, severe forms of human RVF have been reported in Saudi-Arabia and Yemen in 2000 (1603 reported cases/208 deaths), and in various African countries between 2003 and 2016 (3038 reported cases/749 deaths) (4).

In past outbreaks of RVF, mosquito-borne transmissions to humans were associated with mosquito species that normally do not feed on humans. However, during the major outbreak in Egypt 1977-78, mosquitoes that mostly feed on humans (*Culex pipiens*) were associated with transmission, and in this outbreak ~ 200,000 people were infected (7). Since anthropophilic mosquitoes (mosquitoes that prefer to feed on humans) such as *Culex pipiens*, but also *Aedes aegypti* and *Aedes albopictus,* can efficiently transmit RVFV, it is very well conceivable that in future outbreaks, RVFV can be directly transmitted between humans through these mosquito species. There are several examples of which transmission via the mosquito from human to human was not (sufficiently) recognized (Zika virus, Chikungunya, West Nile). The spread of exotic viruses directly from human-to-human is therefore often underestimated, which has become painfully clear due to the recent outbreak of Zika virus.

International health authorities have recognized this potential threat to human health. The WHO has put RVFV on the 2017 list of 13 priority pathogens based on the following prioritizing criteria (3):

- Human transmission
  - Humans can become infected with RVFV through contact with blood, body fluids, or tissues of RVFV-infected animals, mainly livestock. Humans can also be infected with RVFV from bites of infected mosquitoes and, from other biting insects that have the virus on their mouthparts. Spread from person to person has not been documented.
- Medical countermeasures
  - No vaccines are currently available for human RVF vaccination, and no effective treatments for RFV exist
- Severity or case fatality rate

- One to three percent of infected humans develop serious RVF disease. The case fatality rate within that group of patients can be as high as 50%

- The human/animal interface
  - RVF is a zoonotic disease and is transmitted by multiple insect species.
- The public health context of the affected area
  - Because the symptoms of Rift Valley fever are variable and non- specific, clinical diagnosis is often difficult, especially early in the course of the disease. Additionally, RVF is difficult to distinguish from other viral hemorrhagic fevers as well as many other diseases that cause fever, including malaria, shigellosis, typhoid fever, and yellow fever. Definitive diagnosis of RVF requires testing in reference laboratories, and involves hazardous samples that must be handled with extreme care. Such laboratories are not widely found in the Sub-Saharan Africa where RFV is endemic.
- Potential societal impacts
  - Outbreaks of RVF have a dramatic impact on producers and livestock industries, affecting public and animal health, food security and the livelihood of the pastoralist communities. RVF also has an impact on international trade and other agro-industries. The risk of introducing RVF into disease-free countries (Europe) via the importation of an infected animal, infected travelers, or mosquitos is real, and can cause serious human health problems. Additionally, the consequent restriction of access to export markets may induce dramatic economic consequences for national and local economies (5).
- Evolutionary potential
  - Since its discovery in the 1930-ies, RVFV has expanded its geographic range with increasing human disease, caused by several viral lineages. The evolution of RVFV through mutation and re-assortment and the accumulation of these changes over several decades may have changed the disease epidemiology, increasing its geographic distribution and severity in human populations (6).

For agents on this list there is an urgent need for research and development of vaccines and antiviral treatments. For the listed pathogens, the WHO also recognized the value of a One Health approach, i.e. the concept that recognizes that the health of people is connected to the health of animals and the environment. The WHO stresses the importance of working more closely with animal health to identify priority diseases and develop relevant countermeasures. The World Organization for Animal Health (OIE) also endorses the One Health concept (1), and specifically mentions RVF as one of the diseases of animal origin that can be transmitted to humans, and poses a worldwide risk to human health.

The goal of the research described in this project is: 1) to evaluate the efficacy of such 'One Health RVFV vaccines' that are developed to protect both animals and humans against RVFV infections, **2) to test anti-RVFV antibodies fot their potential to cure virus infection.**

The project fits within the above presented WHO strategy, and the proposal will contribute significantly to the One Health approach put forward by WHO and OIE.

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3.4 Research strategy

3.4.1 Provide an overview of the overall design of the project (strategy).

In this project, we will assess the immunogenicity, protective capacity and absence of pathogenicity of novel RVFV One Health-based vaccines**, as well as antiviral antibody therapies** using the marmoset vaccination-challenge model for human RVF.
The evaluation of the **efficacy of a vaccine or antiviral antibodies** against RVFV infection requires that the virus inoculum stock is first tested for its infectivity in marmosets prior to the efficacy testing. Virus infection can be performed by inoculating the animals via various routes; e.g. intradermal, subcutaneous, intravenous, or as an aerosol to the respiratory tract, using different doses, and/or by infection using RVFV virus-infected mosquitoes. Then, the onset of viremia, its duration, and the total virus production in blood are determined. In parallel with the infectivity determination, the disease development and disease symptoms found after experimental infection of marmosets with wild-type RVFV will be documented. A disease symptoms scoring list will be developed that will provide a reference framework for the pathogenicity analysis of, in particular, live-attenuated virus (LAV) vaccines. Absence or presence of residual pathogenicity of LAV-based RVFV vaccines will be assessed by immunizing the animals, followed by analysis of blood samples for presence/absence of vaccine virus, and the monitoring of disease symptoms.
Vaccine efficacy of novel vaccines and antibody therapeutics will be monitored by immunization of the animals, followed by experimental infection of the animals with RVFV. During the immunization, the development of vaccine-induced immune responses will be measured. After infection, the presence/absence of viremia will be monitored as indicator of vaccine efficacy. Tissue samples will be collected and analyzed for absence or presence of vaccine-induced pathology.
In a study focused on therapeutic efficacy antiviral antibodies, animals are first experimentally-infected, followed by the administration of the compound. When applied as viral prophylaxis, the administration of antibodies is followed by viral infection. Similar to the vaccine efficacy studies, the level of viremia will be monitored as indicator of antibody efficacy. Tissue samples will be collected and analyzed for absence or presence of pathology or residual virus.

3.4.2 Provide a basic outline of the different components of the project and the type(s) of animal procedures that will be performed.

1. Experimental infection of common marmosets with RVFV
2. Pathogenicity testing in marmosets of novel RVFV vaccines
3. Immunogenicity and efficacy evaluation of novel RVFV vaccines that are developed for use in humans using the marmoset vaccination-challenge model
4. **Efficacy evaluation of anti-RVFV antibodies that are developed for use in humans using the marmoset challenge model**

Ad.1. The evaluation of the efficacy of a vaccine to protect against infection requires that the challenge inoculum is tested for its infectivity in marmosets, and that the course of viremia, in the nonhuman primate species used, is documented prior to efficacy testing. This is described in Appendix 1. Also,

the infection study will be used to develop a disease symptoms scoring list that will provide a reference framework for the pathogenicity analysis of, in particular, live-attenuated virus (LAV) vaccines.
Ad.2. To evaluate the absence of residual pathogenicity of novel RVFV vaccines, particularly those based on LAV, the animals will be monitored for adverse effects of the vaccine after administration (Appendix 2).
Ad.3. For vaccine immunogenicity and efficacy testing, marmosets will be immunized, and monitored for the development of adaptive immune responses. Next, when adequate immune responses are induced, the vaccine efficacy against infection will be tested by experimental infection with RVFV. A group of non-vaccinated animals will be included as infection controls. (Appendix 2)
Ad.4. **For the efficacy evaluation of novel anti-RVFV neutralizing antibodies, animals will be administered anti-RVFV antibodies prior experimental infection (prophylactic), or after infection (therapeutic or curative use). A group of non-treated animals will be included as infection controls. (Appendix 2).**

3.4.3 Describe the coherence between the different components and the different steps of the project. If applicable, describe the milestones and selection points.

The infection studies of common marmosets with wild-type RVFV (Appendix 1) have a dual purpose. In order to properly address the protective capacity of novel RVFV vaccines **or the efficacy of antiviral antibody-therapies** developed for use in humans, it is essential to have a well-characterized viral inoculum, as absence or reduction of virus replication after experimental vaccination is the main read-out parameter for vaccine or antibody- therapy efficacy (Appendix 2).
To evaluate the absence of residual pathogenicity in LAV-based vaccines, the experimental infection of common marmosets will be used to generate a list of clinical symptoms and changes in virological parameters related to RVF of marmosets. The absence of clinical symptoms in animals vaccinated with the LAV-based RVFV vaccines will be an important read-out parameter on which to conclude that vaccines lack residual pathogenicity (1).
RVFV vaccines that can be used to protect humans against Rift Valley fever are not yet available. Because RVFV is an important zoonotic disease, infecting both wild and domesticated ruminants, and humans, novel vaccines will be developed within the concept of One Health, and should thus be efficacious in host animals as well as in humans. Within this project we will investigate the protective efficacy and absence of pathogenicity of the vaccines **and antibodies** in the marmoset model for human RVF. Importantly, such vaccines **and antibodies** will only be tested in the marmoset model, if they have been shown safe and effective in animal models for RVFV. In vaccine efficacy studies, at the end of the immunization phase, the decision will be made whether to proceed with the experimental infection of the animals with the viral inoculum. This go-no go assessment will be based on vaccine-induced immune responses and the absence of adverse effect of the novel vaccines.

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3.4.4 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	Infection of common marmosets with RVFV
2	Evaluation of RVFV vaccines **and antiviral antibodies** in common marmosets
3	
4	
5	
6	

7	
8	
9	
10	

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 procedure.
- For more information, see 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'.	50200
1.2	Provide the name of the licenced establishment.	Biomedical Primate Research Centre
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 1 Type of animal procedure: Infection of common marmosets with RVFV

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.

In order to evaluate the immunogenicity, absence of pathogenicity, and the efficacy of RVF vaccines that are developed for use in humans, it is necessary to have a well-defined RVFV infection model that best mimics human RVF disease. Virus stocks need to be thoroughly characterized for their viral kinetic profile in marmosets before they can be applied in RVFV vaccine evaluation studies. Also, for the determination of residual pathogenicity, it is necessary to have a comprehensive list of the clinical symptoms caused by RVFV infection in marmosets, in order to assess if any deviations in the clinical picture, general behavior etc., detected in vaccinated animals, are caused by the vaccine or vaccine virus.

In general, the study set-up is as follows: a group of animals will be infected and monitored for clinical symptoms, body temperature, body weight, changes in blood parameters and chemistry, and general behavior. Blood samples will be collected at regular time points to determine if the animals have become infected and developed viremia. Before infection, a telemetric device will be implanted in the abdominal cavity of the animals that enables the continuous monitoring of body temperature and activity.

The primary outcome parameter is:

1. Viremia: start of virus replication, peak virus load, total virus production

Secondary outcome parameters are:

1. Presence/absence of disease symptoms, including changes in body temperature
2. Changes in hematological and chemistry values

3. Changes in activity
4. Pathological changes

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

At least four weeks before infection, a telemetric device will be implanted in the abdominal cavity of the animals that will allow continuous monitoring of body temperature and activity. Then, the animals will be infected by intravenous, intradermal, or subcutaneous inoculation, or may be infected via aerosol. Experimental infection using different numbers of RVFV-infected mosquitoes may also be explored as a natural way of human RVFV infection. At the same time, blood is collected for a zero-value determination. The animals will be monitored daily during the study period for general behavior, appetite, faeces, etc., and at each time-point when the animals are sedated, the body weights will be measured. After infection of the animals, blood will be collected at regular time points for a period of maximally 42 days to monitor the progress of the virus infection and to control for changes in clinical chemistry and hematology parameters.
At the end of the study, the animals will be humanely euthanized and necropsy will be performed for the collection of tissue samples for histopathological and virological tests. The latter will be done to investigate tissue and organ distribution of the virus, to identify potential viral reservoirs, and to perform (histo)-pathological analyses.
The details of each study, regarding the route of infection, dose used, etc., will be submitted for approval to the AWB.

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

Presently, limited literature data are available regarding infection of marmosets with RVFV (1,2). Therefore, initially, the group size is based on experience in other mosquito-borne flavivirus infection models, and on the literature data. The expectation is that with this number of animals (section B) an adequate assessment can be made regarding the reproducibility of infection, and on the variation in viremia. Also, the number of animals used will allow us to set up a clinical scoring list that can be used in the vaccine efficacy studies. On the basis of the data from the experimental infections, a power calculation can be made about the number of animals needed for subsequent virus titration studies, as well as the vaccine efficacy studies.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

The experiments will be performed with common marmosets (*Callithrix jacchus*), n = 24. All marmosets are purpose bred at the institute, or incidentally they will be obtained from a certified supplier in compliance with EU legislation. Both adult male and female animals will be used.
The use of non-human primates (NHP), like common marmosets, for the RVFV vaccine studies is essential:
the evaluation of new vaccines for use in humans requires a test animal model that can be easily infected with RVFV, that shows an infection rate of 100%, and that has an immune system that is comparable to that of humans.
Residual pathogenicity has been observed in current, commercially available live-attenuated virus (LAV) vaccines intended for veterinary use. In order to evaluate the absence of residual pathogenicity of vaccines or vaccine viruses for humans, an animal model is needed that is highly sensitive to RVFV infection, and that faithfully mimics the development of viremia seen in infected humans, and that, all infected animals, shows clinical symptoms and pathology similar to severe

disease symptoms seen in human RVF. Several nonhuman primate species are currently available to study various aspects of RVF disease. Macaques show clear clinical symptoms in only 20% of infected animals and are therefore less suited for the evaluation of residual pathogenicity in novel RVFV vaccines for use in humans.
In contrast, AGM and common marmosets do show signs of severe RVF disease in 100% of infected animals. AGM are not available at the institute, and marmosets have the additional benefit that they are highly susceptible to infection with low dose of virus. In addition, the use of macaques would necessitate larger numbers of animals to obtain statistical significance, and the amount of discomfort caused by RVF in individual animals showing signs of disease will not siggnifically differ between macaques and marmosets.

Of all NHP infection models used in RVFV research, common marmosets best combine high sensitivity for infection, which is necessary for vaccine efficacy testing, and high sensitivity towards development of RVF disease symptoms, which is an essential requirement for the evaluation of absence of residual pathogenicity (1-4).
Based on these features, marmosets are the nonhuman primate species that is selected for this research project.

Assuming 4 animals per virus inoculum, and two doses tested via a particular route of administration, including a follow-up study in 4 animals, 12 animals per inoculum will be needed. We calculate that during the project two different inocula will be evaluated. Thus, over the study period of 5 years, in total 24 animals are the maximum needed for setting up the RFVF infection model, to determine the infectivity of new virus stocks, and to set up a clinical scoring list that can be used in the vaccine efficacy studies.

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C. Re-use

Will the animals be re-used?

☐ No, continue with question D.

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

Animals that will be used in these experiments, might have been used in previous experiments. Animals that have been involved in previous RVFV studies or that have pre-existing antibodies against RVFV are not suitable. In view of the long life 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'?

X No

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

D. 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

Several animal species, primarily rodents and nonhuman primates have been used to study RVFV virus infection of humans. Of these different species, NHPs are the preferred species, because their immune system most closely resembles that of humans. This is important, both for vaccine efficacy evaluation as well as for the infection of the host with RVFV, since these are both strongly affected by the reaction of the innate and adaptive immune system of the host. The proper evaluation of novel vaccines requires adequate infection models in NHP, which is the purpose of the studies proposed here. In addition, the species of choice in these studies, the common marmoset, most faithfully mimics human RVF in viremia, but also shows RFV clinical symptoms and pathology as are also seen in severe human RFV.

Reduction

Limited data are available for RVFV infection in marmosets, and the results of the proposed infection studies will provide the parameters for group-size calculations for subsequent studies. Based on the extensive experience with other viral infection models within the institute where this research will be performed, plus the limited data available from literature, it is expected that four animals per inoculum dose will be sufficient. The animals will also be closely monitored for clinical signs of RVF in order to set up a clinical scoring list for future vaccine efficacy studies. Therefore, no additional animals will be needed for the preparation of the clinical scoring list. On the basis of the outcome of the first study the number of animals needed in follow up experiments can be calculated and less animals may be needed. Only the minimum number of animals needed will be used.

Refinement

The use of telemetric devices to measure body temperature and activity makes it possible to real-time monitor and collect data from the animals, allowing the veterinary staff to take action at the earliest time-point if any of these parameters is influenced by the RVFV infection, even before detected by visual inspection. Thus, adequate action can be taken before an animal reaches its humane endpoint. Placement of the telemetric devices will require surgery, which will be done under anesthesia. Subsequently animals will receive analgesics as long as required. The use of a smaller, novel telemetric device (Anipill 0.1C, DSI™; 17mm x 8 mm; 1.7 grams) than that has been used in previous marmoset studies will cause less discomfort to the animals. The animals are trained to cooperate as much as possible with invasive biotechnical actions, such as giving anesthesia or virus infection. All observations will be documented and added to the clinical scoring form which will be set up as part of the experimental infection studies. A highly sensitive real-time PCR will allow a very accurate determination of the virus load in small blood volumes collected from infected marmosets. Marmosets will be trained to stand on a scale themselves, making it possible to determine changes in body weight without anesthesia. The body weight will then be determined twice a day.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Animals will be housed with a socially compatible animal, whenever possible. There is an extensive program for environmental enrichment in the institute.
During the studies animals will be observed daily by qualified animal caretakers, and changes in body

temperature and activity are monitored continuously using telemetry. Should changes occur in the latter parameters, or in behavior, appetite or stool, they will be documented, and a veterinarian will be informed. Then, if necessary, measures will be taken.
All experimental procedures will be performed under sedation. Each time an animal is sedated, the animal will be weighed, and the animal will be closely examined. The institute uses a customized database that documents all individual animals in the institute. General observations like behavior, appetite and stool are part of this database. This database facilitates early recognition of minor changes in these general parameters. During the study, care will be taken to avoid pain. In case an animal suffers from pain, a veterinarian will be informed, and the animal will receive analgesics to relief the pain, if necessary.
The studies will be performed according the Dutch laws, and will cause no adverse effects on the environment.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Not applicable

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

X No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

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

X No > Continue with question H.

☐ 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.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

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

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

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

For the placement of the telemetric device in the abdomen, the animals will be anesthetized. Subsequently they will receive analgesics for as long as necessary. In previous studies, it was observed that animals can experience some fever during the first days after insertion of the temperature recording device, but have recovered very well within 1 week after the operation.
During the infection phase, the animals will be continuously monitored. Monitoring will be done by observation of the animals by the animal caretakers. As disease may develop rapidly, observation will be done minimally 3 times per day. Also, continuous telemetric monitoring of the changes in body temperature, as well as activity, will allow the early detection of signs of disease. Initially, monitoring of disease symptoms will be based on a clinical scoring list derived from published data. Listed are changes in body temperature (> 5%), changes in body weight (>20%), changes in activity and behavior, as well as changes in appearance (fur) due to dehydration and anorexia. Data obtained from the first infection studies may allow us to expand the scoring list for future use. This will then be communicated with the AWB. In case of symptoms caused by RVFV infection, this can result in distress. To avoid serious discomfort, the animals will then be euthanized.

I. Other aspects compromising the welfare of the animals

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

1. Discomfort because of insertion of the telemetric device.
2. Stress because of recovery from sedation
3. Discomfort due to RVF disease symptoms

Explain why these effects may emerge.

1. The surgery needed for insertion of telemetric device will cause pain and some local inflammation
2. Animals will be repeatedly sedated for virus infection and blood sampling. Nausea can sometimes be observed during recovery from the sedation and hypothermia and disorientation
3. RVFV infection of marmosets can cause disease symptoms

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

1. Surgery will be done under anesthesia and after surgery analgesics will be applied
2. Recovery of the animals is monitored and the veterinarian will intervene if animals do not recover fast enough
3. After infection, the animals are visually monitored minimally three times per day by animal caretakers, and are continuously monitored for changes in body temperature and activity using telemetry. If an animal shows clinical symptoms suggestive for RVF disease like changes in body temperature in combination with abnormal hematology parameters or behavior, the animals will be euthanized.

J. 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 K.

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

Animals are monitored daily by animal caretakers, and are continuously monitored for body temperature, and activity using a telemetric device. A clinical scorings list will be developed and used to assess if an animal shows clinical symptoms suggestive of RVF disease, like changes in body temperature in combination with abnormal biochemical parameters, or abnormal behavior. In such a case, the animals will be euthanized at the earliest time-point in order to avoid unnecessary

suffering. Blood biochemical parameters that are influenced by RVFV in common marmosets, include levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and blood urea nitrogen (BUN), indicators for liver function and kidney function, respectively. Animals may also show neurological signs, like instability and seizures, or show signs of anorexia and dehydration.
A minimal clinical scoring list based on published data will be used to assess RVF in the marmosets. The list includes decreased activity, changes in body temperature and body weight, and changes in blood hematological and biochemistry parameters. In case an animal shows an increase in ALP or ALT levels of > 200%, a loss of body weight of > 20%, or a change in body temperature of > 5%, this will be seen as indicator of severe RVF in marmosets. Then, a veterinarian will be consulted in order to assess if the animal has reached a humane end-point. If this is the case, the animal will be humanely euthanized at an earliest time point.

Indicate the likely incidence.

100%

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

Discomfort is caused by the implantation of the telemetric device. By using this device, the animals can be continuously monitored for body temperature or changes in activity. In combination with frequent observations by animal caretakers, this will facilitate the appropriate intervention by veterinarians at the earliest time-point, and will preclude progression to serious RFV disease. Therefore, the cumulative discomfort will be moderate.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

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

Animals will be euthanized in case they show signs of RVF disease symptoms in order to avoid severe discomfort. To investigate the presence of virus in tissues and organs, and for the investigation of possible tissue damage caused by RFV, it is necessary to euthanize the animals at the end of the study.

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.

X Yes

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 procedure.
- For more information, see 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'.	50200
1.2	Provide the name of the licenced establishment.	Biomedical Primate Research Centre
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 2 Type of animal procedure: Evaluation of RVFV vaccines and antiviral antibodies in common marmosets

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 will use a general study protocol for the evaluation of RVFV vaccines in common marmosets. Before the start of the study a telemetric device is surgically placed in the abdominal cavity that will enable the continuous monitoring of body temperature and activity. Subsequently, the animals are immunized and monitored for 1) the development of vaccine-induced immune responses, and 2) possible adverse effects on behavior and health. All the vaccines that will be evaluated in this project have first been successfully evaluated for safety and efficacy in highly-sensitive target animals, like sheep. Therefore, they are expected to give no, or only very limited adverse effects in marmosets. In addition to the telemetric monitoring of body temperature and activity, the animals will be observed daily for changes in general behavior by animal caretakers, and at each sedation the animals will be visually inspected and the site of immunization will be checked for local reactions. Blood will be drawn to measure clinical chemistry and hematology parameters. Blood collection will be done before, between and after immunizations to measure induction of systemic immune responses. Depending on the specific objective of the vaccine analysis, the immunization may be followed by experimental infection with RVFV in order to evaluate the protective capacity of a vaccine. Then, a group of non-vaccinated animals will be included as infection controls. The infection will be performed as described in Appendix 1. If the primary objective of the study is to investigate absence of pathogenicity, or to evaluate immunogenicity, animals will not be experimentally-infected with RVFV.

The primary outcome parameters for vaccine evaluation are:

- Immunogenicity: induction of adaptive immune responses.
- Efficacy: capacity to protect against viral challenge will be established in terms of absence or reduction of virus replication.
- Absence of clinical symptoms, absence of adverse effects of the vaccine on general behavior, absence of local reactions and changes in blood parameters.

In order to evaluate the efficacy of antiviral antibody therapies to combat, or even cure RVFV infection, we will use the following study set-up: a group of animals will first be experimentally infected with RVFV (as described in Appendix 1). Then, after a defined time-period, the animals will be administered the antibody compound. Blood samples will be collected at regular time points to determine if the viremia is influenced by the therapeutic administration of the monoclonal antibody. A group of animals will not receive the monoclonal antibody, and will be used as controls.
When a monoclonal antibody is evaluated for its prophylactic potential, i.e. to prevent infection, animals will first be administered the compound, and subsequently the animals will be experimentally infected with RVFV. During the study, blood is collected at regular time points and tested for the presence or absence of virus. The primary outcome parameter for the efficacy of RVFV antibody therapies will be the reduction of viral RNA load in plasma

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

Vaccine study

A telemetric device is surgically placed in the abdominal cavity at least 4 weeks before the first immunization takes place. This time frame is necessary for full recovery of the animals from the surgery and to allow adequate body temperature and activity recording during a two to three-week period to establish normal values before immunizations start. Animals will receive one or more immunizations, with 2-6 weeks intervals, although occasionally a longer time frame may be needed between immunizations, when studies in rodent models or natural animal hosts, like sheep or goats, indicate the necessity for longer time-intervals. Immunizations will be done by various routes, like intradermal, intramuscular, subcutaneous, or intravenous injection.
At regular time intervals after every immunization, blood is collected for analysis. If live-attenuated viral vaccines are used, virus replication will also be analyzed as part of the evaluation. The total amount of blood will be less than 1% of the body weight per month and less than 0,7% of body weight per bleeding. This amount can only be exceeded if the specific study requirements leave no other options, specific permission is obtained from the AWB and the veterinarian agrees, based on the health status of the animal. Immune responses measurable after the final immunization will be critical for the decision to continue with viral challenge. If these responses are inadequate then the study will be stopped and animals may be re-used in other non-RVFV virus-related experiments, if allowed. In case of a vaccine immunogenicity study with no efficacy evaluation, animals will be euthanized at this time-point, in order to investigate for possible vaccine-induced pathologies, and, in case of live-attenuated vaccines, for residual vaccine virus in tissues and organs.
Experimental challenge of the animals will be done as described in Appendix 1. Clinical symptoms will be monitored daily during the infection phase. The continuous monitoring of body temperature and activity by using telemetric devices will allow us to quickly respond to early indications of RVF symptoms. Blood is taken to monitor changes in clinical chemistry and hematology parameters, leukocyte subsets and cytokine production. At the same time points the body weight is recorded. The animals will be monitored for a period of maximally 4 weeks for vaccine efficacy. Then, they are humanely euthanized and a full necropsy is performed in order to evaluate pathology and the detection of residual challenge virus. In case an animal should reach the humane endpoint during the

study, it will be immediately humanely euthanized and a full necropsy will be performed. Also, tissues will be collected to determine virus replication in the different organs.
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.

Prophylactic antiviral antibody study:
At the start of the study, the animals will be administered the antiviral antibodies. At the same time blood is collected for a zero-value determination. Then, the animals are experimentally infected with RVFV (as described in Appendix 1). At that time point, a group of animals that did not receive the compound will also be infected, and will act as untreated controls in the study. Typically, after infection of the animals, blood will be collected every other day for a period of maximally 21 days to monitor the progress of the viral infection and to control for changes in clinical chemistry and hematology parameters. This intensive sampling is necessary because in this period significant and rapid changes in the amount of virus in the blood may occur in untreated animals. The total amount of blood will be less than 1% of the body weight per month and less than 0,7% of body weight per bleeding. This amount can only be exceeded if the specific study requirements leave no other options. Specific permission is obtained from the AWB and the veterinarian, and is based on the health status of the animal.
After the untreated control animals have become virus-negative in the PCR for the first time, the groups may be followed for an extra period of 2-4 weeks to confirm absence of the virus and to monitor for sudden re-activations of virus replication in any of the animals. At the end of the study, maximally 6 weeks after the start, the animals will be humanely euthanized and necropsy will be performed for the collection of tissue samples for histopathological and virological tests. The animals will be monitored daily during the study period for general behaviour, appetite, faeces, etc., and at each time-point when the animals are sedated, body weight will be measured.
Therapeutic antiviral antibody study:
The set-up of a therapeutic study using the antiviral antibodies is essentially similar to the prophylactic study. However, in such a study the animals are first infected and then treated with the antiviral compound.

The details of each study, regarding the route of infection, dose used, number of animals used, will be submitted for approval to the AWB.

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

Vaccine immunogenicity calculations take in account the number of animals required to detect significant induction of immune responses compared to unvaccinated controls. The minimal detectable alternative is 1.8 and 1.3 x standard deviations for 6 and 10 animals (based on $\alpha = 0.05$, $\beta = 0.2$ [power = 80%], Student t distribution), respectively.

For vaccine efficacy, calculations are performed to establish the number of animals required to detect vaccine efficacy, defined as: 1. a reduction in the number of infected animals (vaccine efficacy > 85% or 60% for 6 and 10 animals, respectively, analyzed by 2 x 2 contingency tables and Fisher's exact test), and 2. a reduction in serum virus load in the vaccine groups versus the challenge control group (log virus load is approximately normally distributed, statistical comparisons will be done by Student t-test). Like for the immunogenicity testing, the minimal detectable alternative is 1.8 and 1.3 x standard deviations for 6 and 10 animals (based on $\alpha = 0.05$, $\beta = 0.2$ [power = 80%], Student t distribution), respectively.

Only the minimum number of animals per group needed, will be used. When historical data are

available on infection in unvaccinated animals (Appendix 1), usually fewer animals can be used in the non-vaccinated challenge control group than in the vaccine groups. For each individual study, the power analysis will be communicated with the AWB.

The statistical analysis for the NHP antiviral antibody studies will also focus on the plasma viral load (PVL) as primary read-out parameter, and like for vaccine efficacy calculations are performed to establish the number of animals required to detect vaccine efficacy, defined as: 1. a reduction in the number of infected animals (vaccine efficacy > 85% or 60% for 6 and 10 animals, respectively, analyzed by 2 x 2 contingency tables and Fisher's exact test), and 2. a reduction in serum virus load in the vaccine groups versus the challenge control group (log virus load is approximately normally distributed, statistical comparisons will be done by Student t-test). Like for the immunogenicity testing, the minimal detectable alternative is 1.8 and 1.3 x standard deviations for 6 and 10 animals (based on α = 0.05, β = 0.2 [power = 80%], Student t distribution), respectively.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

The experiments will be performed with common marmosets (*Callithrix jacchus*), **maximally n = 108**. All marmosets are purpose bred at the institute, or incidentally they will be obtained from a certified supplier in compliance with EU legislation. Both mature male and female animals can be used.

For veterinary RVFV vaccine efficacy and safety evaluation, sheep are the preferred animal model as they are most sensitive for RVFV infection. Nonhuman primates (NHP) are the preferred species for the efficacy evaluation of novel human vaccines because NHPs have an immune system and physiology that are highly similar to that of humans. In addition, absence of residual pathogenicity of human vaccines is also preferably studied in NHP. NHP show disease symptoms of RVF similar to those found in humans, while ruminants show disease symptoms, like neonatal mortality and increased incidence of abortions and fetal malformations, that are not found in humans.

Historically, rhesus macaques have been used to evaluate potential RVFV vaccines and therapeutics, but recently, a new model for RVF has been described in common marmosets. This model overcomes some of the limitations of the macaque model, as marmosets are more susceptible to low dose challenge with RVFV than rhesus macaques and experience higher rates of morbidity, mortality, and viremia and marked aberrations in hematological and chemistry values. Depending on the route of exposure, these animals exhibit acute-onset hepatitis, delayed-onset encephalitis, and hemorrhagic disease, which are dominant features of human RVF (1,2,3,5).

In the guidelines for nonclinical evaluation of vaccines (4), the WHO states that a product should be characterized in a species sensitive to the biological effects of the vaccine being studied. Ideally, the species chosen should be sensitive to the pathogenic organism, and the animal species used should develop an immune response to the vaccine antigen. Based on the above, the common marmoset is chosen as the animal species for the studies.

The number of animals, requested for RVFV vaccine efficacy evaluation assumes that each study will contain two vaccine groups and 1 control group, with up to 10 animals per group. The group size will be determined per experiment, based on power calculations specific for the experiment. Variation in virus replication between animals has to be such that in a vaccine evaluation study significant protection against infection can be obtained with a limited number of animals per group. For a one dose challenge, usually n=6 per group suffices to reach statistical significance. Probably fewer animals

may be needed in the non-vaccinated challenge control groups if results from infection studies (Appendix 1) are used.

In all, we anticipate performing a maximum of 2 such studies over a 5-year period. Starting from maximally 10 animals per group with a maximum of 2 different vaccine candidates (or different combinations of routes of vaccination) + 1 control group per study (= 2 experimental groups + 1 control group, at n=10/group, with 2 studies, results in a maximum of 60 animals).

Typically, an antiviral efficacy study consists of one control group of max. 10 animals and one treatment group (max. 2 x 10 = 20 animals). In the remaining project period of 3 years we expect to perform a maximum of two antiviral efficacy studies, either prophylactic or therapeutic antibody studies.
In total 40 animals are the maximum needed for the efficacy studies over a period of 5 years.
(4 x 10 animals)

Over the study period of 5 years, we calculate that in total maximally 100 animals are needed for performing RFVF vaccine evaluation and antiviral antibody studies.

1. Hartman, A. L., Powell, D. S., Bethel, L. M., Caroline, A. L., Schmid, R. J., Oury, T., & Reed, D. S. (2014). Aerosolized Rift Valley Fever Virus Causes Fatal Encephalitis in African Green Monkeys and Common Marmosets. *Journal of Virology*, *88*(4), 2235–2245.
2. Smith, D. R., Bird, B. H., Lewis, B., Johnston, S. C., McCarthy, S., Keeney, A., et al. (2012). Development of a Novel Non-human Primate Model for Rift Valley Fever. *Journal of Virology*, *86*(4), 2109–2120.
3. Smith, D. R., Holbrook, M. R., & Gowen, B. B. (2014). Antiviral Research. *Antiviral Research*, *112*(C), 59–79.
4. WHO. (2005). *Annex 1WHO guidelines on nonclinical evaluation of vaccines* (Vol. 927, pp. 32–63). WHO Technical Report Series.
5. Wonderlich, E. R., Caroline, A. L., McMillen, C. M., Walters, A. W., Reed, D. S., Barratt-Boyes, S. M., & Hartman, A. L. (2018). Peripheral Blood Biomarkers of Disease Outcome in a Monkey Model of Rift Valley Fever Encephalitis . *Journal of Virology*, *92*(3), e01662–17.

C. Re-use

Will the animals be re-used?

☐ No, continue with question D.

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

Animals that will be used in these experiments, might have been used in previous experiments. Animals that have been involved in previous RVFV studies or that have pre-existing antibodies against RVFV are not suitable. In view of the long life 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'?

X No

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

D. 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

Nonhuman primates have been used to evaluate RVF vaccines, because NHPs have the advantage that they physiologically and immunologically most closely resemble humans. This has important implications, both for vaccine efficacy evaluation, as well as for the interaction of the host with RVFV, since these are affected both by the physiology and by the reaction of the innate and adaptive immune system. The proper evaluation of novel vaccines requires adequate infection models in NHP, which is the purpose of the studies proposed here. Ideally, the species chosen should be sensitive to the pathogenic organism, and the animal species used should develop an immune response to the vaccine antigen. The species of choice in the proposed studies, the common marmoset, not only faithfully mimics human RVF in viremia, but also shows RFV clinical symptoms and pathology as are also seen in severe human RVF. Based on the above, the common marmoset is chosen as the animal species for our studies.

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 level of protection or reduction in virus load in the circulation between the experimental groups and the challenge control group. Only the minimum number of animals needed will be used. Data will become available on infection in unvaccinated animals (Appendix 1). When using these data usually fewer animals may be used in the challenge control group than in the experimental groups. Furthermore, we aim to evaluate multiple vaccine candidates in a single experiment, so that a single challenge control group can be used.

Refinement

The animals are trained to cooperate as much as possible with the invasive biotechnical handlings, such as receiving a sedation or virus infection. In consultation with our collaborators, the number of blood samplings, and the collected volumes of blood are reduced to a minimum. A highly sensitive real-time PCR will allow a very accurate determination of the virus load in small blood volumes collected from infected marmosets.

The use of telemetric devices to measure body temperature and activity makes it possible to real-time monitor and collect data from the animals, allowing the veterinary staff to take action at the earliest time-point if any of these parameters is influenced by the RVFV infection, even before detected by visual inspection. Thus, adequate action can be taken before an animal reaches its humane endpoint. Placement of the telemetric devices will require surgery, which will be done under anesthesia. Subsequently animals will receive analgesics as long as required. The use of a smaller, novel telemetric device (Anipill 0.1C, DSI™; 17mm x 8 mm; 1.7 grams) than that has been used in previous marmoset studies will cause less discomfort to the animals. The use of a clinical scorings list and **24/7 camera surveillance of the animals that are in experiment**, in combination with the use of temperature/activity telemetric devices makes it possible to continuously monitor and collect data from the animals, allowing the veterinary staff to act as soon as possible when any of these parameters is influenced by the RVFV infection. Marmosets will be trained to stand on a scale themselves, making it possible to determine changes in body weight without anesthesia.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Animals will be socially housed with a socially compatible animal, whenever possible. There is an extensive program for environmental enrichment in the institute.
All experimental procedures will be performed under sedation. Placement of the telemetric devices will require surgery, which will be done under anesthesia. Subsequently animals will receive analgesics as long as required. Animals are trained to cooperate as much as possible with the invasive biotechnical handlings, such as receiving a sedation or virus infection. Each time an animal is sedated for blood collection or immunization, the animal will be weighed, and the animal will be closely examined. The institute uses a customized database that documents all individual animals in the institute. General observations like behavior, appetite and stool are part of this database. This database thus facilitates early recognition of minor changes in these general parameters. During the study, care will be taken to avoid pain. In case an animal suffers from pain, a veterinarian will be informed, and the animal will receive analgesics to relief the pain, if necessary.
The studies will be performed according the Dutch laws, and will cause no adverse effects on the environment.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Not applicable

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

X No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

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

X No > Continue with question H.

☐ 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.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

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

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

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

For the placement of the telemetric device in the abdomen, the animals will be anesthetized. Then, they will receive analgesics for as long as necessary. In previous studies, it was observed that animals can experience some fever during the first days after insertion of the temperature recording device, but have recovered very well within 1 week after the operation.
During the infection phase, the animals will be continuously monitored. Monitoring will be done by observation of the animals by the animal caretakers **and by camera surveillance**. As disease may develop rapidly, observation will be done minimally 3 times per day. Also, continuous telemetric monitoring of the changes in body temperature, as well as activity, will allow the early detection of signs of disease. Monitoring of disease symptoms will be based on a clinical scoring list developed as described in Appendix 1. Listed symptoms include changes in body temperature (> 5%), changes in body weight (>20%), changes in activity and behavior, as well as changes in appearance (ruffed fur) due to dehydration and anorexia. In case of symptoms caused by RVFV infection, this can result in distress. To avoid serious discomfort, the animals will then be euthanized.

I. Other aspects compromising the welfare of the animals

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

1. Discomfort because of insertion of the telemetric device.
2. Discomfort due to administration of vaccines and virus
3. Stress, loss of appetite because of recovery from sedation
4. Discomfort due to RVF disease symptoms

Explain why these effects may emerge.

1. The surgery needed for insertion of the telemetric device will cause pain and some local inflammation
2. Administrations can cause local irritation
3. Animals will be repeatedly sedated for virus infection, immunizations, and blood sampling. Nausea can sometimes be observed during recovery from the sedation
4. RVFV infection of marmosets can cause disease symptoms

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

1. Surgery will be done under anesthesia and pre- and post surgery analgesics will be applied.
2. If local irritation occurs the severity will be minor. Therefore, no extra actions are needed.
3. Recovery of the animals is monitored and the veterinarian will intervene if animals do not recover fast enough.
4. After infection, the animals are visually monitored minimally three times per day by animal caretakers, and are continuously monitored for changes in body temperature and activity using telemetry. If an animal shows clinical symptoms suggestive for RVF disease like changes in body temperature in combination with abnormal hematology parameters or behavior, the animal will be euthanized.

J. 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 K.

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

Animals are monitored daily by animal caretakers, and are continuously monitored for body temperature, and changes in activity using a telemetric device. If an animal shows clinical symptoms suggestive for RVF disease, like changes in body temperature in combination with abnormal

hematology parameters or abnormal behavior, the animals will be euthanized. Blood biochemical parameters that are influenced by RVFV in common marmosets, include levels of alkaline phosphatase and blood urea nitrogen, that are indicators for liver function and kidney function, respectively. Animals may also show neurological signs, like instability and seizures, or show signs of anorexia and dehydration. The use of a clinical scorings list (Appendix 1), will facilitate the early detection of disease symptoms and avoid unnecessary suffering of the animals. Thus, animals can be humanely euthanized at an early time-point of RVF, prior to severe disease symptoms.

Indicate the likely incidence.

100% of infected animals may develop RVFV disease

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

The cumulative amount of discomfort is estimated as moderate. This is mainly caused by the implantation of the telemetric device and development of disease due to infection

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

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

Animals will be euthanized in case they show signs of RVF disease symptoms in order to avoid severe discomfort. To investigate the presence of virus in tissues and organs, and for the investigation of possible tissue damage caused by RFV, it is necessary to euthanize the animals at the end of the study.

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.

X Yes

Van: Info-zbo
Aan: 10.2.e
Cc:
Onderwerp: Ontvangstbevestiging melding AVD5020020174224-1
Datum: donderdag 13 februari 2020 09:47:06
Bijlagen: Ontvangstbevestiging Melding AVD5020020174224-1.pdf

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Geachte 10.2.e

In de bijlage treft u de ontvangstbevestiging van melding aan, waarnaar wij gemakshalve naar verwijzen.

Met vriendelijke groet,

Centrale Commissie Dierproeven **www.centralecommissiedierproeven.nl**
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Onze referentie
Aanvraagnummer
AVD5020020174224-1

Datum 13 februari 2020
Betreft Ontvangstbevestiging Melding projectvergunning dierproeven

Geachte 10.2.e

Wij hebben op 12 februari 2020 een melding ontvangen op uw projectvergunning dierproeven. Het gaat om uw project "Evaluation of Rift Valley Fever Virus vaccines in common marmosets" met aanvraagnummer AVD5020020174224, waarvoor op 19 maart 2018 een vergunning is afgegeven. Uw melding is bij ons geregistreerd onder aanvraagnummer AVD5020020174224-1.

U geeft aan dat zowel het ongerief voor de dieren als het aantal dieren niet verandert.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.